Spider Venoms
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The Venom of Australian Spiders

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Abstract

Australia is home to an estimated 10,000 species of spider, including species from the *Latrodectus* genera and Atracinae family, two of the four widely recognized medically significant spider groups. It is predicted in excess of 5,000 spider bite cases occurring annually in Australia, predominantly by spiders that have not shown any medical relevance. Bites by medically relevant spiders are rare, and of those treatment with antivenom is rarer. Despite extensive publicity and rumor, there is no conclusive evidence that the venom of any Australian spiders is responsible for necrotic arachnidism. The complexity and diversity of spider venoms, combined with potent activity on a range of targets in mammalian and insect systems, have attracted interest in the potential of spider venoms as a source of insecticidal and therapeutic leads. The venom of species of Australian funnel-web spider has received the most attention for study, with more than 75 venom peptides identified from nine toxin families. Recent work has identified venom peptides from the venom of Australian tarantulas with potential as insecticidal and therapeutic leads. This chapter provides an overview of spiders in Australia and their medical and clinical importance and provides a current comprehensive review of the published toxins from Australian spider venoms.

Keywords

Australian spiders; Funnel-web spiders; Redback spiders; Spider bite; Spider venom

Introduction

Spiders (Arthropoda: Arachnida: Araneae) constitute the most successful venomous creature, in terms of speciation and distribution, on the planet and, with the possible exception of predatory beetles, are the most prevalent terrestrial predators (King and Hardy 2013). More than 45,000 species are currently described (World Spider Catalog, version 16.5 (2015)), and estimates predict there are more than 150,000 extant species in total (Coddington and Levi 1991). Australia is thought to be home to 10,000 of these species (Nicholson et al. 2006). Consequently, human interaction with spiders is common, and the number of spider bites in Australia is estimated to exceed 5,000 cases annually (Isbister and White 2004). Worldwide there are four widely recognized groups of spiders that are significantly medically important: members of the Araneomorphae genera *Latrodectus*, *Loxosceles*, and *Phoneutria* and the genera belonging to the mygalomorph family, Hexathelidae. Australia is home to two of these groups, namely, *Latrodectus* and Hexathelidae. While these spiders are widely recognized, other spiders have been identified as potentially medically important (e.g., mouse spiders, *Missulena* spp.), or rumored to be clinically important (e.g., white-tailed spiders, *Lampona* spp., and huntsman spiders, *Neosparassus* spp.).

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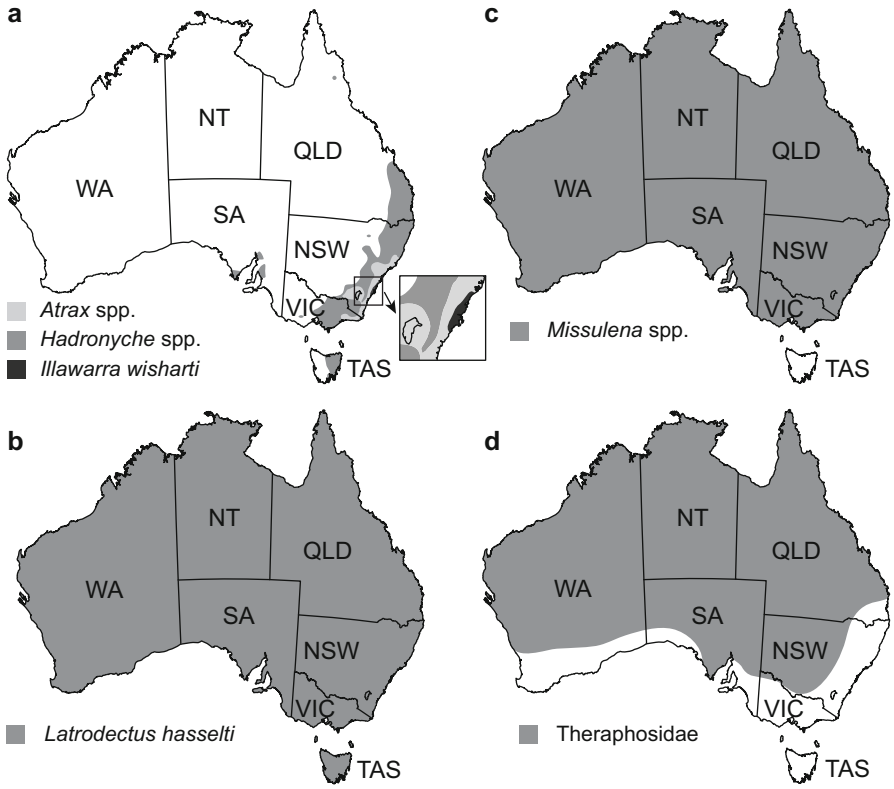

Fig. 1 Distribution maps of Australian spiders. (**a**) Funnel-web spiders (*Atrax* spp., *Hadronyche* spp., and *Illawarra wisharti*), (**b**) redback spider (*Latrodectus hasselti*), (**c**) mouse spiders (*Missulena* spp.), (**d**) Australian tarantulas (Theraphosidae)

The Australian tarantulas have been responsible for a number of bites, but records have shown little effect in humans but more significant effects, including death, in canines.

More recently, work on spider venoms has focused more on the large number and variety of individual molecules present and their potential as bioinsecticides or therapeutic drug leads. Predictions estimate the number of bioactive peptides collectively in spider venoms to exceed ten million, and presently only approximately 0.01 % of this diversity has been characterized (Klint et al. 2012).

