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Profiling hymenopteran venom toxins: Protein families, structural landscape, biological activities, and pharmacological benefits

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ABSTRACT

Hymenopterans are an untapped source of venom secretions. Their recent proteo-transcriptomic studies have revealed an extraordinary pool of toxins that participate in various biological processes, including pain, paralysis, allergic reactions, and antimicrobial activities. Comprehensive and clade-specific campaigns to collect hymenopteran venoms are therefore needed. We consider that data-driven bioprospecting may help prioritise sampling and alleviate associated costs. This work established the current protein landscape from hymenopteran venoms to evaluate possible sample bias by studying their origins, sequence diversity, known structures, and biological functions. We collected all 282 reported hymenopteran toxins (peptides and proteins) from the UniProt database that we clustered into 21 protein families from the three studied clades - wasps, bees, and ants. We identified 119 biological targets of hymenopteran toxins ranging from pathogen membranes to eukaryotic proteases, ion channels and protein receptors. Our systematic study further extended to hymenopteran toxins' therapeutic and biotechnological values, where we revealed promising applications in crop pests, human infections, autoimmune diseases, and neurodegenerative disorders.

1. Introduction

Interactions between venomous wildlife and humans often result in anthropocentric hostility and occasional death. Such interactions have motivated adrenaline-fuelled research studies of the world's deadliest species shaping the hypotheses around venom composition, venom evolution, and the development of anti-venoms, bioinsecticides and pharmaceuticals (Utkin, 2015). Venom research studies have predominantly focused on snakes, spiders, scorpions, and cone snails, leaving out the less threatening arthropods such as ants or butterflies (Sekimura and Nijhout, 2017). With more than 154,000 extant species, hymenopterans represent an untapped source of venom secretions, and possibly an unique chemical diversity with formidable biological properties (Huber, 2017).

The remarkable ecological diversity of hymenopterans across most terrestrial environments along with their complex social behaviours and chemical communication have contributed presumably to the diversity of venom types. For examples, many ants have developed a highly specialised diet, such as Ponerinoid ants *Psalidomyrmex procerus* feeding on earthworms and *Megaponera analis* preying a limited number of termites, suggesting the presence of specialised venom secretions (Dejean et al., 1999; Frank and Linsenmair, 2017). Similar to solitary wasps, some ant species hunt in small groups or alone suggesting that their venom secretions are sufficiently potent to subdue rapidly their prey *i.e.*, caterpillars, crickets or spiders (Cerdá and Dejean, 2011).

Hymenopteran venoms are versatile cocktails of biochemical structures that have been optimised by evolution to select protein targets towards defence against predators or pathogens, prey capture, parasitism and communication (Casewell et al., 2013). With regards to their chemical nature, these venoms are filled with an extraordinary pool of toxins and non-toxic compounds such as salts, sugars, formic acid, biogenic amines, alkaloids, free amino acids, hydrocarbons, peptides and proteins. Earlier scientific evidence suggested that the toxins would participate in an array of biological processes including pain, paralysis,

Abbreviations: AMP, antimicrobial peptide; ICK, inhibitor cystine knot; MCD, mast cell degranulating peptide; PDB, Protein Data Bank; pLDDT, predicted local distance difference test.

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cytolysis, haemolysis, allergic reactions, pro-inflammatory response, insecticidal and antimicrobial activities. In contrast, the non-toxic venom components might be involved in chemical communication as deterrents, aggregators, sex pheromones and trail markers. Here, we analysed 282 hymenopteran venom-derived proteins; their origins (*i.e.* 81 species), their sequences and structures, and their activities against one of the 119 biological targets. We extended our study to 7 therapeutic and biotechnological values of hymenopteran toxins to human and non-human applications.

2. Methods

We screened all sequences from the UniProt database (https://www.UniProt.org/) (The UniProt Consortium, 2021). Alongside their protein sequences, we gathered associated information about accession code, species, protein family, toxin name, toxin length, gene ontology, and key references. We enriched the dataset by adding toxin identification (ID) and family (PFAM). According to the PFAM database, we clustered all toxins into 21 protein families, labelled from *A* to *V* (Mistry et al., 2021). We manually curated each reference to annotate their associated biological activities, plausible targets (*e.g.* microorganisms) and therapeutic applications. We removed all duplicated sequences and those from non-hymenopteran species. Our dataset included the toxins derived from the Aculeatoxin gene superfamily (Robinson et al., 2018) as well as homologous sequences (with conserved signal peptide and propeptide sequences) using the Basic Local Alignment Search Tool (BLAST) program. In total, we listed 282 hymenopteran toxins from 81 species, listed in Supplementary Material Table S1.

We then created all diagrams and plots using the R language (R Core Team, 2021) and Rstudio interface (RStudio Team, 2021). The chord diagram (Fig. 1) was created using the R packages "circlize" (Gu et al., 2014), "colorspace" (Zeileis et al., 2020), and "RcolorBrewer" (Cynthia, 2021). The barplot (Fig. 4A) was created using the R packages "ggplot2" (Wickham, 2009) and "RcolorBrewer" (Zeileis et al., 2020). The sunburst plot (Fig. 4B) was made using the R packages "plotly" (Carson, 2020) and "RcolorBrewer" (Cynthia, 2021). An interactive version of that plot is available in Supplementary Material Fig. 4B. html. To visualise sequence similarity between toxins from the same or distinct protein families (Figs. 2–3), we implemented multiple slow and accurate pairwise sequence alignments using online Clustal W (https://www.genome.jp/tools-bin/clustalw). Those figures also highlighted the structural diversity of hymenopteran toxins and related proteins from other organisms using PyMOL Molecular Graphics System version 2.5.2 (Schrödinger, L. & DeLano, W, 2020. PyMOL, available at: http://www.pymol.org/pymol, professional licence). We either used the reported Protein Data Bank (PDB) structure (*e.g.* 1QNX) or the predicted AlphaFold model (*e.g.* AF-P0DSL6-F1) from the UniProt database. All AlphaFold models were selected with a moderate-high level of confidence (pLDDT >70). In all figures, we added informative texts (*e.g.* labels, representative organisms, PDB entries) with Acrobat Illustrator software version 26.0.1.

3. Results

3.1. The origins and structures of hymenopteran venom toxins

Hymenopterans species are the most diverse venomous order. They are divided into two suborders; Symphyta (sawflies and horntails) and, Apocrita (wasps, bees and ants) (Aili et al., 2014; Branstetter et al., 2017; Senji Laxme et al., 2019). Apocrita suborder splits into two infraorders or clades; the monophyletic Aculeata (stinging wasps) and the now obsolete, paraphyletic Parasitica/Terebrantia (parasitic wasps). The latter includes all Apocrita except for the Aculeata, members of that infraorder are often referred as the terebrant(e)s. The Aculeata clade also includes Formicidae (ants) and Anthophila (bees and bumblebees) (Branstetter et al., 2017). We first mapped out the origins of currently known hymenopteran toxins by gathering sequences from the UniProt database (https://www.UniProt.org/) (The UniProt Consortium, 2021). The different toxin families, their relative abundances and origins are illustrated in Fig. 1. We observed that nearly half (125, 44%) were isolated from terebrants and Aculeata wasps, 112 (40%) were identified from stinging ants (Formicidae), and a small group of proteins 45 (16%) were reported from bees. We then grouped all venom toxins in 21 families or superfamilies (*A-V*) according to their sequence homology.

Toxin (super)family	Code	Toxin diversity
Aculeatoxin	A	63
Myrmeciitoxin	B	2
Allergen 2/4	C	5
Alergen 3/5 (CRISP)	D	18
Ectatomin	E	7
Lipase	F	17
Myrmexin	G	5
Ponericin	H	29
Poneritoxin Ae1	I	7
Unknown	J	58
Glycosyl hydrolase	K	7
Peptidase S	L	6
Phospholipase A2	M	4
Secapin	N	3
Serine protease inhibitor-like	O	3
Kunitz-type	P	5
Bradykinin-related peptide	Q	9
Damage-control phosphatase	R	1
Protease inhibitor	S	1
MCD	T	28
Ntn-hydrolase	U	1
Metalloproteinase (M12B)	V	3

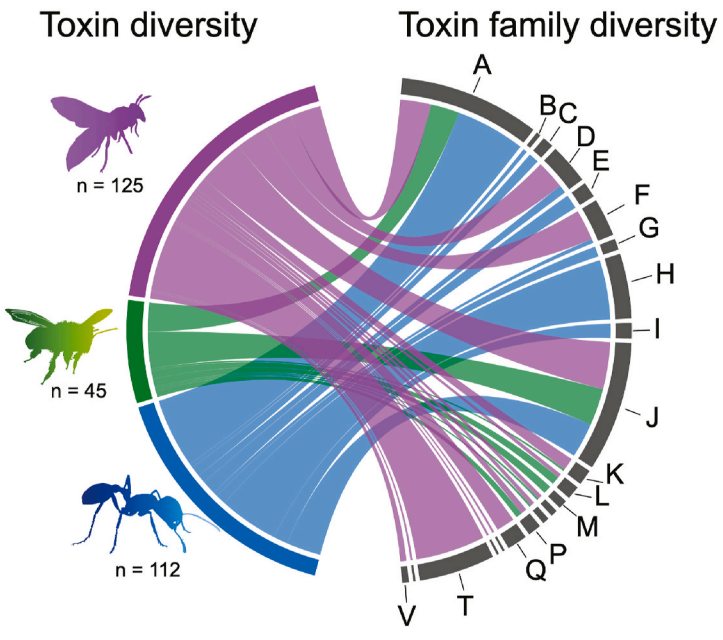

Fig. 1. Origins of hymenopteran toxins. On the left side, the table displays the 21 families or superfamilies (*A–V*) with their respective abundances. On the right side, a chord diagram shows the 282 reviewed hymenopteran toxins from 81 species spread across the three clades; Apocrita and Aculeata wasps (purple), Anthophila species (green), and Formicidae species are depicted in blue.

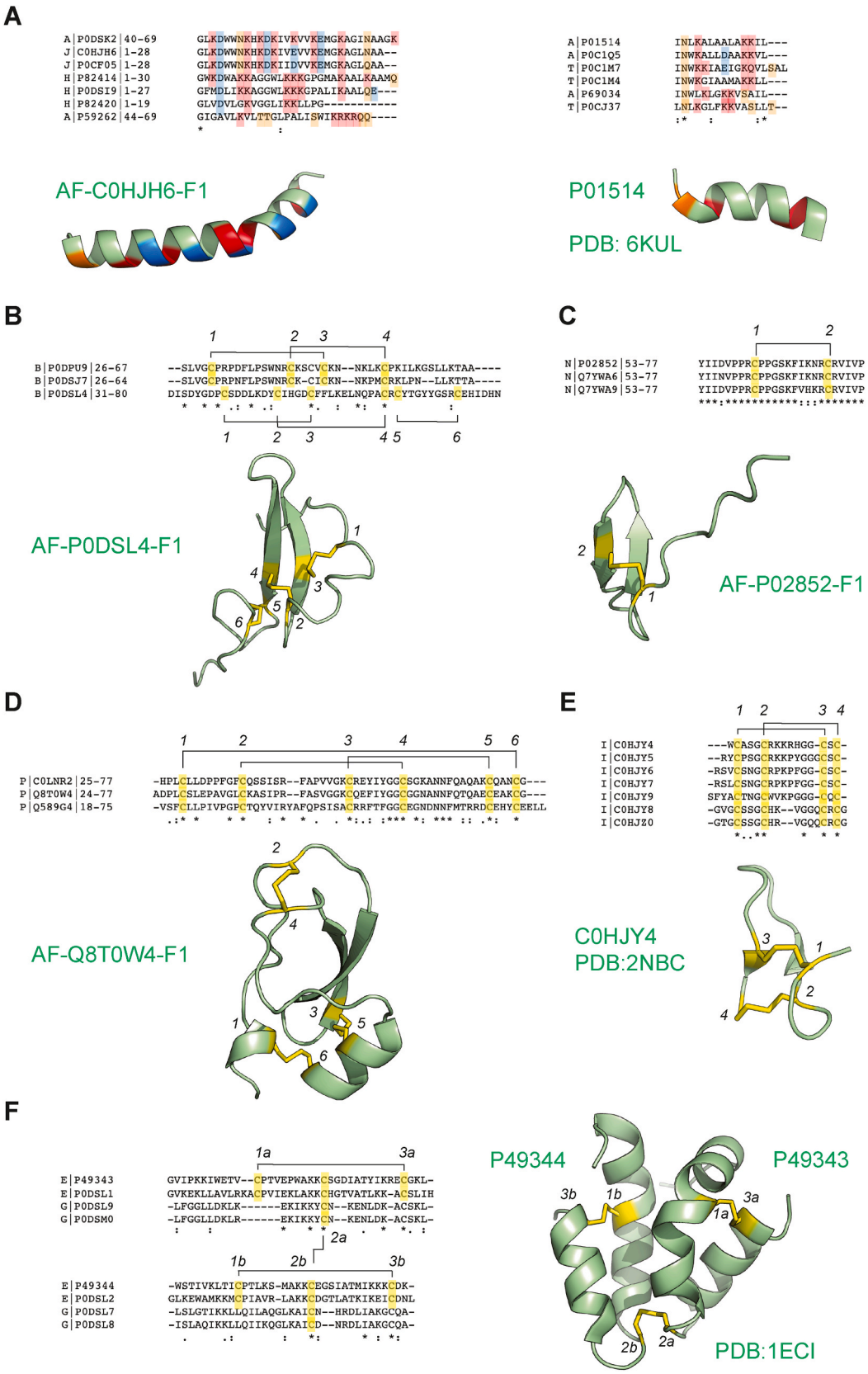

Fig. 2. Multiple sequence alignments and representative toxin structures. (**A**) Amphipathic α-helices: antimicrobial peptides (left) and mast cell degranulating peptides (right). Polar residues, positively charged residues, negatively charged residues are depicted in orange, red and blue, respectively. (**B**) β-hairpins with 2–3 intramolecular disulfide bridges: myrmeciitoxins, (**C**) β-hairpins with a single intramolecular disulfide bridge: secapins, (**D**) Kunitz-type protease inhibitors, (**E**) Small β-hairpins with 2 intramolecular disulfide bridges: poneritoxins and (**F**) Cysteine-rich heterodimers: ectatommins and myrmexins. Disulfide bridges are depicted in yellow and their cysteine connectivities are indicated with numbers *1*–6 and letters *a,b*. All AlphaFold models were selected with a moderate-high level of confidence (pLDDT >70). Slow and accurate multiple sequence alignments were performed using ClustalW.