This chapter will provide an overview of spiders in Australia and their medical and clinical importance and provide a current comprehensive review of the published toxins from Australian spider venoms.

Clinical Implications of Spiders of Medical Significance in Australia

Australian Funnel-Web Spiders (Hexathelidae)

The Australian funnel-web spiders (Araneae: Mygalomorphae: Hexathelidae: Atracinae) are a group of relatively large, highly venomous primitive spiders found primarily along the southeast coast of mainland Australia and Tasmania, with isolated pockets in South Australia and far north Queensland (see Fig. 1a). A recent revision (2010) of the taxonomy divided the Atracinae into three genera and 35 characterized species: *Atrax* (three species), *Hadronyche* (31 species), and *Illawarra* (one species) (Gray 2010). They are arguably the world's most venomous spiders, with the male Sydney funnel-web spider (*Atrax robustus*) (see Fig. 2a, b) responsible for 13 human fatalities prior to the introduction of an antivenom in 1980 (Nicholson et al. 2006). Completely unrelated to the American funnel-web or grass spider (*Agelenopsis aperta*), the Australian funnel-web spiders are relatively large and are typically highly

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Fig. 2 Photographs of Australian spiders. (**a**) Male Sydney funnel-web spider (*Atrax robustus*), (**b**) female Sydney funnel-web spider (*Atrax robustus*), (**c**) female eastern mouse spider (*Missulena bradleyi*), (**d**) male eastern mouse spider (*Missulena bradleyi*), (**e**) female redback spider (*Latrodectus hasselti*), and (**f**) female northern tarantula (*Phlogius crassipes*) (Photographs by Dr. David Wilson)

aggressive when provoked (see Fig. 2a). Of particular interest with the venom is the "selectivity" toward primates, causing only very mild symptoms in other mammals. The reason is still unknown, but proposed ideas from work that showed a purified fraction of nonimmune rabbit serum as an effective antitoxin against male *A. robustus* venom suggest the presence of endogenous inactivating factors in the form of immunoglobulin G (IgG) in the plasma of non-primates that bind to the δ-hexatoxin-1 (δ-HXTX-1) peptide family (the active toxins in the venom) or a general nonspecific binding to immunoglobulins due to the highly basic nature of the toxins (Nicholson et al. 2006). Also of interest are the gender-related differences in venom activity observed for some of the species, including *A. robustus*. Only the venom of the male spider has been responsible for fatalities. Bites are relatively rare, only contributing to ~1 % of the total number of spider bites reported in Australia (Isbister and Gray 2002), and severe envenomation is observed to occur in 10–25 % of funnel-web spider bites (Isbister and Gray 2004b; Miller et al. 2000). The clinical symptoms of severe envenomation include localized pain, salivation, sweating, vomiting, piloerection, lacrimation, skeletal muscle fasciculation, and disturbances in respiration, blood pressure, and heart rate, followed by severe hypotension. Death can occur due to respiration and circulatory failure or from increased intracranial pressure resulting from cerebral edema (Miller et al. 2000).

No deaths have been recorded from Australian funnel-web envenomation since the introduction of a purified rabbit IgG antivenom, raised against the venom of male *A. robustus*, by Prof. Struan Sutherland in

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1980 (Nicholson et al. 2006). The antivenom has also been reported in case studies to reverse the envenomation syndrome of other species of funnel-web spiders, including *H. formidabilis*, *H. versuta*, *H. infensa*, *H. cerberea*, *H. nimoola* (previously *H.* sp.7), and *H. macquariensis* (previously *H.* sp.14) (Gray 2010; Miller et al. 2000). In vitro studies showed the ability of funnel-web spider antivenom to reverse and neutralize venom from male and female specimens of several species of *Hadronyche*, *Atrax*, and *Illawarra* (Graudins et al. 2002a).

Redback and Widow Spiders (Theridiidae)

The widow, or comb-footed, spiders (Araneae: Araneomorphae: Theridiidae), termed theridiids, can be considered the most clinically relevant spiders in the world. This is due to a worldwide distribution of the primary clinically significant genus, *Latrodectus*. In Australia, the most infamous theridiid is the redback spider, *Latrodectus hasselti* (Fig. 2e). However, other Australian Theridiidae spiders from the genera *Steatoda* and *Archaearanae* have also shown clinical relevance (Isbister and Gray 2003c). In Australia alone, a gross approximation estimates that there are in excess of 5,000 bites by theridiid spiders per year (Isbister and White 2004). The true incidence of envenomation by these spiders worldwide is largely unknown. Some studies exist for envenomation incidence in particular countries.

The clinical symptoms experienced from envenomation by spiders of the genus *Latrodectus* are collectively termed latrodectism. These symptoms include local and regional pain that can be prolonged for days, associated with diaphoresis, malaise, lethargy, nausea, vomiting, headache, fever, hypertension, and tremor, and are responsible for significant morbidity and infrequent mortality (Isbister and Gray 2003c). In a prospective study of redback spider bites in Australia, the majority of bites were shown to cause significant effects, with pain identified as the primary symptom. Persistent pain was reported in 66 % of cases, and one-third experienced severe pain that prevented sleep within the first 24 h (Isbister and Gray 2003b). Envenomation by the genera *Steatoda* and *Archaearanae* was shown to exhibit similar symptoms to latrodectism. In severe cases of envenomation by *Steatoda* ("steatodism"), the clinical effects have been reported as almost indistinguishable from latrodectism, although diaphoresis was not present. In cases of envenomation by *Archaearanae*, the associated pain was reported as similar to latrodectism (Isbister and Gray 2003c).

The treatment of bites by theridiid spiders is problematic and the subject of significant controversy. Antivenom is only available in some countries, and clinical practices vary worldwide. Australia has had access for more than 60 years to a highly purified equine antivenom raised against the redback spider, *L. hasselti*. This antivenom has been shown to prevent both in vitro and in vivo toxicity from venoms of numerous *Latrodectus* species and α-latrotoxin, the primary toxic component in the venom, in mice (Graudins et al. 2001). In addition, the redback antivenom has been reported to have successfully treated a clinical case of steatodism and demonstrated the ability to reverse the effects of *Steatoda* spp. venom in vitro (Graudins et al. 2002b). The effectiveness of redback spider antivenom in the clinical setting has come into question after three randomized controlled trials in Australia and one in the USA. Two of the Australian studies showed no evidence of a difference between administration of the antivenom intravenously and intramuscularly. The third study demonstrated that the addition of redback spider antivenom to standardized analgesia treatment of patients suffering latrodectism did not significantly improve pain or systemic effects. The results of this study support the results of the only other placebo-controlled randomized trial of widow spider antivenom, performed in the USA. Collectively, these studies support the idea that widow spider antivenom may not be effective. Further and larger studies involving different widow spiders and antivenom are required before a definitive conclusion can be reached (Isbister et al. 2014).

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Other Australian Spiders

The Australian mouse spiders (Araneae: Mygalomorphae: Actinopodidae) belong to the genus *Missulena* and are primitive ground-burrowing spiders (see Fig. 2c, d). The 16 known species (World Spider Catalog, version 16.5 (2015)) in Australia are distributed across all states except Tasmania (see Fig. 1c). They are often confused with the Australian funnel-web spiders (Isbister and Gray 2004b). Serious bites from these spiders are rare, with only one report of a serious bite occurring in a 19-month-old child (*Missulena bradleyi*) (Isbister and Gray 2004b). The child experienced a number of symptoms resembling those observed for Australian funnel-web spider bites (muscle fasciculation, dyspnea, hypertension, heavy perspiration, and tachycardia). The condition was reversed by administration of Australian funnel-web spider antivenom (Isbister and Gray 2004b). Isbister and Gray (2004b) reviewed confirmed mouse spider bite cases and identified 40 records from three species (*M. bradleyi*, *M. occatoria*, and *M. pruinosa*) (Isbister and Gray 2004b). Minor local neurotoxic effects, including paresthesia, numbness, and diaphoresis, were evident in six records of bites by *M. bradleyi*. Five cases reported minor systemic effects (headache and nausea). Mouse spider bites were concluded to have the potential to result in severe envenomation in rare cases and have been concluded to not pose a major medical problem (Isbister and Gray 2004b).

A number of genera of Australian tarantulas (Araneae: Mygalomorphae: Theraphosidae) (see Fig. 2f), referred to as theraphosids, are distributed across the warmer tropical and temperate regions of the continent (Isbister et al. 2003) (see Fig. 1d). Presently, the taxonomy of Australian theraphosids is incomplete and makes definitive identification of specimens difficult. The current genera include *Coremiocnemis*, *Selenotholus*, *Selenotypus*, and *Selenocosmia* (World Spider Catalog, version 16.5 (2015)); however, recent references in the literature also refer to *Phlogiellus* (Raven 2005) and *Phlogius,* a synonym replacing the Australian *Selenocosmia* genera (Chow et al. 2015; Raven and Covacevich 2012; Raven 2005) (Dr. Robert Raven, personal communication). Bites and envenomation in humans by these spiders are rare. Isbister et al. (2003) noted only nine confirmed reports of human envenomation over the 25-year period from 1978 to 2002 (Isbister et al. 2003). No reports of major effects were evident in any of the case reports. Local pain was the most common symptom, and mild systemic effects were reported in one case. Raven and Covacevich (2012) reported one further case by *Phlogius crassipes* that resulted in pain and swelling, but no systemic effects (Raven and Covacevich 2012). The venom of Australian theraphosids has shown significant selectivity toward different mammalian systems (Isbister et al. 2003). In contrast to the primate-specific activity of the Australian funnel-web spiders, case studies of seven confirmed bites on canines (weighing up to ~50 kg weight) by identified Australian theraphosids reported that the bites were rapidly fatal in all cases and highlight the selectivity of the venom components to some mammalian systems other than humans (Isbister et al. 2003). Given that bites to canines up to the weight of a small human are rapidly fatal and that most bites to humans result in local pain only, it has been concluded that the Australian theraphosids pose no significant medical problem (Isbister et al. 2003).

A study of 750 definite spider bite cases over a 27-month period from three Australian states showed that the most common spider bite encountered is from members of the Sparassidae (huntsman) family (22.9 %), with members of the Araneidae (orb weavers) second (21.4 %). Only 6 % of the total bites were medically significant, and of the medically significant bites, 84 % were attributed to the redback spider (*Latrodectus hasselti*), five bites were from Australian funnel-web spiders (Atracinae family), and one bite was from an Araneidae (Isbister and Gray 2002). An important note of significance from this study was the occurrence of 16 % of the total bites by white-tailed spiders (Lamponidae family), commonly attributed to and believed to cause necrotic arachnidism (Isbister and Gray 2004a). No necrotic lesions were reported from any of the definite spider bite cases.