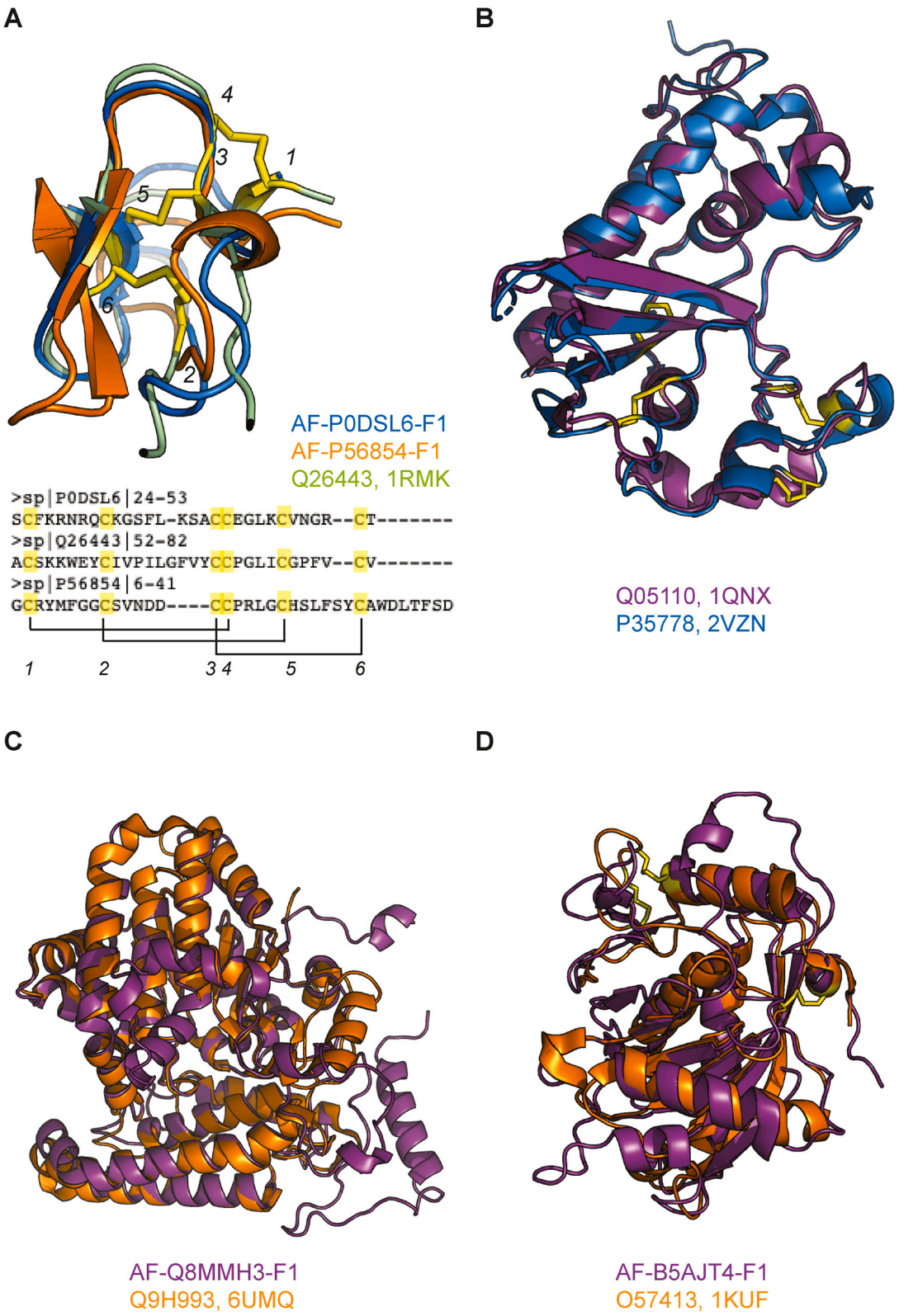

Fig. 3. Structural alignments between hymenopteran toxins and proteins from other organisms. (**A**) Conserved inhibitory cystine knot from ant *Dinoponera quadriceps* U1-PONTX-Dq5a (Entry: P0DSL6, AF model: AF-P0DSL6-F1, blue), from tarantula *Hysterocrates gigas* ω-theraphotoxin-Hg1a (P56854, AF-P56854-F1, orange) and from cone snail *Conus marmoreus* μ-conotoxin MrVIB (Q26443, PDB: 1RMK, green), (**B**) Allergens from wasp *Vespa vulgaris* (Q05110, 1QNX, purple) and ant *Solenopsis invicta* (P35778, 2VZN, blue), (**C**) Damage-control phosphatases from wasp *Pimpla hypochondriaca* (Q8MMH3, AF-Q8MMH3-F1, purple) and *Homo sapiens* (Q9H993, 6UMQ, orange) and (**D**) Overlapping segments of M12B metalloproteases from wasp *Eulophus pennicornis* (B5AJT4, AF-B5AJT4-F1, purple) and snake *Protobothrops mucrosquamatus* (O57413, 1KUF, orange). Disulfide bridges are depicted in yellow. All AlphaFold models were selected with a moderate-high level of confidence (pLDDT >70). Slow and accurate multiple sequence alignment was performed using ClustalW.

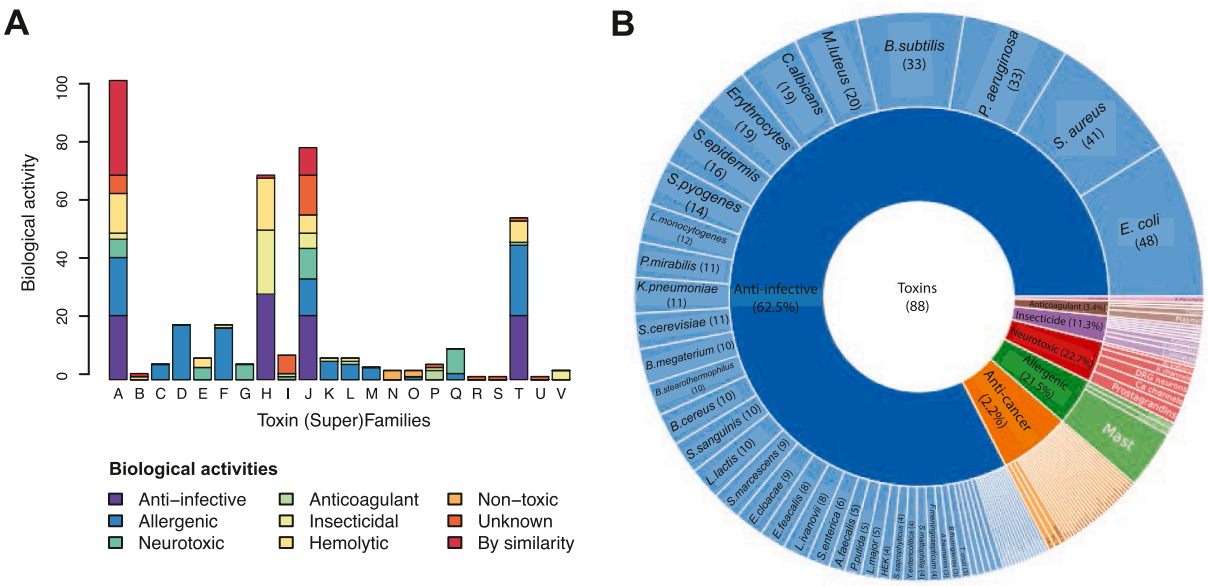

Fig. 4. Biological activities and applications of hymenopteran venoms. (**A**) The stacked barplot displays the 9 most common biological activities (colour-coded) in relation to each of the 21 hymenopteran toxin superfamilies (*A*–*V*). (**B**) Sunburst plot showing the biological applications for 88 hymenopteran toxins and their targets. An interactive version of that plot is available in Supplementary Material Fig. 4B. html.

The jointed table, on the left, summarised their relative abundances. In contrast, the chord diagram, on the right, indicated the same 21 toxin superfamilies spread across the three clades - ants (blue), bees (green), and wasps (purple).

3.1.1. *Toxin families shared between the three clades*

The aculeatoxin protein family (*A*) was the most abundant protein family overall, counting 63 out of 282 sequences, from which more than half (34) belong to ant venoms (Fig. 1). The other half was isolated from wasp and bee venom components. These toxins are believed to dominate the ant venom gene pool (Robinson et al., 2018) along with the ponericins (*H*). The hymenopteran Aculeata gene superfamily encoded for all 63 toxins, which possess similar signal peptides and a repetitive highly anionic propeptide sequence. Their mature sequences typically fold into α-helical peptides with amphipathic characters (Fig. 2A, *left*). Among the most representative examples of aculeatoxins, we could mention the membranolytic melittin (P59262) identified in various bees and wasps, and U-MIITX$_1$-Mg1a (P0DSJ4) from the painful red bull ant *Myrmecia gulosa*.

The second-largest proportion of toxins (*J*) did not constitute a protein family *per se*, with 58 representatives identified across all three clades. This cluster consisted of non-homologous proteins, ranging from 8 to 246 residues. Some *J* toxins, like the M-PONTX-Da4b from neotropical ant *Dinoponera australis* and ampulexins (*e.g.* A0A1W6EVM7) from the venom of the parasitic emerald wasp *Ampulex compressa*, presented high sequence homology to the melittin as mentioned earlier (P59262, *A*) and likely fold into α-helical structures (Fig. 2A, *left*). Of note, ampulexins could be related to the parasitic activity that allows wasps to turn cockroaches into zombies (Moore et al., 2018). Other *J* toxins displayed sequence similarity to toxins reported from other venomous species. For example, U1-PONTX-Dq5a (P0DSL6) is a toxin from Brazilian ant *Dinoponera quadriceps* with an inhibitor cystine knot (ICK) motif exhibiting neurotoxic activity (Torres et al., 2014). The toxin shares structural similarity to the ω-theraphotoxin-Hg1a (P56854) from Cameroon red baboon tarantula *Hysterocrates gigas* and the μ-conotoxin MrVIB (Q26443) from cone snail *Conus marmoreus* (Aili et al., 2014). Their conserved ICK motif containing cysteine connectivities 1–4, 2–5, 3–6 is depicted in Fig. 3A. This motif is a common protein scaffold spread across fungi, plants, marine molluscs and arthropods, particularly among spider toxins (Craik et al., 2001; Pallaghy et al., 1994). Undheim and coworkers considered the well-conserved structure a valuable molecular probe to study venom toxin evolution (Undheim et al., 2016). In 2020, the King group further supported that claim revealing that the many ICK-rich toxins (e.g δ-hexatoxins) within funnel-web spiders toxic arsenals originated from the duplication and diversification of a single knottin gene (Herzig et al., 2020; Pineda et al., 2020). Its remarkable stability against temperature and pH changes, and proteolysis, has also translated into the development of highly stable bioactive proteins (González-Castro et al., 2020; Wang and Craik, 2018). The rest of the cluster included toxins lacking structural and biological information.

3.1.2. *Toxin families shared between two clades*

Ant and wasp venoms contained 17–18 proteins from the CRiSP [Cysteine Rich Secretory Proteins] and lipase families (*D*, *F*). CRiSP members are cysteine-rich proteins with high sequence homology that share a C-terminal CAP domain (cd00168: cysteine-rich proteins, antigen 5, pathogen-related 1 proteins), found in prokaryotes and eukaryotes. In hymenopterans, toxins from the CRiSP family are almost exclusively allergens 5 from wasp venoms and 3 from ants venoms (Fig. 3B). Both proteic groups were reported to cause allergy leading to possible anaphylactic events or even death (Blank et al., 2020). Ants and wasps also share lipases (*F*) with type 1 phospholipid hydrolysis activity. These enzymes hold a highly conserved catalytic site, yet their loops and cysteine frameworks differ. Lipase-derived envenomation led to an allergic reaction by cross-reaction of immunoglobulin E (IgE) antibodies and hydrolysis of phosphatidylcholine (Hoffman et al., 2005; Torres et al., 2014).

Bees and wasps shared toxins from glycosyl hydrolase (*K*), peptidase S (*L*), secapin (*N*), serine protease inhibitor (*O*), and Kunitz-type protease inhibitor (*P*) families. The remaining protein families belong to a single clade. The hymenopteran *K* family contains highly conserved enzymes called glycosyl hydrolases/hyaluronidases. The crystal structure of wasp-derived hyaluronidase A (PDB: 2ATM, unshown) displays a globular 313-residue protein with multiple α-helices, β-sheets and two distant disulfide bridges (Skov et al., 2006). Sensitised patients to hyaluronidases might exhibit an IgE reaction to other hymenopteran hyaluronidase-like proteins (Kolarich et al., 2005). Bees and wasps also

secreted peptidases S (*L* protein family), which are proteases including a trypsin-like domain (S1) with variable structural loops and a dipeptidyl peptidase-type (S9) similar to the human dipeptidyl peptidase 4. These enzymes have also reacted with IgE (Blank et al., 2010; Winningham et al., 2004). Alongside enzymes, the hymenopterans produce ~25 residue-long peptides named secapins (*N*). Secapins are multifunctional peptides folding into small β-hairpins that are stabilised by a single disulfide bridge (Lee et al., 2016), like the AlphaFold model AF-P02852-F1 in Fig. 2C. The serine protease inhibitor family (*O*) is a group of cysteine-rich toxins with a trypsin inhibitor-like domain marked by a single conservative framework of five disulfide bridges (Michel et al., 2012; Parkinson et al., 2004). Finally, the Kunitz-type family (*P*) are 58 residue-long peptides from bumblebee and wasp venoms that contain a Kunitz-like domain. These peptides consist of α-helices and β-sheets, stabilised by three disulfide bridges with cysteine connectivities 1–6, 2–4, 3–5 (AF-Q8T0W4–F1, Fig. 2D). These peptides were also known to inhibit serine proteases (Choo et al., 2012; Yang et al., 2009). Like the ICK motif, Kunitz-type domain is a highly stable protein domain found in serine protease inhibitors across microbes, plants and animals. The protease inhibitors have acted as potential allergens, immunomodulators or anticoagulant factors (de Magalhães et al., 2018; Ranasinghe and McManus, 2013).

3.1.3. Bee-specific toxins

Bees and bumblebees were the sole producers of phospholipase A2 enzymes (*M*), PLA2 for short. These are stable and highly conserved cysteine-rich enzymes, including a calcium-dependent domain for phosphoglycolipid hydrolysis (Shipolini et al., 1974; Kawakami et al., 2017). Ferreira-Junior and co-workers observed that the concentrations of PLA2 enzymes in total venom would vary seasonably in Africanized honey bees *Apis mellifera* (Ferreira-Junior et al., 2010). These phospholipases share similarities to those found in arachnids, insects, snake venoms or mammals (Kuchler et al., 1989; Nicolas et al., 1997). More recently, in 2020, Aili and co-workers reported the presence of PLA2 enzymes in bullet ants *Paraponera clavata* (Aili et al., 2020). These enzymes were initially absent from the UniProt database and were not included in our dataset for analysis.