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Isbister and Hirst (2003) conducted a prospective study over 27 months on bites from the Sparassidae family, the most prevalent source of spider bites in Australia (Isbister and Gray 2002). The Sparassidae family (Araneae: Araneomorphae: Sparassidae) are large spiders found on most continents in tropical and temperate regions of the world. Bites were recorded from six genera: *Isopeda*, *Isopedella*, *Neosparassus*, *Heteropoda*, *Delena*, and *Holconia*. Bites by these spiders were predominantly characterized by immediate pain with a duration averaging 5 min, and associated with bleeding and/or puncture marks and local redness. Severe pain was reported in a small number of cases, and the incidence of local effects, including local redness and itchiness, and systemic effects was less than for bites by other spiders. No clinical effects consistent with an envenomation syndrome were evident. The study concluded that bites from spiders of the Sparassidae family cause only minor effects and these spiders are not dangerous to humans. It also showed that there are no differences between bites from different genera within the family, refuting previous reports that *Neosparassus* spp. can cause severe effects and should be considered dangerous (Isbister and Gray 2002).

One clinically important aspect of spider bite in Australia that would be remiss not to mention due to the debate and publicity it has received relates to necrotic arachnidism. A number of Australian spider species have been suspected of causing necrotic ulcers including black house spiders (*Badumna* spp.), wolf spiders (family Lycosidae), and the most infamous suspects, white-tailed spiders (*Lampona* spp.) (Isbister and Gray 2004a). In prospective studies of 750 spider bites (Isbister and Gray 2002), 130 definite bites by white-tailed spider species (Isbister and Gray 2003a) and black house spider bites (Isbister and Gray 2004a), Isbister and colleagues showed that there was no evidence of necrotic arachnidism. Given the lack of evidence of confirmed necrotic arachnidism in Australia, it is unlikely that necrotic arachnidism is a real problem in Australia.

Australian Spider Venom Components

Research into the components of Australian spider venoms has focused on four primary areas: identification and characterization of the primary toxic components of clinically relevant venoms (Nicholson et al. 1996), discovery of insecticidal components with potential commercial application (Hardy et al. 2013; Windley et al. 2012), discovery of potential therapeutic leads (Chow et al. 2015), and use of venom component fingerprinting as a taxonomic tool (Palagi et al. 2013; Wilson and Alewood 2004, 2006). The identification and characterization of the primary toxic components of clinically relevant venoms (Nicholson et al. 1996) have been undertaken to understand the mechanism of action and develop and understand the action of relevant antivenoms. As one of the most successful insect predators on the planet, and possessing a vast library of natural and highly evolved insecticidal components, spiders present an excellent source of novel insecticidal molecules with potential commercial application (Hardy et al. 2013; Windley et al. 2012). Numerous spider venom components possess mammalian activity and offer a great resource for the discovery of potential leads for desirable and relevant therapeutic targets (Chow et al. 2015). For recent reviews on the potential insecticidal and therapeutic application of spider venoms, see Kalia et al. (2015), King and Hardy (2013), Pineda et al. (2014b), and Smith et al. (2013). In the analysis of spider venom for the identification, characterization, and discovery of the venom components, correct species identification is crucial to ensure relatively consistent venom composition and activity. Spider venoms are complex mixtures of different components, dominated by disulfide-rich peptides (King and Hardy 2013). Venom component fingerprinting has been shown to be highly effective as a taxonomic tool for the Australian funnel-web spiders (Palagi et al. 2013; Wilson and Alewood 2004, 2006) and in identifying intersexual species differences in venom composition (Herzig and Hodgson 2009; Herzig et al. 2008).

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Australian Funnel-Web Spiders (Hexathelidae)

The venom of the Australian funnel-web spiders is by far the most extensively studied Australian spider venom. This is most likely due to the inherent toxicity of the venom and clinical impact of envenomation and the relative ease of collection of large venom samples. Additionally, the discovery of potentially useful insecticidal peptides within the venom has driven further investigation into the composition of these venoms. There are currently 75 funnel-web spider toxin records, classified into six groups (δ-HXTX-1, ω-HXTX-1, ω-HXTX-2, κ-HXTX, U_1-HXTX, and U_2-HXTX) from eight species (*H. versuta*, *H. infensa*, *A. robustus*, *H. venenata*, *A. sutherlandi, A*. sp. (*Illawarra*), *H. modesta*, and *I. wisharti*), listed in the ArachnoServer 2.0 database (Herzig et al. 2011).