3.1.4. Ant-specific toxins

Ant venoms contained exclusively myrmeciitoxins (*B*), venom allergens 2/4 (*C*), ectatomins (*E*), myrmexins (*G*), ponericins (*H*), and poneritoxins Ae1 (*I*). They are low molecular weight peptides with additional features (*i.e.*, disulfide bridges, exempt of propeptide sequence), grouped into four subfamilies based on their structural similarities. Ant toxins U-MIITX$_1$-Mg4a (P0DPU9) and U-MIITX$_1$-Mg4b (P0DSJ7) are two peptides belonging to the myrmeciitoxin family (MIITX), identified from the giant red bull ant *Myrmecia gulosa* (Robinson et al., 2018). Numerous ant toxins (*i.e.* pilosulins, pilosulin-like peptides, dinoponera-like toxins and related peptides) share similar signal peptide and propeptide sequences suggesting that most might belong to the single aculeatoxin gene superfamily (*A)* (Robinson et al., 2018). In contrast, both myrmeciitoxins, and the third toxin MIITX$_2$-Mg1a (P0DSL4), lack that propeptide sequence; together, they form the distinct *B* protein family. Their predicted structures, illustrated in Fig. 2B with the AlphaFold model AF-P0DSL4-F1, describe myrmeciitoxins as β-hairpins surrounded by two loose ends, all tightened with 2 or 3 disulfide bridges (cysteine connectivities 1–3, 2–4, and occasionally 5–6). Eagles and co-workers recently reported the solution structure of MIITX$_2$-Mg1a (P0DSL4) in the Protein Data Bank under the code 7R6P (Eagles et al., 2022). Its signal and propeptide sequences were also reported in the UniProt database, repositioning the toxin among aculeatoxins (*A*). In addition to these three toxins, ants secreted the venom allergens 2 and 4, mostly isolated from the venom of fire ants *Solenopsis* sp, representing the *C* protein family. These proteins own a conserved cysteine framework called venom allergen Sol i II (P35775) and likely originate from the same gene family. Venom allergens 2 are well-conserved, sharing 77% sequence homology and some 40% sequence similarity with allergens 4. Unlike other venom allergens such as phospholipases, venom allergens 2 and 4 do not belong to the CAP allergens. They are devoid of phospholipase-like activity (Hoffman, 1993). Next, two related peptide heterodimers were ectatomins (*E*) and myrmexins (*G*). The ectatomins (*E*), isolated from ant *Ectatomma tuberculatum*, comprised 34 and 37 residue-long subunits. The myrmexins, found in ant *Pseudomyrmex triparius*, consisted of two shorter subunits of 29 and 33 amino acids. The tridimensional PDB structure 1ECI in Fig. 2F illustrates the general structure adopted by these heterodimers. The two antiparallel α-helical subunits (P49343, P49344) of *E* toxin ω/M-ECTX-Et1a are connected *via* the intermolecular disulfide bridge *2a-2b* (Pan and Hink, 2000; Pluzhnikov et al., 1999). That figure also indicated that ectatomins (*E*) included two additional disulfide bridges (*1a-3a*, *1b–3b*) within their antiparallel α-helices. Finally, ponericins (*H*) and poneritoxins Ae1 (*I*) are amphipathic peptides containing 14 to 30 residues. The ponericins commonly fold into α-helical structures and cluster into subfamilies based on sequence homology with other amphipathic peptides such as melittins, cecropins, and dermaseptins (Aili et al., 2014; Touchard et al., 2016) - Fig. 2A (*left*). The poneritoxins Ae1 form β-hairpins constrained by two disulfide bridges with the four-cysteine connectivities 1–3 and 2–4 (PDB: 2NBC, Fig. 2E).

3.1.5. Wasp-specific toxins

Wasp venoms included both low molecular weight peptides (*i.e.* bradykinin-related peptides (*Q*), protease inhibitors (*S*), and mast cell degranulating [MCD] peptides (*T*)) and larger enzymes (*i.e.* damage-control phosphatases (*R*), Ntn-hydrolases (*U*), and M12B metalloproteinases (*V*)). Bradykinin-related toxins (*Q*) are linear peptides of 15 residues in length on average with a conserved bradykinin-like domain (Čeřovský et al., 2007; Mendes and Palma, 2006; Rocchi et al., 2009; Yoshida et al., 1975). Biochemical and recombinant DNA techniques permitted the discovery of bradykinin and its related peptides across many venomous frogs, snakes and wasps (Lameu et al., 2013). Screening the UniProt database, we did not find any structural information for these linear *Q* peptides. AlphaFold predicted disordered folds with low-moderate levels of confidence for the following wasp-derived peptides; waspkinin (P83660), vespulakinins (P57672), protopolybiakinins (P0DM70/P0DM71), and Cd-146 (P83660). The protease inhibitor cvp4 (Q8T0W2) from the *S* family is a toxin from the parasitic wasp *Pimpla hypochondriaca*, which belongs to the family pacifastin-like protease inhibitors. AlphaFold's predicted structure of cvp4 (AF-Q8T0W2–F1) unveiled three spaced inhibitor domains rich in β-sheets. Three disulfide bridges in a six-cysteine rearrangement 1–4, 2–6, and 3–5 tighten each domain (Parkinson et al., 2004; Simonet et al., 2002). The third group of wasp-derived peptides are the so-called mast cell degranulating [MCD] peptides, forming the *T* protein family. The MCD toxins are the main peptide venom components of the Vespidae and Polistinae wasp families (Baek and Lee, 2010). Some MCD toxins share sequence and structural similarity with Mastoparan members of the aculeatoxin protein family (*A*) (P0C1Q5, P01514, P69034), as depicted in Fig. 2A (*right*). The bumblebee bombolitins are also MCD peptides that conserve the signal peptide and pro-peptide sequences from the aculeatoxin protein family where they currently belong (Robinson et al., 2018). The NMR solution structure of Mastoparan-L (PDB: 6KUL), in Fig. 2A (*right*), portrayed that these short linear amphipathic peptides generally fold into α-helical structures. The damage-control phosphatase ARMT1 (Q8MMH3) is the unique representative of the *R* protein family. It was initially found from the cDNA (gene *vpr 2*) of the venomous gland of the parasitic wasp *Pimpla hypochondriaca*. This protein shares structural homology (Fig. 3C) with the human metal-dependent phosphatase ARMT1 (Q9H993), also involved in metabolite damage control (Parkinson et al., 2003). Ntn-hydrolase (*U*) is an enzyme from the venom of the parasitic wasp *Asobara tabida*, consisting of two subunits of 30 kDa (α-subunit) and 18 kDa (β-subunit). Both subunits showed sequence and structural similarity to reported

aspartylglucosaminidases (AGAs), suggesting an AGA-like domain in the insect venom (Moreau et al., 2004). The last protein family is the M12B metalloproteases (*V*), zinc-dependent peptidases with a molecular weight similar to snake type II reprolysins (Fig. 3D). They were present in the venom of the parasitic wasp *Eulophus pennicornis*. They contain a sandwich-type domain of α-helix/β-sheet/α-helix, and a homologous catalytic site to metalloproteases from other insects (Price et al., 2009).

3.2. The biological activities and applications of hymenopteran toxins

Hymenopteran venom compounds are versatile biochemical weapons associated with various ecological roles such as hunting, defence, parasitism, competition and communication (Aili et al., 2014; Fry et al., 2009; Senji Laxme et al., 2019). Recently, several proteo-transcriptomic studies were conducted on hymenopteran venoms, followed by their biological evaluations (Robinson et al., 2018; Aili et al., 2020; Jensen et al., 2021). Here, we manually curated all possible biological functions associated with each hymenopteran toxin and its corresponding protein family. We summarised the diversity of biological activities per hymenopteran toxin superfamilies in Fig. 4A. We then uncovered the main biological targets per reported biological activity for 88 hymenopteran toxins in a sunburst plot (Fig. 4B).

3.2.1. Anti-infective peptides

Antimicrobial resistance (AMR) represents a significant threat to patient and population health. The emergence of resistant pathogens is outpacing the development of conventional antibiotics, and the development of novel and effective drugs is critical (Hoffman, 2020). Antimicrobial peptides (AMPs) offer promising opportunities as they display direct antibacterial and antifungal activities with low risks of pharmacoresistance (Haney et al., 2019; Magana et al., 2020). The search for novel and potent AMPs has reached all kingdoms of life, including insects (Manniello et al., 2021; Mylonakis et al., 2016). Some of these AMPs would exhibit growth inhibition against bacteria, fungi, and parasites; therefore, we used the term "anti-infective" to include those with antiparasitic activity. In Fig. 4A, we could narrow most anti-infective peptides to the four most prominent protein families - *A*, *H*, *J* and *T*. In Fig. 4B, we reported 88 hymenopteran toxins with fully characterised biological activities, each against at least one of 544 different targets. Two-thirds of these toxins (62.5%) exhibited anti-infective properties; most inhibited the growth of bacterias, predominantly against *Escherichia coli* (48 tested peptides), *Staphylococcus aureus* (41), *Bacillus subtilis* (33) and *Pseudomonas aeruginosa* (33). The peptides also displayed antifungal activities against nine species, including *Candida albicans* (19), and they demonstrated antiparasitic activity, notably against *Trypanosoma cruzi* (Chagas disease).

Melittin (P59262, Fig. 2A, left) is the major venom component of honey bee *Apis mellifera* and the prototypical example of the aculeatoxin protein family that can kill many pathogens (*i.e.*, bacteria, yeasts, viruses) by disrupting their lipid membranes, a process known as membranolysis. Its mechanism of action has been extensively reviewed (Askari et al., 2021; Boigegrain et al., 1992; Leandro et al., 2015; Memariani et al., 2020; Morgan and Montague, 1984; Ribeiro et al., 2004; Van Den Bogaart et al., 2008). M-MIITX$_1$-Tb1a (W8GNV3) from *Tetramorium bicarinatum*, commonly called bicarinalin, is another membranolytic antimicrobial peptide exhibiting moderate to high minimal inhibitory concentrations against gram-negative bacteria, gram-positive bacteria, fungi and parasite *Leishmania infantum*. The peptide did not show cytotoxic activity against human lymphocytes between ranges (0.06–8.5 μM) and limited haemotoxicity (Rifflet et al., 2012; Téné et al., 2016). A third example of the aculeatoxin protein family is the peptide named M-PONTX-Dq4e (P0DSK2) from the venom of South American ant *Dinoponera quadriceps*. The peptide displayed antitrypanosomal activity, reducing the number of its three forms (epimastigote, trypomastigote and amastigote) (Lima et al., 2018). To date, numerous aculeatoxins remain without any biological profiles; myrmicitoxins (Bouzid et al., 2013), poneritoxins (Kazuma et al., 2017) and myrmeciitoxins (Robinson et al., 2018) are all suspected of exhibiting antimicrobial or haemolytic activities for folding into a linear cationic α-helix, a typical structure among AMPs.

The ponericins (*H*) were the third-largest toxin family with associated biological activities. Like aculeatoxins mentioned above, these peptides could destroy bacteria, fungi, and red blood cells by breaking their membranes apart. For example, M-ECTX-Eb2a (C0HK45) and homologs from *Ectatomma brunneum* ants inhibited the growth of Gram-negative bacteria *E. coli* and *P. aeruginosa* at submicromolar concentrations (Pluzhnikov et al., 2014). Several poneritoxins from ant genus Neoponera (*N. apicalis*, *N. goeldii*, *N. inversa*) demonstrated a broad spectrum of activity against Gram-positive and Gram-negative bacteria and yeast *Saccharomyces cerevisiae* (Aili et al., 2014; Orivel et al., 2001).

The *J* protein family represented the second largest cluster of non-homologous toxin sequences. Like the protein families above, these toxins demonstrated antimicrobial activities. For example, poneritoxins M-PONTX-Dq4b (C0HJH6) and M-PONTX-Da4b (P0CF05) were identified from the venom secretions of ants *Dinoponera quadriceps* and *D. australis* respectively. Both toxins exhibited antibacterial activity against *Bacillus amyloliquefaciens*, *Listeria monocytogenes*, *S. aureus*, *Pseudomonas putida*, *P. aeruginosa*, and *E. coli*, anti-yeast activity against *Saccharomyces cerevisiae* and *Rhodotonda mucilaginosa*, and the fungus *Cladosporium cucumerinum* (Cologna et al., 2013). The toxin M-PONTX-Dq3a (P0DSK0) from *D. quadriceps* is a promising anti-trypanosomal activity with a mean lethality of 4.7 μM and low cytotoxic activity (25.7 μM), in addition to its selectivity towards the parasites and infected cells (Lima et al., 2018).

The amphipathic α-helical structures of some MCD toxins (T protein family) conferred the peptides with membranolytic activity against bacteria, fungi, parasites, and erythrocytes. As such, eumenitin and eumenine toxins from solitary wasp genera *Eumenes* (*E. fraterculus*, *E. rubrofemoratus*, *E. rubronotatus*) all demonstrated moderate anti-infective properties. Eumenitin-R (P0CJ36) was the most potent peptide reported against Gram-positive bacteria *Bacillus subtilis* and yeast *Candida albicans* with MIC values of 7.5 μM or less (Konno et al., 2006; Rangel et al., 2011). Two other families of antimicrobial MCD peptides, called mastoparan-like peptides and vespid chemotactic peptides, from the venom of hornet wasp *Vespa magnifica*, displayed moderate-high minimal inhibitory concentrations against *E. coli*, *S. aureus* and *C. albicans* (Xu et al., 2006a; 2006b). The MCD family represents a promising source for mast cell rolls therapeutics; the peptides demonstrated various activities, including allergy, angiogenesis, haemostasis, blood pressure, and innate immune response linked to diseases like atherosclerosis or celiac disease (Krystel-Whittemore et al., 2016; Lüddecke et al., 2019; Radlović, 2013; Xu and Chen, 2015).

These antimicrobial mechanisms are believed to participate in the hymenopteran natural immune system against pathogens (Aili et al., 2014). Such peptides represent promising antibiotic solutions to combat antimicrobial resistance (Manniello et al., 2021). However, most AMPs presented in preclinical/clinical applications are limited to topical administration due to their haemolytic activity. We previously estimated that roughly 70% of all 3081 natural peptides in the Antimicrobial Peptide Database APD3 might be hemotoxins, meaning they could break down erythrocytes (Plisson et al., 2020). In Fig. 4B, we counted 19 AMPs with haemolytic activity. Among them, mastoparans from the Lesser paper wasp *Parapolybia indica* (P42716) and the Brazilian wasp *Protonectarina sylveirae* (P0C1Q5) were two reported haemolytic aculeatoxins with their respective HC_{50} values of 34 and 37 μM in rat blood cells (Dohtsu et al., 1993; Toki et al., 1988). In contrast, related mastoparan-like peptides (*T*) also showed limited haemolytic toxicity against human blood red cells (Xu et al., 2006a; 2006b).

3.2.2. Neurotoxins

Similarly to antimicrobial peptides, venomous neurotoxins have evolved in plants, animals and microbes to serve defensive and

predatory roles by inducing pain and paralysis through a specialised envenomation apparatus (*e.g.* sting). The peptides target a wide variety of ion channels and protein receptors with high selectivity and affinity (Fry et al., 2015; Lewis and Garcia, 2003). We identified several neurotoxins in the protein families *A*, *E*, *G*, *J* and *Q* (Fig. 4A).

Among aculeatoxins (*A*), the α-pompilidotoxin (P69391) from solitary wasp *Anoplius samariens* impaired voltage-gated sodium channels (Konno et al., 1998, 2000; Schiavon et al., 2010). The U-MIITX$_1$-Mg1a (P0DSJ4) from the Australian red bulldog ant *Myrmecia gulosa* modulated the calcium influx in murine Dorsal Root Ganglion neurons (Fig. 4B) (Robinson et al., 2018).