The δ-HXTX-1 Family

The toxin responsible for the envenomation syndrome observed for bites from male *A. robustus* was isolated and determined to be a 42-residue peptide, δ-HXTX-Ar1a, containing an unusual cysteine framework with four disulfide bonds (Nicholson et al. 2006). In a study of genomics and cDNA from *H. infensa*, δ-HXTX-Hi1a was shown to be encoded by an intronless gene (Pineda et al. 2012). The unusual cysteine framework comprises disulfide-bonded N- and C-terminal cysteines and three contiguous cysteines ($Cys^{14,15,16}$) involved in disulfide bonds. Two similar peptides were identified in the venom of *H. versuta*, δ-HXTX-Hv1a and δ-HXTX-Hv1b. Studies of venom gland cDNA libraries from *A. robustus*, *H. valida*, *H. infensa*, *H. versuta*, and *I. wisharti* identified a number of orthologous peptides (see Fig. 3a) and confirmed that some venoms contain more than one δ-HXTX-1 ortholog (Escoubas et al. 2006). The difference in toxicity observed between the venom of male and female *A. robustus* spiders is apparent upon liquid chromatography/mass spectrometry (LC/MS) analysis of the venom, where the primary toxic component δ-HXTX-Ar1a is a dominant component of the venom profile of the male spider but not evident at all in the venom profile of the female spider (see Fig. 4) (Wilson and Alewood 2004). The three-dimensional solution structures of δ-HXTX-Ar1a and δ-HXTX-Hv1a were determined using nuclear magnetic resonance (NMR) spectroscopy. The structures consist of a triple-stranded antiparallel β-sheet core that conforms to the inhibitor cystine knot (ICK) motif and a C-terminal 3_{10} helix (δ-HXTX-Hv1a) or a series of C-terminal interlocking γ-turns (δ-HXTX-Ar1a) (Fletcher et al. 1997; Pallaghy et al. 1997) (see Fig. 5a, b).

Early biological activity studies identified the target site and mode of action of the δ-HXTXs as site 3 on the tetrodotoxin-sensitive (TTX) voltage-gated sodium channel in both mammals and insects, resulting in a slowing of the sodium current inactivation (Little et al. 1998). δ-HXTX-Hv1b has 67 % and 62 % identity with δ-HXTX-Hv1a and δ-HXTX-Ar1a, respectively. Activity studies found δ-HXTX-Hv1b to be 15–30-fold less active in mammalian assays and completely lack insecticidal activity in crickets (*Acheta domesticus*) (Szeto et al. 2000b). The activity of δ-HXTX-Iw1a, which differs in sequence from δ-HXTX-Ar1a by a Thr-Ser substitution, was also tested in patch clamp recordings on rat dorsal root ganglion neurons and showed similar sodium channel gating and kinetics to the other δ-HXTXs tested (Nicholson et al. 2004). Studies into the structure-function relationships of the δ-HXTX-1 peptides have been severely limited by the inability to efficiently produce a correctly folded synthetic or recombinant peptide. Consequently, the δ-HXTX pharmacophore remains to be determined and confirmed.

The ω-HXTX-1 Family

The ω-HXTX-1 peptide family was the first insect-specific peptide toxin family identified in the venom of the Australian funnel-web spiders (Atkinson et al. 1993) and the first toxin family discovered of the Shiva superfamily (Pineda et al. 2014a). These 36–37 residue peptides containing three disulfide bonds (see Fig. 3b) are lethally active across a number of arthropod orders including Acarina, Coleoptera, Dictyoptera, Diptera, Hemiptera, Lepidoptera, and Orthoptera. They cause an irreversible spastic

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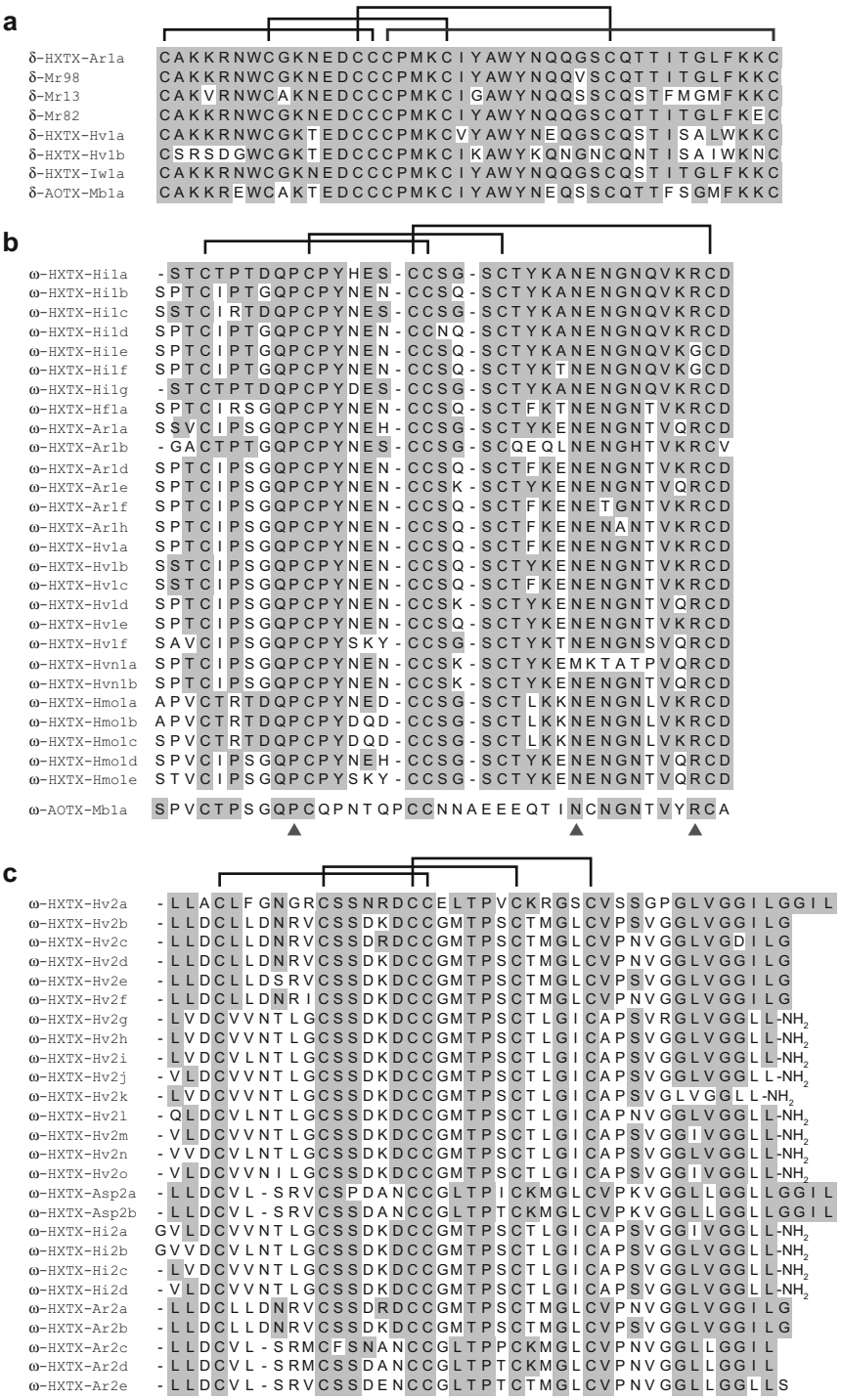

Fig. 3 Delta/omega amino acid sequences from Australian funnel-web spiders. (**a**) δ-HXTX-1 family, (**b**) ω-HXTX-1 family, and (**c**) ω-HXTX-2 family. Identical residues are boxed in *gray*, and the disulfide bonds are shown. The key functional residues, where known, are highlighted by *triangles* below the sequences. Included in (**a**) is the sequence of δ-actinopoditoxin-Mb1a (δ-AOTX-Mb1a), and included in (**b**) is the sequence of ω-AOTX-Mb1a from the eastern mouse spider, *Missulena bradleyi*

paralysis that precedes a flaccid paralysis and death in insects; however, no toxic effects have been reported from studies on vertebrate preparations. The three-dimensional solution structure of ω-HXTX-Hv1a, the first member of this family to be structurally characterized, demonstrates a disulfide-rich core

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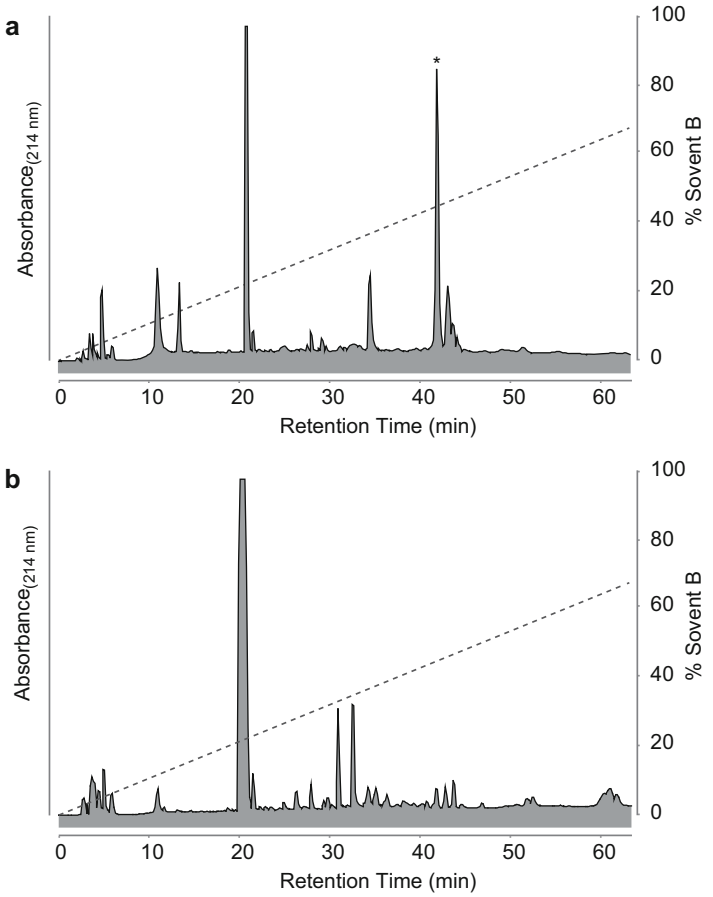

Fig. 4 Reversed-phase high-performance liquid chromatography (*HPLC*) chromatograms of crude venom from (**a**) male *Atrax robustus* and (**b**) female *Atrax robustus* (Vydac C_{18} 4.6 × 250mm, 300 Å pore size, 5 µm particle size column, 1 % gradient solvent B [90 % acetonitrile/10 % H_2O/0.09 % trifluoroacetic acid] @ 1 mL/min; absorbance 214 nm). * denotes the peak of δ-HXTX-Ar1a, present only in the venom of male specimens and confirmed by liquid chromatography/mass spectrometry (*LC/MS*) analysis of both venoms (Wilson and Alewood 2004)