Neurotoxic protein families included the small peptide dimers ectatomins (*E*) from the *Ectatomma tuberculatum* venom (Pluzhinikov et al., 1994) and myrmexins (*G*) secreted by *Pseudomyrmex triplarinus* ants (Pan and Hink, 2000). The latter group is believed to inhibit carrageenan-induced oedema, probably by neuronal stimulation for the synthesis of prostaglandins (Pan and Hink, 2000). The toxin ω/M-ECTX-Et1a (P49343/P49344, Fig. 2F), also known as ectatomin, is the sole heterodimer of all ectatomins with reported biological activity; the peptide inhibited cardiac L-type calcium currents in isolated rat cardiac ventricular myocytes (Fig. 4B). Its lethality was 6.8 μg/kg in mammals and 2.1 μg/g (Pluzhnikov et al., 1999). The helical heterodimer δ-Myrtoxin-Mp1a, also described as M-myrmeciitoxin-Mp1 or Mp1a, was identified from Myrmecia pilosula venom gland. The peptide demonstrated antimicrobial, membrane-disrupting and nociceptive activities (Dekan et al., 2017). Touchard and co-workers reported the toxin with insecticidal activity (Touchard et al., 2020) whereas Nixon et al. described Mp1a as a calcium-realising peptide in DRG cells (Nixon et al., 2020). Its monomeric precursor labelled Q07932 was originally classified in our analysis as an aculeatoxin (*A*).

Among the toxins from the *J* protein family with neurotoxic profiles, we noted that the bullet ant *Paraponera clavata* δ-PPOTX-Pc1a (P41736) blocked the nicotinic synaptic transmission in insect nervous system (Hendrich et al., 2002), the β-pompilidotoxin (P69392) from the tarantula hawk *Batozonellus maculifrons* affected neuromuscular junction in rats targeting voltage-gated sodium channels (Schiavon et al., 2010), and the tertiapin (P56587) and its derivative tertiapin-Q from the honeybee *Apis mellifera* could modulate various types of potassium channels (Drici et al., 2000; Hashimoto et al., 2006; Jin et al., 1999; Jin et al., 1999; Kitamura et al., 2000). Among these potassium channels, tertiapin-Q blocked the calcium-activated large conductance potassium channel (BK) leading to its potential therapeutic uses to treat pain, multiple sclerosis and rheumatoid arthritis (Kanjhan et al., 2005). At last, apamines (*e.g.* P01500) were potent neurotoxins from bee venom *Apis mellifera* blocking calcium-activated potassium ion channels (also named SK channels) (Labbé-Jullié et al., 1991) and could represent a possible treatment for Parkinson's disease (Faber and Sah, 2007).

Finally, the bradykinin-related peptides (*Q*) from wasps venoms were reported with various activities, including presynaptic block irreversible paralysis, hyperalgesia in mammals, mast cell degranulation, hypertension, and muscle contraction (Mendes and Palma, 2006; Picolo et al., 2010). Their pharmacological activities are mainly mediated by binding to B1 and B2 receptors (Fig. 4B) (Lameu et al., 2013). Some wasp-derived bradykinin-related peptides also exhibited inhibitory properties against the angiotensin-converting enzyme (ACE), leading to hypotensive effects. In 1970, Ferreira and coworkers reported the earliest bradykinin-related peptides from pit viper *Bothrops jararaca* with ACE inhibition (Ferreira et al., 1970) guiding the development of the first commercial site-directed ACE inhibitor, *i.e.* Captopril for the treatment of human hypertension (McCleary et al., 2015). Besides hypertension, bradykinin-related peptides were proposed to treat cardiovascular disorders, inflammation, asthma, angiogenesis, pain and inflammation (Lameu et al., 2013). In contrast, the ecological roles of these toxins remain uncertain (Mendes and Palma, 2006).

3.2.3. Allergens

Allergens were commonly found in bees and wasps venoms targeting mast cells and basophils either provoking degranulation (Argiolas and Pisano, 1985; Kawakami et al., 2017; Mendes et al., 2004; Müller, 2011), reacting with immunoglobulin E (IgE) (Ollert and Blank, 2015) or with other circulatory system disorders (*i.e.* leukocytes, granulocytes) (Fig. 4B). It is thought that such allergens participate in nest defence against vertebrates. We could trace allergens to the following protein families: *A*, *C*, *D*, *F*, *J*, *K*, *L*, *M*, and *T* (Fig. 4A).

The bombolitins I–V from the venom of bumblebee *Megabombus pennsylvanicus* were allergenic aculeatoxins (*A*) that could degranulate mast cells (Argiolas et al., 1985; Argiolas and Pisano, 1985; Favreau et al., 2006). The *J* toxin family also housed venom allergens that could degranulate mast cells, including the non-haemolytic sylverin (P0C1R2) from Brazilian wasp *Protonectarina sylveirae* (Dohtsu et al., 1993), polybine-1 and polybine-2 (P84388, P84389) from the venom of swarm-founding polistine wasp *Polybia paulista* (Ribeiro et al., 2004), or the potent anti-inflammatory MCD peptide (Q6H2Z4) from the Eastern honey bee *Apis cerena* (Shi et al., 2003). Other venom allergens like icarapin (Q5EF78, *A. mellifera carnica*) induced IgE reaction among beekeepers (Peiren et al., 2006). The fourth-largest *T* protein family includes the MCD peptides known to the degranulate mast cells (Baek and Lee, 2010; Čeřovský et al., 2007; De Souza et al., 2004; Konno et al., 2006; Mendes et al., 2004; Rangel et al., 2011; Torres et al., 2018; Turillazzi et al., 2006; Xu et al., 2006a). Some peptides mentioned above from the *J* family also induced mast cell degranulation. Still, they could not be assimilated as *T* protein family members as they lacked the signal peptide or propeptide signatures.

Six minor toxin superfamilies gave out similar allergen activity by IgE reaction, including venom allergens 2/4 (*C* family) and 3/5 (CRiSP, *D* family), as well as enzymes like lipases (*F*), glycosyl hydrolases (*K*), peptidases S (*L*) and phospholipases A2 (*M*). Venom allergens 2/3/4 were predominantly isolated from the fire ant genera *Solenopsis* (*S. invicta*, *S. saevissima*, *S. richteri* and *S. geminata*). In contrast, all venom allergens 5 were identified from various vespid venoms; including parasitoid wasp *Microctonus hyperodae*, potter wasp *Rhynchium brunneum* and eusocial wasp *Polybia paulista*, polistine wasps *(Polistes dominula*, *P. gallicus, P. exclamans, P. fuscatus, P. annularis*), and yellow-jackets (*Vespula maculifrons, V. squamosa, V. vulgaris, V. pensylvanica*). Venom allergens are considered to be responsible for most human allergic responses recognising immunoglobulin E (IgE) (Bazon et al., 2017; Dos Santos-Pinto et al., 2014; Dos Santos et al., 2010; Lu et al., 1993; Pantera et al., 2003; Zhang et al., 2003), in particular in the US southern states (Hoffman, 1993; Hoffman et al., 1990). Among the allergenic enzymes, members of the lipase family (*F*) are phospholipases A1 (EC 3.1.1.32), sometimes referred to as venom allergens 1, and could catalyse the hydrolysis of phosphatidylcholine (*e.g.* P0DSI2, Torres et al., 2014). These proteins also induced allergic reactions in humans (Moawad et al., 2005; Rungsa et al., 2018; Sukprasert et al., 2013; Yang et al., 2008) and released cross-reactivity with IgE antibodies in mice or patients sensitised to hymenopteran venoms (Hoffman et al., 2005; King et al., 1996; Pantera et al., 2003). The hyaluronidases (*K* protein family, EC 3.2.1.35) mainly were identified from vespid venoms; they participated in the hydrolysis of hyaluronic acid leading to skin allergy in humans (Shi et al., 2003). The prototypical example of the *K* protein family was α,α-trehalase (Q8MMG9, EC 3.2.1.2) from the parasitoid wasp *Pimpla hypochondriaca*. The enzyme catalysed the production and storage of glucose in the hemolymph of insects (Parkinson et al., 2003). Among members of the *L* protein family, the venom protease P0CH88 was considered an essential allergen to the bumblebee *Bombus terrestris* (Hoffman et al., 2001). Moreover, the venom dipeptidyl peptidase 4, also called Ves-v-3 (B1A4F7), from the venom of the yellow jacket *Vespula vulgaris*, showed allergenic activity by activation of basophils in the serum of sensitised patients (Blank et al., 2010). Finally, some PLA2 enzymes (*M* protein family) in bees and bumblebees venoms were allergens such as Q7M4I6 from *Bombus pensylvanicus* and P00630 from

Apis mellifera. Both toxins are reactive with serum from patients allergic to bee and bumblebee envenomation (Hoffman and Jacobson, 1996; Weber et al., 1987). Interestingly, the PLA2 toxin P00630 showed some similarities to the phospholipase A2 (P04362, structural overlay not shown) found in the venom of Mexican beaded-lizard *Heloderma horridum* (Sosa et al., 1986) and genetically more similar to that of the bovine pancreas (Kuchler et al., 1989).

3.2.4. Anticoagulant peptides

Anticoagulants inhibit pathways of coagulation cascade and unwanted blood clot formation. They are notably used for treating thrombotic disorders (*e.g.* stroke, coronary artery disease, heart malfunctions, pulmonary embolism) and during procedures such as blood transfusion and dialysis (Harter et al., 2015). Venomous animals, particularly snakes, secrete anticoagulant proteins to specifically target critical enzymes of blood circulation during predation, *i.e.* factor Xa and thrombin (Khan et al., 2018; Kini, 2006). In Fig. 4A, we observed that members of the two protein families, *P* and *V*, have been evaluated for their anticoagulant properties.

Antifibrinolytic drugs are increasingly used to reduce oral bleeding during dental procedures (van Galen et al., 2019). Kunitz-type toxins (*P* protein family) are peptides with antifibrinolytic activity. The serine protease inhibitors Bt-KTI (D8KY58) and Bi-KTI (G3LH89), identified from the respective bumblebee *B. terrestris* and *B. ignitus* venoms, prevented the activation of plasminogen to plasmin, inhibiting fibrinolysis. In contrast, these toxins did not inhibit thrombin and factor Xa (Choo et al., 2012; Qiu et al., 2013). Another Kunitz-type toxin, bicolin (C0LNR2, Fig. 2D), was found in the venom of Black shield wasp *Vespa bicolor*; the peptide displayed anticoagulant activity, inhibiting thrombin (Yang et al., 2009).

Venom M12B metalloproteinases (*V*) 1, 2, and 3 (B5AJT2, B5AJT3, B5AJT4) from the venom of the parasitic wasp *Eulophus pennicornis* are toxins with gelatinase activity (Price et al., 2009). Such proteases are similar to a metalloprotease from the saliva of black-legged tick *Ixodes scapularis*, which prevents host-clotting while feeding. Likewise, the metalloprotease B5AJT4 (Fig. 4D) presented anticoagulant activity akin to the hemorrhagic activity of snake venom (Francischetti et al., 2003).

3.2.5. Anticancer peptides

In 2018, the World Health Organisation declared that cancer was the second cause of death globally, reaching about 9.6 million deaths and an estimated 18.1 million new cases (Bray et al., 2018; Ferlay et al., 2019). New treatments with greater selectivity towards cancer cells that could evade the common multidrug resistance mechanisms are sought after (Hoskin and Ramamoorthy, 2008). Anticancer peptides (ACPs) are generally small amphiphilic and cationic peptides in nature due to a high proportion of basic and hydrophobic residues (Gaspar et al., 2013). In Fig. 4A and B, 33 toxins from protein families *A* and *T* uncovered anticancer properties against one of 30 cancer cell lines.

The aculeatoxin melittin (P59262, Fig. 2A) from *Apis mellifera* has been extensively studied for its anticancer activities, such as leukemic cell line U937, in a dose-dependent manner (Lyu et al., 2018; Moon et al., 2008). Likewise, the MCD toxin Polybia-CP (Polybia chemotactic peptide) (P0C1R0) demonstrated moderate inhibitory activity (IC_{50}) against prostate cancer cells (CP-3) and bladder cancer cells (Biu 87) (Wang et al., 2011). In addition, the toxin (P0C1R0) has low activity against mast cells and lacks hemolytic activity (Souza et al., 2005); it might be an excellent anti-carcinogenic candidate.

3.2.6. Anti-diabetic peptides

Melittin (P59262, Fig. 2A) also showed antidiabetic properties by inducing changes in the membrane integrity of pancreatic ß cells, increasing insulin levels (Morgan and Montague, 1984). In addition, melittin and PLA2 enzymes reduced glucose levels in the blood (Hossen et al., 2017).

3.2.7. Bioinsecticides

Pests such as insects and mites are responsible for destroying roughly 15% of the world's annual crop production and transmitting many pathogens. Considering that many venomous species feed on insects, they represent a valuable source for insecticidal agents (Windley et al., 2012). Like spiders, hymenopterans secrete venom peptides with insecticidal activity as part of their hunting process. In Fig. 4A, the protein families *A*, *H*, *J* and *V* with reported insecticidal peptides.

We first observed that few acuelatoxins (*A*) were reported for their insecticidal activities. One rare example is the broad-spectrum antimicrobial toxin called M-MIITX-Mp2b (P0C023) from the venom of the Jack jumper ant *Myrmecia pilosula* (Dekan et al., 2017). The peptide displayed paralytic activity against fruit flies *Drosophila melanogaster* with a mean lethal dose (LD_{50}) of 260 pmol/g (Nixon et al., 2020).

In contrast, numerous α-helical ponericins (*H*) such as M-PONTX-Ng3a (P82414, Fig. 2A) from the venom of ant *Neoponera goeldii* demonstrated insecticidal properties against cricket larvae in addition to their broad-spectrum antimicrobial properties (see 3.2.1) (Orivel et al., 2001). Another ponericin, M-PONTX-Na1b from *Neoponera apicalis* venom, was recently reported as the most anthelmintic peptides against parasitic nematodes (*Haemonchus contortus*, *Brugia malayi*) from a pool of *Neoponera* ponericins, at μM concentration. The pool also encased potent insecticidal peptides, like M-PONTX-Nc3a from *N. commutata*, against adult sheep blowflies *Lucilia cuprina* with a lethal dose of 3.5 nmol/g (Nixon et al., 2021).

The *J* protein family included U1-MYRTX-Mr1a (P0DSL0) from the venom of ruby ant *Myrmica rubra* that showed insecticidal activity against aphids *Acyrthosiphon pisum* at a concentration of 500 μg/mL *via* oral administration. Unlike acuelatoxins and ponericins, P0DSL0 did not inhibit the growth of bacteria, fungi or the larval development of parasites (Heep et al., 2019). In addition, the peptide caused greater sensitivity towards other insecticides such Imidacloprid™ and Methomyl™.

Finally, the venom of the parasitic wasp *Eulophus pennicornis* expresses three reprolysin-like toxins (*V*, Fig. 3D) with insecticidal activity against tomato moth larvae *Lacanobia oleracea*. These metalloproteinases might be related to the successful parasitism by the host management (Price et al., 2009).