region forming an ICK motif where the β-hairpin protrudes and a structurally disordered N-terminus (see Fig. 5c). Site-directed mutagenesis and sequence truncation studies of ω-HXTX-Hv1a have identified the key residues involved in binding to the insect target site (insectophore). On one face of the peptide surface, residues Pro^{10}, Asn^{27}, and Arg^{35} form a small, contiguous patch and constitute the primary insectophore (Tedford et al. 2004). Residues Gln^{9} and Tyr^{13} are reported to be of minor functional importance in orthopterans and dictyopterans, but not dipterans (Chong et al. 2007). Both ω-HXTX-Hv1a and ω-HXTX-Ar1a were found to block the mid- to low-voltage-activated (M-LVA) and high-voltage-activated (HVA) Ca_v channels in cockroach neurons, with minor activity toward Na_v, but no activity on K_v channels. The block of the channels observed was voltage independent and did not alter the voltage dependence of Ca_v channel activation, implying that the toxins are pore blockers rather than gating modifiers. ω-HXTX-Hv1a has been reported to demonstrate oral activity against ticks (Mukherjee et al. 2006) and mosquitoes (Chong et al. 2007). It has also been trialed, and shown promise, as a novel biopesticide via expression of a toxin transgene in tobacco plants (*Nicotiana tabacum*) and expression as a fusion protein in *E. coli*. Interestingly, the thioredoxin-ω-HXTX-Hv1a fusion protein expressed in *E. coli* caused paralysis and death in *Helicoverpa armigera* and *Spodoptera littoralis* caterpillars when applied topically. Expression of the ω-HXTX-Hv1a toxin transgene in tobacco plants resulted in effective

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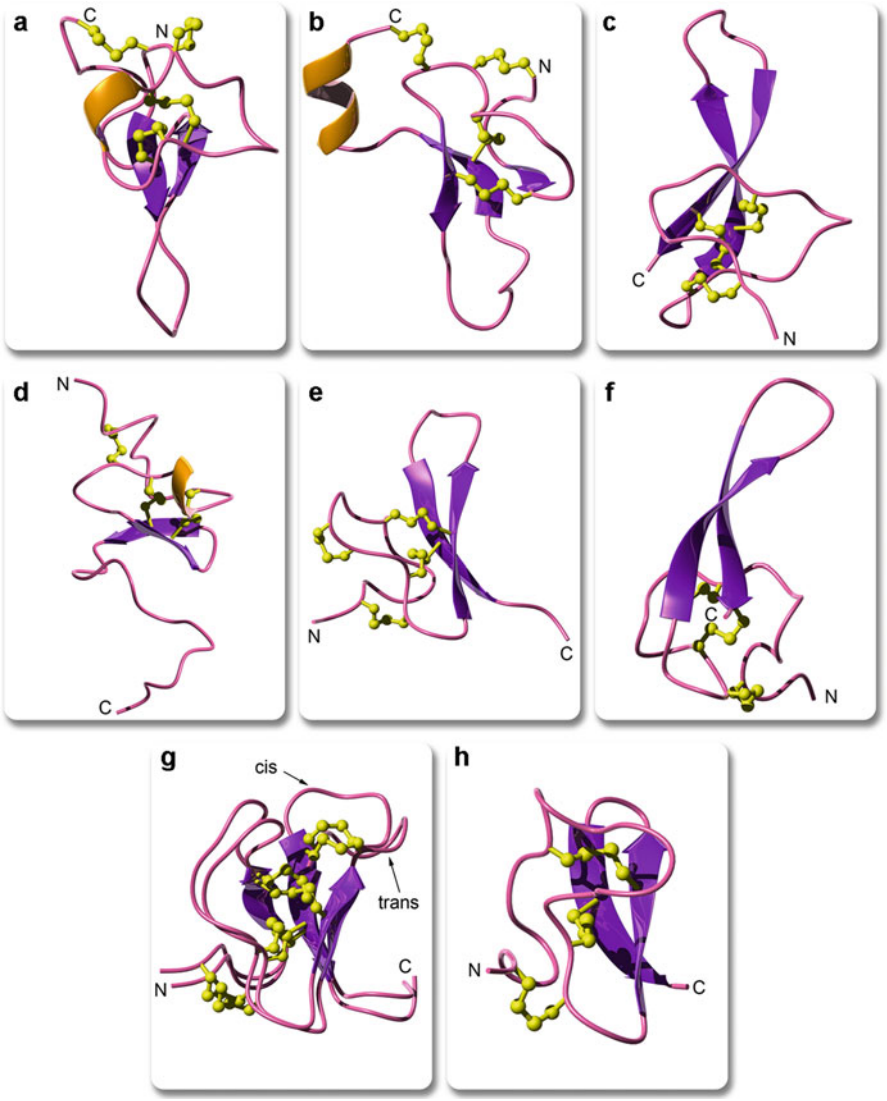

Fig. 5 Solution structures of Australian spider venom peptides. (**a**) δ-HXTX-Ar1a (PDB file 1QDP) from male *Atrax robustus*; (**b**) δ-HXTX-Hv1a (PDB file 1VTX) from *Hadronyche versuta*; (**c**) ω-HXTX-Hv1a (PDB 1AXH) from *Hadronyche versuta*; (**d**) ω-HXTX-Hv2a (PDB 1G9P) from *Hadronyche versuta*; (**e**) κ-HXTX-Hv1c (PDB 1DL0) from *Hadronyche versuta*; (**f**) ω/κ-HXTX-Hv1a (PDB 2H1Z) from *Hadronyche versuta*; (**g**) *cis*/*trans* U_2-HXTX-Hi1a (PDB *cis* 1KQH, *trans* 1KQI) from *Hadronyche infensa*. *Cis*/*trans* isomerism occurs at the bond preceding Pro^{30}. and (**h**) U_1-TRTX-Spl1a (PDB 2LL1) from *Selenotypus plumipes*. β-strands are shown as *purple arrows*, helices are shown in *orange*, and the disulfide bonds are represented in *yellow*. (Figures generated using MOLMOL (Koradi et al. 1996))

protection from *H. armigera* and *S. littoralis* larvae, causing 100 % mortality within 48 h and 93–100 % mortality of *H. armigera* larvae within 72 h when under phloem-specific expression (Khan et al. 2006; Shah et al. 2011).