4. Discussion

Bioprospecting campaigns of selected venomous species have introduced important sources of biologically active peptides and proteins that could modulate a wide variety of ion channels and protein receptors (Lewis and Garcia, 2003). Research efforts have predominantly focused on venom composition and toxin evolution among snakes (McCleary et al., 2015), spiders (Smith et al., 2015), scorpions (Rodriguez De La Vega et al., 2015) and cone snails (Teichert et al., 2015). The combined or intertwined usage of genomic, proteomic, transcriptomic and bioinformatic techniques over venomous species, referred to as the umbrella term "venomics", has supported our understanding of venom protein diversity and protein evolution, even from smaller species (Dutertre et al., 2015). Our meta-analysis over hymenopteran venom protein compositions using the UniProt database has leveraged 81 producers, representing less than 0.1% of the hymenopteran species (81:154,067) (Huber, 2017), across three clades; ants (21), wasps (44) and bees (16). Most studied species have been selected from different regions of the World.

We reported 282 peptides and proteins spread across 21 families using the PFAM database from the limited sample of hymenopteran diversity. The most populated protein families (*A*, *H*, *J* and *T*) comprise short α-helical amphipathic peptides (*i.e.* aculeatoxins, ponericins, ampulexins, mastoparan-like peptides) capable of killing various pathogens (*e.g.* bacteria, fungi, virus), parasites or crop pests by disrupting their membranes. Their similar physicochemical characteristics with other AMPs (*e.g.* amphibian brevinins, insect cecropins, bovine

cathelicidins) (Wang et al., 2016) have triggered that many hymenopteran venom peptides (55 out of 88, 62.5%) to be investigated for anti-infective properties against many microorganisms (Fig. 4A–B). The growing biological results for microbial growth inhibition indicated that research efforts should focus on bioprospecting first formicoid venoms to source novel antibiotics. Alternatively, these α-helical amphipathic peptides exhibited insecticidal and anticancer activities. Like other host-defence peptides, they are believed to protect their hosts from pathogens (Haney et al., 2019). In contrast, hymenopterans utilise neurotoxins to inflict pain, repel predators and paralyse preys. Our systematic review suggested that novel venom-derived peptides as possible pharmacological tools or therapeutics to treat ailments such as chronic pain, multiple sclerosis, rheumatoid arthritis, or neurodegeneration should be sourced from any three clades, indifferently. Indeed, we identified several cysteine-rich neurotoxins from either ants (*i.e.* *E*: ectatomins, *G*: myrmexins, *J*: poneritoxin), wasps (*i.e.* *A*, *J*: pompilidotoxins, *Q*: bradykinin-related peptides) or bees (*i.e.* *J*: tertiapins and apamines). The unique difference is structural; two formicoid neurotoxin families (*E*, *G*) fold into protein heterodimers, whereas other neurotoxins from wasps and bees are cysteine-rich monomers (Figs. 2F and 3A). All hymenopterans produce sheer amounts of allergenic peptides and enzymes. Finally, venom-derived anticoagulant peptides could primarily be isolated from wasp venoms as possible treatments against thrombotic disorders (*e.g.* stroke) or excessive oral bleeding.

Hymenopteran venoms are therefore valuable to yield antimicrobial peptides, neurotoxins or bioinsecticides. The peptides might eventually present biological profiles unrelated to their natural defensive or predatory functions, *e.g.* anticancer or anti-diabetic activity. Some of the venom-derived protein families like aculeatoxins (Robinson et al., 2018) or ponericins (Nixon et al., 2021) have been well-characterised for their biological activities as opposed to protein families such as damage-control phosphatase (*R*), protease inhibitor (*S*), and Ntn-hydrolase (*U*). In some cases, their biological activities have been inferred from sequence or structural similarity with other reported peptides and proteins ("By similarity" Fig. 2A). For example, the biological role of the pacifastin-like protease inhibitor cvp4 (Q8T0W2) (*S*) from parasitic wasp *Pimpla hypochondriaca* is still unknown. The β-sheets-rich domains of this protein resemble those of the protease inhibitor P80060 from migratory locust *Locusta migratoria* (Parkinson et al., 2004), which was reported as a chymotrypsin and elastase inhibitor present in the lobster hemolymph (Boigegrain et al., 1992). To date, numerous hymenopteran toxins remain with unknown biological activity or function, particularly within protein families *A* and *J*.

5. Conclusion remarks

Our meta-analysis and systematic review have revealed that hymenopteran venoms were valuable sources of peptide-based treatments like aculeatoxins (*A*) or ponericins (*H*) implicated in growth inhibition towards model microorganisms, including viruses (Herpesviridae, Penumoviridae, Retroviridae), gram-positive bacteria (*Arthrobacter globiformis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Lactococcus garvieae*), gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *P. putida*), fungi (*Candida albicans*, *Cryptococcus neoformans*, *Saccharomyces cerevisiae*), and parasites (*Trypanosoma cruzi*, *Leishmania major*). Hymenopterans often secrete neurotoxins to paralyse preys or repel predators, which led to studying their toxins towards targets in the nervous system, in particular the dorsal root ganglia neurons, and cation channels (*e.g.* sodium, calcium, potassium, transient receptor potential). We could notably cite the example of tertiapin-Q (*J*) to treat pain, multiple sclerosis and rheumatoid arthritis, and the example of bradykinin-related peptides (*Q*) to reduce human hypertension. Beyond pathogen infection and neurotoxicity, hymenopteran toxins such as Kunitz-type protease inhibitors (*P*) and metalloproteinases (*V*) were evaluated for their capacity to perturb cell homeostasis involving intermediaries such as fibrin, fibrinogen, thrombin, and specific cells (*i.e.* erythrocytes). Finally, sting envenomation often provokes allergic reactions due to its large quantity of allergens involving IgE, mast cells degradation, and other circulatory system disorders (*i.e.* leucocytes, granulocytes).

Credit author statement

Juan Carlos Guido-Patiño: Formal analysis, Investigation, Data curation, Writing – original draft, Visualisation, Writing – review & editing. **Fabien Plisson**: Conceptualization, Writing – review & editing, Visualisation, Supervision, Project administration, and Funding acquisition.

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Human and animal rights

The authors declare that the work described has not involved experimentation on humans or animals.

Informed consent and patient details

The authors declare that the work described does not involve patients or volunteers.

Disclosure of interest

The authors declare that they have no known competing financial or personal relationships that could be viewed as influencing the work reported in this paper.

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Declaration of competing interest

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Appendix A. Supplementary data

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References

Aili, S.R., Touchard, A., Escoubas, P., Padula, M.P., Orivel, J., Dejean, A., Nicholson, G. M., 2014. Diversity of peptide toxins from stinging ant venoms. Toxicon 92, 166–178. https://doi.org/10.1016/j.toxicon.2014.10.021.

Aili, S.R., Touchard, A., Hayward, R., Robinson, S.D., Pineda, S.S., Lalagüe, H., Mrinalini, Vetter I., Undheim, E.A.B., Kini, R.M., Escoubas, P., Padula, M.P., Myers, G.S.A., Nicholson, G.M., 2020. An integrated proteomic and transcriptomic analysis reveals the venom complexity of the bullet ant *Paraponera clavata*. Toxins 12. https://doi.org/10.3390/toxins12050324.

Argiolas, A., Herring, P., Pisano, J.J., 1985. Amino acid sequence of bumblebee MCD peptide: a new mast cell degranulating peptide from the venom of the bumblebee *Megabombus pennsylvanicus*. Peptides 6, 431–436. https://doi.org/10.1016/0196-9781(85)90410-3.

Argiolas, A., Pisano, J.J., 1985. Bombolitins, a new class of mast cell degranulating peptides from the venom of the bumblebee *Megabombus pennsylvanicus*. J. Biol. Chem. 260, 1437–1444.

Askari, P., Namaei, M.H., Ghazvini, K., Hosseini, M., 2021. In vitro and in vivo toxicity and antibacterial efficacy of melittin against clinical extensively drug-resistant bacteria. BMC Pharmacol. Toxicol. 22, 1–12. https://doi.org/10.1186/s40360-021-00503-z.

Baek, J.H., Lee, S.H., 2010. Isolation and molecular cloning of venom peptides from *Orancistrocerus drewseni* (Hymenoptera: eumenidae). Toxicon 55, 711–718. https://doi.org/10.1016/j.toxicon.2009.10.023.

Bazon, M.L., Perez-Riverol, A., Dos Santos-Pinto, J.R.A., Fernandes, L.G.R., Lasa, A.M., Justo-Jacomini, D.L., Palma, M.S., De Lima Zollner, R., Brochetto-Braga, M.R., 2017. Heterologous expression, purification and immunoreactivity of the antigen 5 from *Polybia paulista* wasp venom. Toxins 9. https://doi.org/10.3390/toxins9090259.

Blank, S., Bazon, M.L., Grosch, J., Schmidt-Weber, C.B., Brochetto-Braga, M.R., Bilò, M. B., Jakob, T., 2020. Antigen 5 allergens of hymenoptera venoms and their role in diagnosis and therapy of venom allergy. Curr. Allergy Asthma Rep. 20 https://doi.org/10.1007/s11882-020-00954-0.

Blank, S., Seismann, H., Bockisch, B., Braren, I., Cifuentes, L., McIntyre, M., Rühl, D., Ring, J., Bredehorst, R., Ollert, M.W., Grunwald, T., Spillner, E., 2010. Identification, recombinant expression, and characterization of the 100 kDa high molecular weight hymenoptera venom allergens api m 5 and ves v 3. J. Immunol. 184, 5403–5413. https://doi.org/10.4049/jimmunol.0803709.

Boigegrain, R.-A., Mattras, H., Brehélin, M., Paroutaud, P., Coletti-Previero, M.-A., 1992. Insect immunity: two proteinase inhibitors from hemolymph of Locusta migratoria. Biochem. Biophys. Res. Commun. 189 (2), 790–7993. https://doi.org/10.1016/0006-291X(92)92271-X.

Bouzid, W., Klopp, C., Verdenaud, M., Ducancel, F., Vétillard, A., 2013. Profiling the venom gland transcriptome of *Tetramorium bicarinatum* (Hymenoptera: Formicidae): the first transcriptome analysis of an ant species. Toxicon 70, 70–81. https://doi.org/10.1016/j.toxicon.2013.03.010.

Branstetter, M.G., Danforth, B.N., Pitts, J.P., Faircloth, B.C., Ward, P.S., Buffington, M.L., Gates, M.W., Kula, R.R., Brady, S.G., 2017. Phylogenomic insights into the evolution of stinging wasps and the origins of ants and bees. Curr. Biol. 27, 1019–1025. https://doi.org/10.1016/j.cub.2017.03.027.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A., Jemal, A., 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Ca - Cancer J. Clin. 68, 394–424. https://doi.org/10.3322/caac.21492.

Carson, S., 2020. Interactive Web-Based Data Visualization with R, Plotly, and Shiny. Chapman and Hall/CRC.

Casewell, N.R., Wüster, W., Vonk, F.J., Harrison, R.A., Fry, B.G., 2013. Complex cocktails: the evolutionary novelty of venoms. Trends Ecol. Evol. 28, 219–229. https://doi.org/10.1016/j.tree.2012.10.020.

Cerdá, X., Dejean, A., 2011. Predation by ants on arthropods and other animals. In: Predation in the Hymenoptera: an Evolutionary Perspective. National Academy of Sciences, Washington, DC, USA, ISBN 978-81-7895-530-8.

Čeřovský, V., Pohl, J., Yang, Z., Alam, N., Attygalle, A.B., 2007. Identification of three novel peptides isolated from the venom of the neotropical social wasp *Polistes major major*. J. Pept. Sci. 16–26. https://doi.org/10.1002/psc.860.

Choo, Y.M., Lee, K.S., Yoon, H.J., Qiu, Y., Wan, H., Sohn, M.R., Da Sohn, H., Jin, B.R., 2012. Antifibrinolytic role of a bee venom serine protease inhibitor that acts as a plasmin inhibitor. PLoS One 7, 1–5. https://doi.org/10.1371/journal.pone.0032269.

Cologna, C.T., Cardoso, J. dos S., Jourdan, E., Degueldre, M., Upert, G., Gilles, N., Uetanabaro, A.P.T., Costa Neto, E.M., Thonart, P., de Pauw, E., Quinton, L., 2013. Peptidomic comparison and characterization of the major components of the venom of the giant ant *Dinoponera quadriceps* collected in four different areas of Brazil. J. Proteonomics 94, 413–422. https://doi.org/10.1016/j.jprot.2013.10.017.

Craik, D.J., Daly, N.L., Waine, C., 2001. The cystine knot motif in toxins and implications for drug design. Toxicon 39, 43–60. https://doi.org/10.1016/S0041-0101(00)00160-4.

Cynthia, A., 2021. Brewer [WWW Document]. Geogr. Pennsylvania State Univ (accessed 12.19.21). http://www.colorbrewer.org.

de Magalhães, M.T.Q., Mambelli, F.S., Santos, B.P.O., Morais, S.B., Oliveira, S.C., 2018. Serine protease inhibitors containing a Kunitz domain: their role in modulation of host inflammatory responses and parasite survival. Microb. Infect. 20, 606–609. https://doi.org/10.1016/j.micinf.2018.01.003.

De Souza, B.M., Ribeiro Marques, M., Tomazela, D.M., Nogueira Eberlin, M., Mendes, M. A., Palma, M.S., 2004. Mass spectrometric characterization of two novel inflammatory peptides from the venom of the social wasp *Polybia paulista*. Rapid Commun. Mass Spectrom. 18, 1095–1102. https://doi.org/10.1002/rcm.1452.

Dejean, A., Schatz, B., Orivel, J., Beugnon, G., 1999. Prey capture behavior of *Psalidomyrmex procerus* (Formicidae: ponerinae), a specialist predator of earthworms (Annelida). Sociobiology 34, 545–554.

Dekan, Z., Headey, S.J., Scanlon, M., Baldo, B.A., Lee, T., Aguilar, M., Deuis, J.R., Vetter, I., Elliott, A.G., Amado, M., 2017. Δ-Myrtoxin-Mp1a is a helical heterodimer from the venom of the jack jumper ant that has antimicrobial, membrane-disrupting, and nociceptive activities. Angew. Chem. 56 (29), 8495–8499.

Dohtsu, K., Okumura, K., Hagiwara, K., Palma, M.S., Nakajima, T., 1993. Isolation and sequence analysis of peptides from the venom of *Protonectarina sylveirae* (hymenoptera-vespidae). Nat. Toxins 1, 271–276. https://doi.org/10.1002/nt.2620010503.

Dos Santos-Pinto, J.R.A., Dos Santos, L.D., Andrade Arcuri, H., Castro, F.M., Kalil, J.E., Palma, M.S., 2014. Using proteomic strategies for sequencing and post-translational modifications assignment of antigen-5, a major allergen from the venom of the social wasp *Polybia paulista*. J. Proteome Res. 13, 855–865. https://doi.org/10.1021/pr4008927.

Dos Santos, L.D., Santos, K.S., Pinto, J.R.A., Dias, N.B., Souza, B.M. De, Dos Santos, M.F., Perales, J., Domont, G.B., Castro, F.M., Kalil, J.E., Palma, M.S., 2010. Profiling the proteome of the venom from the social wasp *Polybia paulista*: a clue to understand the envenoming mechanism. J. Proteome Res. 9, 3867–3877. https://doi.org/10.1021/pr1000829.

Drici, M.D., Diochot, S., Terrenoire, C., Romey, G., Lazdunski, M., 2000. The bee venom peptide tertiapin underlines the role of I(KACh) in acetylcholine-induced atrioventricular blocks. Br. J. Pharmacol. 131, 569–577. https://doi.org/10.1038/sj.bjp.0703611.

Dutertre, S., Undheim, E.A.B., Pineda, S.S., Jin, A.H., Lavergne, V., Fry, B.G., Lewis, R.J., Alewood, P.F., King, G.F., 2015. Venoms-based drug discovery: proteomic and transcriptomic approaches. In: King, G.F. (Ed.), Venoms to Drugs: Venom as a Source for the Development of Human Therapeutics. The Royal Society of Chemistry, pp. 80–96. https://doi.org/10.1039/9781849737876-00080.

Eagles, D.A., Saez, N.J., Krishnarjuna, B., Bradford, J.J., Chin Yanni, K.-Y., Starobova, H., Mueller, A., Reichelt, M.E., Undheim, E.A.B., Norton, R.S., Thomas, W.G., Vetter, I., King, G.F., Robinson, S.D., 2022. A peptide toxin in ant venom mimics vertebrate EGF-like hormones to cause long-lasting hypersensitivity in mammals. Proc. Natl. Acad. Sci. Unit. States Am. 119 (7), e2112630119 https://doi.org/10.1073/pnas.2112630119.

Faber, E.S.L., Sah, P., 2007. Functions of SK channels in central neurons. Clin. Exp. Pharmacol. Physiol. 34, 1077–1083. https://doi.org/10.1111/j.1440-1681.2007.04725.x.

Favreau, P., Menin, L., Michalet, S., Perret, F., Cheneval, O., Stöcklin, M., Bulet, P., Stöcklin, R., 2006. Mass spectrometry strategies for venom mapping and peptide sequencing from crude venoms: case applications with single arthropod specimen. Toxicon 47, 676–687. https://doi.org/10.1016/j.toxicon.2006.01.020.

Ferlay, J., Colombet, M., Soerjomataram, I., Mathers, C., Parkin, D.M., Piñeros, M., Znaor, A., Bray, F., 2019. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int. J. Cancer 144, 1941–1953. https://doi.org/10.1002/ijc.31937.

Ferreira Junior, R.S., Sciani, J.M., Marques-Porto, R., Junior, A.L., Orsi, R. de O., Barraviera, B., Pimenta, D.C., 2010. Africanized honey bee (*Apis mellifera*) venom profiling: seasonal variation of melittin and phospholipase A2 levels. Toxicon 56, 355–362. https://doi.org/10.1016/j.toxicon.2010.03.023.

Ferreira, S.H., Greene, L.J., Alabaster, V.A., Bakhle, Y.S., Vane, J.R., 1970. Activity of various fractions of bradykinin potentiating factor against angiotensin I converting enzyme. Nature 225, 379–380. https://doi.org/10.1038/225379a0.

Francischetti, I.M.B., Mather, T.N., Ribeiro, J.M.C., 2003. Cloning of a salivary gland metalloprotease and characterization of gelatinase and fibrin(ogen)lytic activities in the saliva of the Lyme disease tick vector *Ixodes scapularis*. Biochem. Biophys. Res. Commun. 305, 869–875. https://doi.org/10.1016/S0006-291X(03)00857-X.

Frank, E.T., Linsenmair, K.E., 2017. Individual versus collective decision making: optimal foraging in the group-hunting termite specialist *Megaponera analis*. Anim. Behav. 130, 27–35. https://doi.org/10.1016/j.anbehav.2017.06.010.

Fry, B.G., Koludarov, I., Jackson, T.N.W., Holford, M., Terrat, Y., Casewell, N.R., Undheim, E.A.B., Vetter, I., Alia, S.A., Low, D.H.W., Sunagar, K., 2015. Seeing the woods for the trees: understanding venom evolution as a guide for biodiscovery. In: King, G.F. (Ed.), Venoms to Drugs: Venom as a Source for the Development of Human Therapeutics. The Royal Society of Chemistry, Cambridge, UK, pp. 1–36. https://doi.org/10.1039/9781849737876-00001.

Fry, B.G., Roelants, K., Champagne, D.E., Scheib, H., Tyndall, J.D.A., King, G.F., Nevalainen, T.J., Norman, J.A., Lewis, R.J., Norton, R.S., Renjifo, C., de la Vega, R.C. R., 2009. The toxicogenomic multiverse: convergent recruitment of proteins into animal venoms. Annu. Rev. Genom. Hum. Genet. 10, 483–511. https://doi.org/10.1146/annurev.genom.9.081307.164356.

Gaspar, D., Salomé Veiga, A., Castanho, M.A.R.B., 2013. From antimicrobial to anticancer peptides. A review. Front. Microbiol. 4, 1–16. https://doi.org/10.3389/fmicb.2013.00294.

González-Castro, R., Gómez-Lim, M.A., Plisson, F., 2020. Cysteine-rich peptides: hyperstable scaffolds for protein engineering. Chembiochem 22, 961–973. https://doi.org/10.1002/cbic.202000634.

Gu, Z., Gu, L., Eils, R., Schlesner, M., Brors, B., 2014. Circlize implements and enhances circular visualization in R. Bioinformatics 30, 2811–2812. https://doi.org/10.1093/bioinformatics/btu393.

Haney, E.F., Straus, S.K., Hancock, R.E.W., 2019. Reassessing the host defense peptide landscape. Front. Chem. 7, 1–22. https://doi.org/10.3389/fchem.2019.00043.

Harter, K., Levine, M., Henderson, S.O., 2015. Anticoagulation drug therapy: a review. West. J. Emerg. Med. 16, 11–17. https://doi.org/10.5811/westjem.2014.12.22933.

Hashimoto, N., Yamashita, T., Tsuruzoe, N., 2006. Tertiapin, a selective IKACh blocker, terminates atrial fibrillation with selective atrial effective refractory period prolongation. Pharmacol. Res. 54, 136–141. https://doi.org/10.1016/j.phrs.2006.03.021.

Heep, J., Klaus, A., Kessel, T., Seip, M., Vilcinskas, A., Skaljac, M., 2019. Proteomic analysis of the venom from the Ruby ant *Myrmica rubra* and the isolation of a novel insecticidal decapeptide. Insects 10, 1–17. https://doi.org/10.3390/insects10020042.

Hendrich, A.B., Mozrzymas, J.W., Konopinska, D., Scuka, M., 2002. The effect of poneratoxin on neuromuscular transmission in the rat diaphragm. Cell. Mol. Biol. Lett. 7, 195–202.

Herzig, V., Sunagar, K., Wilson, D.T.R., Pineda, S.S., Israel, M.R., Dutertre, S., McFarland, B.S., Undheim, E.A.B., Hodgson, W.C., Alewood, P.F., Lewis, R.J., Bosmans, F., Vetter, I., King, G.F., Fry, B.G., 2020. Australian funnel-web spiders evolved human-lethal δ-hexatoxins for defense against vertebrate predators. Proc. Natl. Acad. Sci. U.S.A. 117, 24920–24928. https://doi.org/10.1073/pnas.2004516117.

Hoffman, D.R., 1993. Allergens in hymenoptera venom XXIV: the amino acid sequences of imported fire ant venom allergens Sol i II, Sol i III, and Sol i IV. J. Allergy Clin. Immunol. 91, 71–78. https://doi.org/10.1016/0091-6749(93)90298-T.

Hoffman, D.R., El-Choufani, S.E., Smith, M.M., Groot, H. De, 2001. Occupational allergy to bumblebees: allergens of *Bombus terrestris*. J. Allergy Clin. Immunol. 108, 855–860. https://doi.org/10.1067/mai.2001.119029.

Hoffman, D.R., Jacobson, R.S., 1996. Allergens in hymenoptera venom XXVII: bumblebee venom allergy and allergens. J. Allergy Clin. Immunol. 97, 812–821. https://doi.org/10.1016/S0091-6749(96)80159-X.

Hoffman, D.R., Sakell, R.H., Schmidt, M., 2005. Sol i 1, the phospholipase allergen of imported fire ant venom. J. Allergy Clin. Immunol. 115, 611–616. https://doi.org/10.1016/j.jaci.2004.11.020.

Hoffman, D.R., Smith, A.M., Schmidt, M., Moffitt, J.E., Guralnick, M., 1990. Allergens in Hymenoptera venom. XXII. Comparison of venoms from two species of imported fire ants, *Solenopsis invicta* and richteri. J. Allergy Clin. Immunol. 85, 988–996. https://doi.org/10.1016/0091-6749(90)90042-3.

Hoffman, P.S., 2020. Antibacterial discovery: 21st century challenges. Antibiotics 9. https://doi.org/10.3390/antibiotics9050213.

Hoskin, D.W., Ramamoorthy, A., 2008. Studies on anticancer activities of antimicrobial peptides. Biochim. Biophys. Acta Biomembr. 1778, 357–375. https://doi.org/10.1016/j.bbamem.2007.11.008.

Hossen, M.S., Gan, S.H., Khalil, M.I., 2017. Melittin, a potential natural toxin of crude bee venom: probable future arsenal in the treatment of diabetes mellitus. J. Chem. 2017, 1–7. https://doi.org/10.1155/2017/4035626, 4035626.

Huber, J.T., 2017. Biodiversity of hymenoptera. In: Foottit, R.G., Adler, P.H. (Eds.), Insect Biodiversity: Science and Society. Wiley-Blackwell, pp. 303–323. https://doi.org/10.1002/9781444308211.ch12.

Jensen, T., Walker, A.A., Nguyen, S.H., Jin, A.H., Deuis, J.R., Vetter, I., King, G.F., Schmidt, J.O., Robinson, S.D., 2021. Venom chemistry underlying the painful stings of velvet ants (Hymenoptera: mutillidae). Cell. Mol. Life Sci. 78, 5163–5177. https://doi.org/10.1007/s00018-021-03847-1.

Jin, W., Klem, A.M., Lewis, J.H., Lu, Z., 1999. Mechanisms of inward-rectifier K+ channel inhibition by tertiapin-Q. Biochemistry 38, 14294–14301. https://doi.org/10.1021/bi991206j.

Kanjhan, R., Coulson, E.J., Adams, D.J., Bellingham, M.C., 2005. Tertiapin-Q blocks recombinant and native large conductance K+ channels in a use-dependent manner. J. Pharmacol. Exp. Ther. 314, 1353–1361. https://doi.org/10.1124/jpet.105.085928.

Kawakami, H., Goto, S.G., Murata, K., Matsuda, H., Shigeri, Y., Imura, T., Inagaki, H., Shinada, T., 2017. Isolation of biologically active peptides from the venom of Japanese carpenter bee, *Xylocopa appendiculata*. J. Venom. Anim. Toxins Incl. Trop. Dis. 23, 1–11. https://doi.org/10.1186/s40409-017-0119-6.

Kazuma, K., Masuko, K., Konno, K., Inagaki, H., 2017. Combined venom gland transcriptomic and venom peptidomic analysis of the predatory ant *Odontomachus monticola*. Toxins 9. https://doi.org/10.3390/toxins9100323.

Khan, S., Gul, A., Noreen, R., Ahmad, S., Ashraf, M., Awan, M.S.B., Niazi, Z.R., Khan, N., 2018. Potential applications of venom peptides as anti-thrombotic agents for management of arterial and deep-vein thrombosis. Protein Pept. Lett. 25, 677–687. https://doi.org/10.2174/0929866524666180614100101.

King, T.P., Lu, G., Gonzalez, M., Qian, N., Soldatova, L., 1996. Yellow jacket venom allergens, hyaluronidase and phospholipase: sequence similarity and antigenic cross-reactivity with their hornet and wasp homologs and possible implications for clinical allergy. J. Allergy Clin. Immunol. 98, 588–600. https://doi.org/10.1016/S0091-6749(96)70093-3.

Kini, R.M., 2006. Anticoagulant proteins from snake venoms: structure, function and mechanism. Biochem. J. 397, 377–387. https://doi.org/10.1042/BJ20060302.

Kitamura, H., Yokoyama, M., Akita, H., Matsushita, K., Kurachi, Y., Yamada, M., 2000. Tertiapin potently and selectively blocks muscarinic K(+) channels in rabbit cardiac myocytes. J. Pharmacol. Exp. Ther. 293, 196. LP – 205.

Kolarich, D., Léonard, R., Hemmer, W., Altmann, F., 2005. The N-glycans of yellow jacket venom hyaluronidases and the protein sequence of its major isoform in *Vespula vulgaris*. FEBS J. 272, 5182–5190. https://doi.org/10.1111/j.1742-4658.2005.04841.x.

Konno, K., Hisada, M., Itagaki, Y., Naoki, H., Kawai, N., Miwa, A., Yasuhara, T., Takayama, H., 1998. Isolation and structure of pompilidotoxins, novel peptide neurotoxins in solitary wasp venoms. Biochem. Biophys. Res. Commun. 250, 612–616. https://doi.org/10.1006/bbrc.1998.9299.

Konno, K., Hisada, M., Naoki, H., Itagaki, Y., Fontana, R., Rangel, M., Oliveira, J.S., Cabrera, M.Pdoss., Neto, J.R., Hide, I., Nakata, Y., Yasuhara, T., Nakajima, T., 2006. Eumenitin, a novel antimicrobial peptide from the venom of the solitary eumenine wasp *Eumenes rubronotatus*. Peptides 27, 2624–2631. https://doi.org/10.1016/j.peptides.2006.04.013.

Konno, K., Hisada, M., Naoki, H., Itagaki, Y., Yasuhara, T., Nakata, Y., Miwa, A., Kawai, N., 2000. Molecular determinants of binding of a wasp toxin (PMTXs) and its analogs in the Na+ channels proteins. Neurosci. Lett. 285, 29–32. https://doi.org/10.1016/S0304-3940(00)01017-X.

Krystel-Whittemore, M., Dileepan, K.N., Wood, J.G., 2016. Mast cell: a multi-functional master cell. Front. Immunol. 6, 1–12. https://doi.org/10.3389/fimmu.2015.00620.

Kuchler, K., Gmachl, M., Sippl, M.J., Kreil, G., 1989. Analysis of the cDNA for phospholipase A2 from honeybee venom glands. The deduced amino acid sequence reveals homology to the corresponding vertebrate enzymes. Eur. J. Biochem. 184, 249–254. https://doi.org/10.1111/j.1432-1033.1989.tb15014.x.

Labbé-Jullié, C., Granier, C., Albericio, F., Defendini, M.-L., Ceard, B., Rochat, H., Van Rietschoten, J., 1991. Binding and toxicity of apamin: characterization of the active site. Eur. J. Biochem. 196, 639–645. https://doi.org/10.1111/j.1432-1033.1991.tb15860.x.

Lameu, C., Márcia, N., Hayashi, M.A.F., 2013. Venom bradykinin-related peptides (BRPs) and its multiple biological roles. In: Rádis-Baptista, G. (Ed.), An Integrated View of the Molecular Recognition and Toxinology - from Analytical Procedures to Biomedical Applications. IntechOpen. https://doi.org/10.5772/52872.

Leandro, L.F., Mendes, C.A., Casemiro, L.A., Vinholis, A.H.C., Cunha, W.R., De Almeida, R., Martins, C.H.G., 2015. Antimicrobial activity of apitoxin, melittin and phospholipase A2 of honey bee (*Apis mellifera*) venom against oral pathogens. An. Acad. Bras. Cienc. 87, 147–155. https://doi.org/10.1590/0001-3765201520130511.

Lee, K.S., Kim, B.Y., Yoon, H.J., Choi, Y.S., Jin, B.R., 2016. Secapin, a bee venom peptide, exhibits anti-fibrinolytic, anti-elastolytic, and anti-microbial activities. Dev. Comp. Immunol. 63, 27–35. https://doi.org/10.1016/j.dci.2016.05.011.

Lewis, R.J., Garcia, M.L., 2003. Therapeutic potential of venom peptides. Nat. Rev. Drug Discov. 2, 790–802. https://doi.org/10.1038/nrd1197.

Lima, D.B., Mello, C.P., Bandeira, I.C.J., De Menezes, R.R.P.P.B., Sampaio, T.L., Falcão, C.B., Morlighem, J.É.R.L., Rádis-Baptista, G., Martins, A.M.C., 2018. The dinoponeratoxin peptides from the giant ant *Dinoponera quadriceps* display in vitro antitrypanosomal activity. Biol. Chem. 399, 187–196. https://doi.org/10.1515/hsz-2017-0198.

Lu, G., Villalba, M., Coscia, M.R., Hoffman, D.R., King, T.P., 1993. Sequence analysis and antigenic cross-reactivity of a venom allergen, antigen 5, from hornets, wasps, and yellow jackets. J. Immunol. 150, 2823–2830.

Lüddecke, T., Vilcinskas, A., Lemke, S., 2019. Phylogeny-guided selection of priority groups for venom bioprospecting: harvesting toxin sequences in tarantulas as a case study. Toxins 11. https://doi.org/10.3390/toxins11090488.

Lyu, C., Fang, F., Li, B., 2018. Anti-tumor effects of melittin and its potential applications in clinic. Curr. Protein Pept. Sci. 20, 240–250. https://doi.org/10.2174/1389203719666180612084615.

Magana, M., Pushpanathan, M., Santos, A.L., Leanse, L., Fernandez, M., Ioannidis, A., Giulianotti, M.A., Apidianakis, Y., Bradfute, S., Ferguson, A.L., Cherkasov, A., Seleem, M.N., Pinilla, C., de la Fuente-Nunez, C., Lazaridis, T., Dai, T., Houghten, R. A., Hancock, R.E.W., Tegos, G.P., 2020. The value of antimicrobial peptides in the age of resistance. Lancet Infect. Dis. 20, e216–e230. https://doi.org/10.1016/S1473-3099(20)30327-3.

Manniello, M.D., Moretta, A., Salvia, R., Scieuzo, C., Lucchetti, D., Vogel, H., Sgambato, A., Falabella, P., 2021. Insect antimicrobial peptides: potential weapons to counteract the antibiotic resistance. Cell. Mol. Life Sci. 78, 4259–4282. https://doi.org/10.1007/s00018-021-03784-z.

McCleary, R.J.R., Kang, T.S., Kini, R.M., 2015. Reptile venoms as a platform for drug development. In: King, G.F. (Ed.), Venoms to Drugs: Venom as a Source for the Development of Human Therapeutics. The Royal Society of Chemistry, Cambridge, UK, pp. 1507–1508. https://doi.org/10.1007/s10337-015-2962-3.

Memariani, H., Memariani, M., Moravvej, H., Shahidi-Dadras, M., 2020. Melittin: a venom-derived peptide with promising anti-viral properties. Eur. J. Clin. Microbiol. Infect. Dis. 39, 5–17. https://doi.org/10.1007/s10096-019-03674-0.

Mendes, M.A., De Souza, B.M., Dos Santos, L.D., Palma, M.S., 2004. Structural characterization of novel chemotactic and mastoparan peptides from the venom of the social wasp *Agelaia pallipes pallipes* by high-performance liquid chromatography/electrospray ionization tandem mass spectrometry. Rapid Commun. Mass Spectrom. 18, 636–642. https://doi.org/10.1002/rcm.1382.

Mendes, M.A., Palma, M.S., 2006. Two new bradykinin-related peptides from the venom of the social wasp *Protopolybia exigua* (Saussure). Peptides 27, 2632–2639. https://doi.org/10.1016/j.peptides.2006.05.018.

Michel, Y., McIntyre, M., Ginglinger, H., Ollert, M., Cifuentes, L., Blank, S., Spillner, E., 2012. The putative serine protease inhibitor Api m 6 from *Apis Mellifera* venom: recombinant and structural evaluation. J Investig. Allergol. Clin. Immunol. 22, 476–484.

Mistry, J., Chuguransky, S., Williams, L., Qureshi, M., Salazar, G.A., Sonnhammer, E.L.L., Tosatto, S.C.E., Paladin, L., Raj, S., Richardson, L.J., Finn, R.D., Bateman, A., 2021. Pfam: the protein families database in 2021. Nucleic Acids Res. 49, D412–D419. https://doi.org/10.1093/nar/gkaa913.

Moawad, T.I., Hoffman, D.R., Zalat, S., 2005. Isolation, cloning and characterization of *Polistes dominulus* venom phospholipase A1 and its isoforms. Acta Biol. Hung. 56, 261–274. https://doi.org/10.1556/ABiol.56.2005.3-4.9.

Moon, D.O., Park, S.Y., Choi, Y.H., Kim, N.D., Lee, C., Kim, G.Y., 2008. Melittin induces Bcl-2 and caspase-3-dependent apoptosis through downregulation of Akt phosphorylation in human leukemic U937 cells. Toxicon 51, 112–120. https://doi.org/10.1016/j.toxicon.2007.08.015.

Moore, E.L., Arvidson, R., Banks, C., Urenda, J.P., Duong, E., Mohammed, H., Adams, M. E., 2018. Ampulexins: a new family of peptides in venom of the emerald jewel wasp, *Ampulex compressa*. Biochemistry 57, 1907–1916. https://doi.org/10.1021/acs.biochem.7b00916.

Moreau, S.J.M., Cherqui, A., Doury, G., Dubois, F., Fourdrain, Y., Sabatier, L., Bulet, P., Saarela, J., Prévost, G., Giordanengo, P., 2004. Identification of an aspartylglucosaminidase-like protein in the venom of the parasitic wasp *Asobara tabida* (Hymenoptera: braconidae). Insect Biochem. Mol. Biol. 34, 485–492. https://doi.org/10.1016/j.ibmb.2004.03.001.

Morgan, N.G., Montague, W., 1984. Stimulation of insulin secretion from isolated rat islets of Langerhans by melittin. Biosci. Rep. 4, 665–671. https://doi.org/10.1007/BF01121020.

Müller, U.R., 2011. Hymenoptera venom proteins and peptides for diagnosis and treatment of venom allergic patients. Inflamm. Allergy - Drug Targets 10, 420–428. https://doi.org/10.2174/187152811797200704.

Mylonakis, E., Podsiadlowski, L., Muhammed, M., Vilcinskas, A., 2016. Diversity, evolution and medical applications of insect antimicrobial peptides. Philos. Trans. R. Soc. B Biol. Sci. 371 https://doi.org/10.1098/rstb.2015.0290.

Nicolas, J.P., Lin, Y., Lambeau, G., Ghomashchi, F., Lazdunski, M., Gelb, M.H., 1997. Localization of structural elements of bee venom phospholipase A2 involved in N-type receptor binding and neurotoxicity. J. Biol. Chem. 272, 7173–7181. https://doi.org/10.1074/jbc.272.11.7173.

Nixon, S.A., Dekan, Z., Robinson, S.D., Guo, S., Vetter, I., Kotze, A.C., Alewood, P.F., King, G.F., Herzig, V., 2020. It takes two: dimerization is essential for the broad-spectrum predatory and defensive activities of the venom peptide Mp1a from the jack jumper ant *Myrmecia pilosula*. Biomedicines 8, 1–13. https://doi.org/10.3390/biomedicines8070185.

Nixon, S.A., Robinson, S.D., Agwa, A.J., Walker, A.A., Choudhary, S., Touchard, A., Undheim, E.A.B., Robertson, A., Vetter, I., Schroeder, C.I., Kotze, A.C., Herzig, V., King, G.F., 2021. Multipurpose peptides: the venoms of Amazonian stinging ants contain anthelmintic ponericins with diverse predatory and defensive activities. Biochem. Pharmacol. 192, 114693. https://doi.org/10.1016/j.bcp.2021.114693.

Ollert, M., Blank, S., 2015. Anaphylaxis to insect venom allergens: role of molecular diagnostics. Curr. Allergy Asthma Rep. 15 https://doi.org/10.1007/s11882-015-0527-z.

Orivel, J., Redeker, V., Le Caer, J.P., Krier, F., Revol-Junelles, A.M., Longeon, A., Chaffotte, A., Dejean, A., Rossier, J., 2001. Ponericins, new antibacterial and insecticidal peptides from the venom of the ant pachycondyla goeldii. J. Biol. Chem. 276, 17823–17829. https://doi.org/10.1074/jbc.M100216200.

Pallaghy, P.K., Norton, R.S., Nielsen, K.J., Craik, D.J., 1994. A common structural motif incorporating a cystine knot and a triple-stranded β-sheet in toxic and inhibitory polypeptides. Protein Sci. 3, 1833–1839. https://doi.org/10.1002/pro.5560031022.

Pan, J., Hink, W.F., 2000. Isolation and characterization of myrmexins, six isoforms of venom proteins with anti-inflammatory activity from the tropical ant. Pseudomyrmex triplarinus. Toxicon 38, 1403–1413. https://doi.org/10.1016/S0041-0101(99)00233-0.

Pantera, B., Hoffman, D.R., Carresi, L., Cappugi, G., Turillazzi, S., Manao, G., Severino, M., Spadolini, I., Orsomando, G., Moneti, G., Pazzagli, L., 2003. Characterization of the major allergens purified from the venom of the paper wasp *Polistes gallicus*. Biochim. Biophys. Acta Gen. Subj. 1623, 72–81. https://doi.org/10.1016/j.bbagen.2003.07.001.

Parkinson, N.M., Conyers, C., Keen, J., MacNicoll, A., Smith, I., Audsley, N., Weaver, R., 2004. Towards a comprehensive view of the primary structure of venom proteins from the parasitoid wasp *Pimpla hypochondriaca*. Insect Biochem. Mol. Biol. 34, 565–571. https://doi.org/10.1016/j.ibmb.2004.03.003.

Parkinson, N.M., Conyers, C.M., Keen, J.N., MacNicoll, A.D., Smith, I., Weaver, R.J., 2003. cDNAs encoding large venom proteins from the parasitoid wasp *Pimpla hypochondriaca* identified by random sequence analysis. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 134, 513–520. https://doi.org/10.1016/S1532-0456(03)00041-3.

Peiren, N., de Graaf, D.C., Brunain, M., Bridts, C.H., Ebo, D.G., Stevens, W.J., Jacobs, F.J., 2006. Molecular cloning and expression of icarapin, a novel IgE-binding bee venom protein. FEBS Lett. 580, 4895–4899. https://doi.org/10.1016/j.febslet.2006.08.005.

Picolo, G., Hisada, M., Moura, A.B., Machado, M.F.M., Sciani, J.M., Conceição, I.M., Melo, R.L., Oliveira, V., Lima-Landman, M.T.R., Cury, Y., Konno, K., Hayashi, M.A. F., 2010. Bradykinin-related peptides in the venom of the solitary wasp *Cyphononyx fulvognathus*. Biochem. Pharmacol. 79, 478–486. https://doi.org/10.1016/j.bcp.2009.08.020.

Pineda, S.S., Chin, Y.K.Y., Undheim, E.A.B., Senff, S., Mobli, M., Dauly, C., Escoubas, P., Nicholson, G.M., Kaas, Q., Guo, S., Herzig, V., Mattick, J.S., King, G.F., 2020. Structural venomics reveals evolution of a complex venom by duplication and diversification of an ancient peptide-encoding gene. Proc. Natl. Acad. Sci. U.S.A. 117 https://doi.org/10.1073/pnas.1914536117.

Plisson, F., Ramírez-Sánchez, O., Martínez-Hernández, C., 2020. Machine learning-guided discovery and design of non-hemolytic peptides. Sci. Rep. 10, 1–19. https://doi.org/10.1038/s41598-020-73644-6.

Pluzhinikov, K.A., Nol'de, D.E., Tertyshnikova, S.M., Sukhanov, S.V., Sobol', A.G., Torgov, M.I., Filippov, A.K., Arsen'ev, A.S., Grishin, E.V., 1994. Structure-activity study of the basic toxic component of venom from the ant *Ectatomma tuberculatum*. Bioorg. Khim. 20, 857–871.

Pluzhnikov, K., Nosyreva, E., Shevchenko, L., Kokoz, Y., Schmalz, D., Hucho, F., Grishin, E., 1999. Analysis of ectatomin action on cell membranes. Eur. J. Biochem. 262, 501–506. https://doi.org/10.1046/j.1432-1327.1999.00426.x.

Pluzhnikov, K.A., Kozlov, S.A., Vassilevski, A.A., Vorontsova, O.V., Feofanov, A.V., Grishin, E.V., 2014. Linear antimicrobial peptides from *Ectatomma quadridens* ant venom. Biochimie 107, 211–215. https://doi.org/10.1016/j.biochi.2014.09.012.

Price, D.R.G., Bell, H.A., Hinchliffe, G., Fitches, E., Weaver, R., Gatehouse, J.A., 2009. A venom metalloproteinase from the parasitic wasp *Eulophus pennicornis* is toxic towards its host, tomato moth (Lacanobia oleracae). Insect Mol. Biol. 18, 195–202. https://doi.org/10.1111/j.1365-2583.2009.00864.x.

Qiu, Y., Lee, K.S., Choo, Y.M., Kong, D., Yoon, H.J., Jin, B.R., 2013. Molecular cloning and antifibrinolytic activity of a serine protease inhibitor from bumblebee (*Bombus terrestris*) venom. Toxicon 63, 1–6. https://doi.org/10.1016/j.toxicon.2012.11.004.

R Core Team, 2021. R: A Language and Environment for Statistical Computing.

Radlović, N., 2013. Celiac disease. Srp. Arh. Celok. Lek. 141, 122–126. https://doi.org/10.2298/SARH1302122R.

Ranasinghe, S., McManus, D.P., 2013. Structure and function of invertebrate Kunitz serine protease inhibitors. Dev. Comp. Immunol. 39, 219–227. https://doi.org/10.1016/j.dci.2012.10.005.

Rangel, M., dos Santos Cabrera, M.P., Kazuma, K., Ando, K., Wang, X., Kato, M., Nihei, K. ichi, Hirata, I.Y., Cross, T.J., Garcia, A.N., Faquim-Mauro, E.L., Franzolin, M.R., Fuchino, H., Mori-Yasumoto, K., Sekita, S., Kadowaki, M., Satake, M., Konno, K., 2011. Chemical and biological characterization of four new linear cationic α-helical peptides from the venoms of two solitary eumenine wasps. Toxicon 57, 1081–1092. https://doi.org/10.1016/j.toxicon.2011.04.014.

Ribeiro, S.P., Mendes, M.A., dos Santos, L.D., de Souza, B.M., Marques, M.R., de Azevedo Jr., W.F., Palma, M.S., 2004. Structural and functional characterization of N-terminally blocked peptides isolated from the venom of the social wasp Polybia paulista. Peptides 25 (12), 2069–2078. https://doi.org/10.1016/j.peptides.2004.08.019.

Rifflet, A., Gavalda, S., Téné, N., Orivel, J., Leprince, J., Guilhaudis, L., Génin, E., Vétillard, A., Treilhou, M., 2012. Identification and characterization of a novel antimicrobial peptide from the venom of the ant *Tetramorium bicarinatum*. Peptides 38, 363–370. https://doi.org/10.1016/j.peptides.2012.08.018.

Robinson, S.D., Mueller, A., Clayton, D., Starobova, H., Hamilton, B.R., Payne, R.J., Vetter, I., King, G.F., Undheim, E.A.B., 2018. A comprehensive portrait of the venom of the giant red bull ant, *Myrmecia gulosa*, reveals a hyperdiverse hymenopteran toxin gene family. Sci. Adv. 4, 1–13. https://doi.org/10.1126/sciadv.aau4640.

Rocchi, R., Biondi, L., Filira, F., Scolaro, P.B., 2009. Synthesis, conformation, and biological activity of the carbohydrate-free vespulakinin 1. Int. J. Pept. Protein Res. 30, 240–256. https://doi.org/10.1111/j.1399-3011.1987.tb03332.x.

Rodriguez De La Vega, R.C., Corzo, G., Possani, L.D., 2015. Scorpion venoms as a platform for drug development. In: King, G.F. (Ed.), Venoms to Drugs: Venom as a Source for the Development of Human Therapeutics. The Royal Society of Chemistry, Cambridge, UK, pp. 204–220. https://doi.org/10.1039/9781849737876-00204.

RStudio Team, 2021. RStudio. Integrated Development for R.

Rungsa, P., Peigneur, S., Daduang, S., Tytgat, J., 2018. Purification and biochemical characterization of VesT1s, a novel phospholipase A1 isoform isolated from the venom of the greater banded wasp *Vespa tropica*. Toxicon 148, 74–84. https://doi.org/10.1016/j.toxicon.2018.03.015.

Schiavon, E., Stevens, M., Zaharenko, A.J., Konno, K., Tytgat, J., Wanke, E., 2010. Voltage-gated sodium channel isoform-specific effects of pompilidotoxins. FEBS J. 277, 918–930. https://doi.org/10.1111/j.1742-4658.2009.07533.x.

Sekimura, T., Nijhout, H.F., 2017. Diversity and evolution of butterfly wing patterns: an integrative approach 1–321. https://doi.org/10.1007/978-981-10-4956-9. ISBN 9789811049569.

Senji Laxme, R.R., Suranse, V., Sunagar, K., 2019. Arthropod venoms: biochemistry, ecology and evolution. Toxicon 158, 84–103. https://doi.org/10.1016/j.toxicon.2018.11.433.

Shi, W.-J., Zhang, S.-F., Zhang, C.-X., Cheng, J.-A., 2003. Cloning and comparative analysis of the venom prepromelittin genes from four wasp species. Yi Chuan Xue Bao 30, 555–559.

Shipolini, R.A., Callewaert, G.L., Cottrel, R.C., Vernon, C.A., 1974. The amino-acid sequence and carbohydrate content of phospholipase A2 from bee venom. Eur. J. Biochem. 48, 465–476. https://doi.org/10.1111/j.1432-1033.1974.tb03787.x.

Simonet, G., Claeys, I., Broeck, J. Vanden, 2002. Structural and functional properties of a novel serine protease inhibiting peptide family in arthropods. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 132, 247–255. https://doi.org/10.1016/S1096-4959(01)00530-9.

Skov, L.K., Seppälä, U., Coen, J.J.F., Crickmore, N., King, T.P., Monsalve, R., Kastrup, J. S., Spangfort, M.D., Gajhede, M., 2006. Structure of recombinant Ves v 2 at 2.0 Å resolution: structural analysis of an allergenic hyaluronidase from wasp venom. Acta Crystallogr. Sect. D Biol. Crystallogr. 62, 595–604. https://doi.org/10.1107/S0907444906010687.

Smith, J.J., Ho Yee Lau, C., Herzig, V., Ikonomopoulou, M.P., Rash, L.D., 2015. Therapeutic applications of spider-venom peptides. In: King, G.F. (Ed.), Venoms to Drugs: Venom as a Source for the Development of Human Therapeutics. The Royal Society of Chemistry, Cambridge, UK, pp. 221–244.

Sosa, B.P., Alagón, A.C., Martin, B.M., Possani, L.D., 1986. Biochemical characterization of the phospholipase A2 purified from the venom of the Mexican beaded lizard (*Heloderma horridum horridum wiegmann*). Biochemistry 25, 2927–2933. https://doi.org/10.1021/bi00358a029.

Souza, B.M., Mendes, M.A., Santos, L.D., Marques, M.R., César, L.M.M., Almeida, R.N.A., Pagnocca, F.C., Konno, K., Palma, M.S., 2005. Structural and functional characterization of two novel peptide toxins isolated from the venom of the social wasp *Polybia paulista*. Peptides 26, 2157–2164. https://doi.org/10.1016/j.peptides.2005.04.026.

Sukprasert, S., Rungsa, P., Uawonggul, N., Incamnoi, P., Thammasirirak, S., Daduang, J., Daduang, S., 2013. Purification and structural characterisation of phospholipase A1 (Vespapase, Ves a 1) from Thai banded tiger wasp (*Vespa affinis*) venom. Toxicon 61, 151–164. https://doi.org/10.1016/j.toxicon.2012.10.024.

Teichert, R.W., Olivera, B.M., Michael McIntosh, J., Bulaj, G., Horvath, M.P., 2015. The molecular diversity of conoidean venom peptides and their targets: from basic research to therapeutic applications. In: King, G.F. (Ed.), Venoms to Drugs: Venom as a Source for the Development of Human Therapeutics. The Royal Society of Chemistry, Cambridge, UK, pp. 163–203. https://doi.org/10.1039/9781849737876-00163.

Téné, N., Bonnafé, E., Berger, F., Rifflet, A., Guilhaudis, L., Ségalas-Milazzo, I., Pipy, B., Coste, A., Leprince, J., Treilhou, M., 2016. Biochemical and biophysical combined study of bicarinalin, an ant venom antimicrobial peptide. Peptides 79, 103–113. https://doi.org/10.1016/j.peptides.2016.04.001.

The UniProt Consortium, 2021. UniProt: the universal protein knowledgebase in 2021. Nucleic Acids Res. 49 (D1), D480–D489. https://doi.org/10.1093/nar/gkaa1100.

Toki, T., Yasuhara, T., Nakajima, T., 1988. Isolation and sequential analysis of peptides on the venom sac of *Parapolybia indica*. Med. Entomol. Zool. 39, 105–111. https://doi.org/10.7601/mez.39.105.

Torres, A.F., Huang, C., Chong, C.M., Leung, S.W., Prieto-da-Silva, Á.R.B., Havt, A., Quinet, Y.P., Martins, A.M.C., Lee, S.M.Y., Rádis-Baptista, G., 2014. Transcriptome analysis in venom gland of the predatory giant ant *Dinoponera quadriceps*: insights into the polypeptide toxin arsenal of hymenopterans. PLoS One 9. https://doi.org/10.1371/journal.pone.0087556.

Torres, M.D.T., Pedron, C.N., Higashikuni, Y., Kramer, R.M., Cardoso, M.H., Oshiro, K.G. N., Franco, O.L., Silva Junior, P.I., Silva, F.D., Oliveira Junior, V.X., Lu, T.K., de la Fuente-Nunez, C., 2018. Structure-function-guided exploration of the antimicrobial peptide polybia-CP identifies activity determinants and generates synthetic therapeutic candidates. Commun. Biol. 1, 1–16. https://doi.org/10.1038/s42003-018-0224-2.

Touchard, A., Brust, A., Cardoso, F.C., Chin, Y.K.Y., Herzig, V., Jin, A.H., Dejean, A., Alewood, P.F., King, G.F., Orivel, J., Escoubas, P., 2016. Isolation and characterization of a structurally unique β-hairpin venom peptide from the predatory ant *Anochetus emarginatus*. Biochim. Biophys. Acta Gen. Subj. 1860, 2553–2562. https://doi.org/10.1016/j.bbagen.2016.07.027.

Touchard, A., Mendel, H.C.H.C., Boulogne, I., Herzig, V., Braga Emidio, N., King, G.F., Triquigneaux, M., Jaquillard, L., Beroud, R., De Waard, M., Delalande, O., Dejean, A., Muttenthaler, M., Duplais, C., 2020. Heterodimeric insecticidal peptide provides new insights into the molecular and functional diversity of ant venoms. ACS Pharmacology & Translational Science 3 (6), 1211–1224. https://doi.org/10.1021/acsptsci.0c00119.

Turillazzi, S., Mastrobuoni, G., Dani, F.R., Moneti, G., Pieraccini, G., La Marca, G., Bartolucci, G., Perito, B., Lambardi, D., Cavallini, V., Dapporto, L., 2006. Dominulin A and B: two new antibacterial peptides identified on the cuticle and in the venom of the social paper wasp *Polistes dominulus* using MALDI-TOF, MALDI-TOF/TOF, and ESI-ion trap. J. Am. Soc. Mass Spectrom. 17, 376–383. https://doi.org/10.1016/j.jasms.2005.11.017.

Undheim, E.A.B., Mobli, M., King, G.F., 2016. Toxin structures as evolutionary tools: using conserved 3D folds to study the evolution of rapidly evolving peptides. Bioessays 38, 539–548. https://doi.org/10.1002/bies.201500165.

Utkin, Y.N., 2015. Animal venom studies: current benefits and future developments. World J. Biol. Chem. 6, 28. https://doi.org/10.4331/wjbc.v6.i2.28.

Van Den Bogaart, G., Guzmán, J.V., Mika, J.T., Poolman, B., 2008. On the mechanism of pore formation by melittin. J. Biol. Chem. 283, 33854–33857. https://doi.org/10.1074/jbc.M805171200.

van Galen, K.P., Engelen, E., Mauser-Bunschoten, E.P., van Es, R.J., Schutgens, R.E., 2019. Antifibrinolytic therapy for preventing oral bleeding in patients with haemophilia or von willebrand disease undergoing minor oral surgery or dental extractions. Cochrane Database Syst. Rev. 32 https://doi.org/10.1002/14651858.CD011385.pub3.

Wang, C.K., Craik, D.J., 2018. Designing macrocyclic disulfide-rich peptides for biotechnological applications perspective. Nat. Chem. Biol. 14, 417–427. https://doi.org/10.1038/s41589-018-0039-y.

Wang, G., Li, X., Wang, Z., 2016. APD3: the antimicrobial peptide database as a tool for research and education. Nucleic Acids Res. 44, D1087–D1093. https://doi.org/10.1093/nar/gkv1278.

Wang, K., Yan, J., Liu, X., Zhang, J., Chen, R., Zhang, B., Dang, W., Zhang, W., Kai, M., Song, J., Wang, R., 2011. Novel cytotoxity exhibition mode of polybia-CP, a novel antimicrobial peptide from the venom of the social wasp *Polybia paulista*. Toxicology 288, 27–33. https://doi.org/10.1016/j.tox.2011.06.014.

Weber, A., Schröder, H., Thalberg, K., Marz, L., 1987. Specific interaction of IgE antibodies with a carbohydrate epitope of honey bee venom phospholipase A2. Allergy 42, 464–470. https://doi.org/10.1111/j.1398-9995.1987.tb00364.x.

Wickham, H., 2009. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, Media.

Windley, M.J., Herzig, V., Dziemborowicz, S.A., Hardy, M.C., King, G.F., Nicholson, G. M., 2012. Spider-venom peptides as bioinsecticides. Toxins 4, 191–227. https://doi.org/10.3390/toxins4030191.

Winningham, K.M., Fitch, C.D., Schmidt, M., Hoffman, D.R., 2004. Hymenoptera venom protease allergens. J. Allergy Clin. Immunol. 114, 928–933. https://doi.org/10.1016/j.jaci.2004.07.043.

Xu, X., Li, J., Lu, Q., Yang, H., Zhang, Y., Lai, R., 2006a. Two families of antimicrobial peptides from wasp (*Vespa magnifica*) venom. Toxicon 47, 249–253. https://doi.org/10.1016/j.toxicon.2005.10.015.

Xu, X., Yang, H., Yu, H., Li, J., Lai, R., 2006b. The mastoparanogen from wasp. Peptides 27, 3053–3057. https://doi.org/10.1016/j.peptides.2006.09.003.

Xu, Y., Chen, G., 2015. Mast cell and autoimmune diseases. Mediat. Inflamm. 2015, 1–8. https://doi.org/10.1155/2015/246126. Article ID 246126.

Yang, H., Xu, X., Ma, D., Zhang, K., Lai, R., 2008. A phospholipase A1 platelet activator from the wasp venom of *Vespa magnifica* (Smith). Toxicon 51, 289–296. https://doi.org/10.1016/j.toxicon.2007.10.003.

Yang, X., Wang, Y., Lu, Z., Zhai, L., Jiang, J., Liu, J., Yu, H., 2009. A novel serine protease inhibitor from the venom of *Vespa bicolor Fabricius*. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 153, 116–120. https://doi.org/10.1016/j.cbpb.2009.02.010.

Yoshida, H., Geller, R.G., Pisano, J.J., 1975. Vespulakinins: new carbohydrate-containing bradykinin derivativest. Biochemistry 15, 61–64. https://doi.org/10.1021/bi00646a010.

Zeileis, A., Fisher, J.C., Hornik, K., Ihaka, R., McWhite, C.D., Murrell, P., Stauffer, R., Wilke, C.O., 2020. Colorspace: a toolbox for manipulating and assessing colors and palettes. J. Stat. Software 96, 1–49. https://doi.org/10.18637/jss.v096.i01.

Zhang, S.-F., Shi, W.-J., Cheng, J.-A., Zhang, C.-X., 2003. Cloning and comparison of the genes encoding preproapamin from the venom of 2 honeybee and 4 wasp species. Insect Sci. 10, 21–26. https://doi.org/10.1111/j.1744-7917.2003.tb00360.x.