The ω-HXTX-2 Family

A second family of potent insect-selective peptides, ω-HXTX-2, that block the insect voltage-gated calcium channel was identified during screening of the venom of *H. versuta*. Homologous peptides were identified from cDNA libraries of female *H. infensa*, female *H. versuta*, and male *A. sutherlandi* (King and Sollod 2007). A total of 26 ω-HXTX-2 peptide sequences are listed in the ArachnoServer 2.0 database (see Fig. 3c). These 41–45-residue peptides possess a highly structured, three disulfide-rich core and a

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structurally disordered C-terminal extension that is critical for channel blocking activity (see Fig. 5d) (King and Sollod 2007; Wang et al. 2001). In a study of genomic and cDNA from *H. infensa*, ω-HXTX-Hi2a was shown to be encoded by an intronless gene (Pineda et al. 2012). Weak structural and functional homology was observed for ω-HXTX-Hv2a, from the venom of *H. versuta*, with ω-agatoxin-Aa4a/b (ω-AGTX-Aa4a/b), an inhibitor of P-type calcium channels from the venom of *A. aperta*, and may be indicative of a similar mechanism of action. ω-HXTX-Hv2a was shown to exhibit exceptional phylogenetic specificity, displaying at least a 10,000-fold preference for insect calcium channels over vertebrate channels (Wang et al. 2001). The peptide was inactive in vertebrate smooth and skeletal nerve-muscle preparations and did not cause any adverse effects upon injection into newborn mice. In bee brain neurons, ω-HXTX-Hv2a inhibited calcium currents with an EC_{50} of approximately 130 pM. In contrast, the peptide had little effect on calcium currents in mouse sensory neurons and did not show any effect on bee brain neuron sodium and potassium currents or mouse sensory sodium currents. A further study showed that injection of ω-HXTX-Hv2a into the lone star tick (*Amblyomma americanum*) is lethal and induces a pronounced phenotype characterized by an unusual gait, followed by paralysis and death (Mukherjee et al. 2006).

The κ-HXTX-1 Family

In an early study looking at the potential of Australian funnel-web spider venoms to harbor insecticidal molecules, a 37-residue insecticidal peptide with a novel sequence and four-disulfide bond novel cysteine framework was identified in the venom of *H. formidabilis* (Atkinson et al. 1993). Later studies, including cDNA and transcriptome work, identified a further five 36–37-residue orthologs in the venom of *H. versuta*, κ-HXTX-Hv1a-c, and a 36-residue ortholog from *H. modesta*, κ-HXTX-Hmo1a (see Fig. 6a) (Pineda et al. 2014a; Wang et al. 2000). The cDNA and transcriptome studies identified these peptides as the second toxin family of the Shiva superfamily (Pineda et al. 2014a). Determination and analysis of the three-dimensional structure revealed that these peptides adopt an ICK motif and possess an extremely rare and functionally critical vicinal disulfide bond (see Fig. 5e) (Wang et al. 2000). The κ-HXTX-Hv1 peptides were found to be highly insecticidal via injection in crickets (A. domesticus), with LD_{50} values in the range 167–303 pmol g^{-1}, but had no effect on vertebrate smooth (rat vas deferens) and skeletal (chick biventer cervicis) muscle preparations, or in newborn mice. The most potent insecticidal peptide, κ-HXTX-Hv1c, was shown to be an excitatory neurotoxin by direct application to the cockroach (*Periplaneta americana*) metathoracic ganglion which caused spontaneous, uncoordinated movement. Mutagenesis studies identified that the pharmacophore consists of seven residues that form a bipartite surface patch on one face of the molecule. The primary pharmacophore was found to incorporate just five residues, comprising Arg^{8}, Pro^{9}, Tyr^{31}, and the Cys^{13}-Cys^{14} vicinal disulfide (Maggio and King 2002). Using patch clamp analysis of cockroach dorsal unpaired median neurons, κ-HXTX-Hv1c was found to be a high-affinity blocker of insect large-conductance Ca^{2+}-activated K^{+} channel currents and did not affect Na_v, Ca_v, and K_v channel currents (Gunning et al. 2008).

The ω/κ-HXTX-1 Family

A study of cDNA libraries constructed from the venom glands of single specimens of female *H. versuta* and female *A. robustus* identified nine novel sequences encoding five mature 38–39-residue peptides, ω/κ-HXTXs (see Fig. 6b) (King and Sollod 2006). Together with the ω-HXTX-1 and κ-HXTX groups, these peptides constitute the third group of peptides characterized in the Shiva toxin superfamily (the three peptide groups share an almost completely conserved signal peptide sequence). Of note was the finding that the C-terminal proteolytic recognition signal of the propeptide sequence is completely conserved across all families (Arg-Arg), as are the cysteine residues of the mature sequences that direct the three-dimensional fold of the toxins. The three-dimensional solution structure of a recombinant version of

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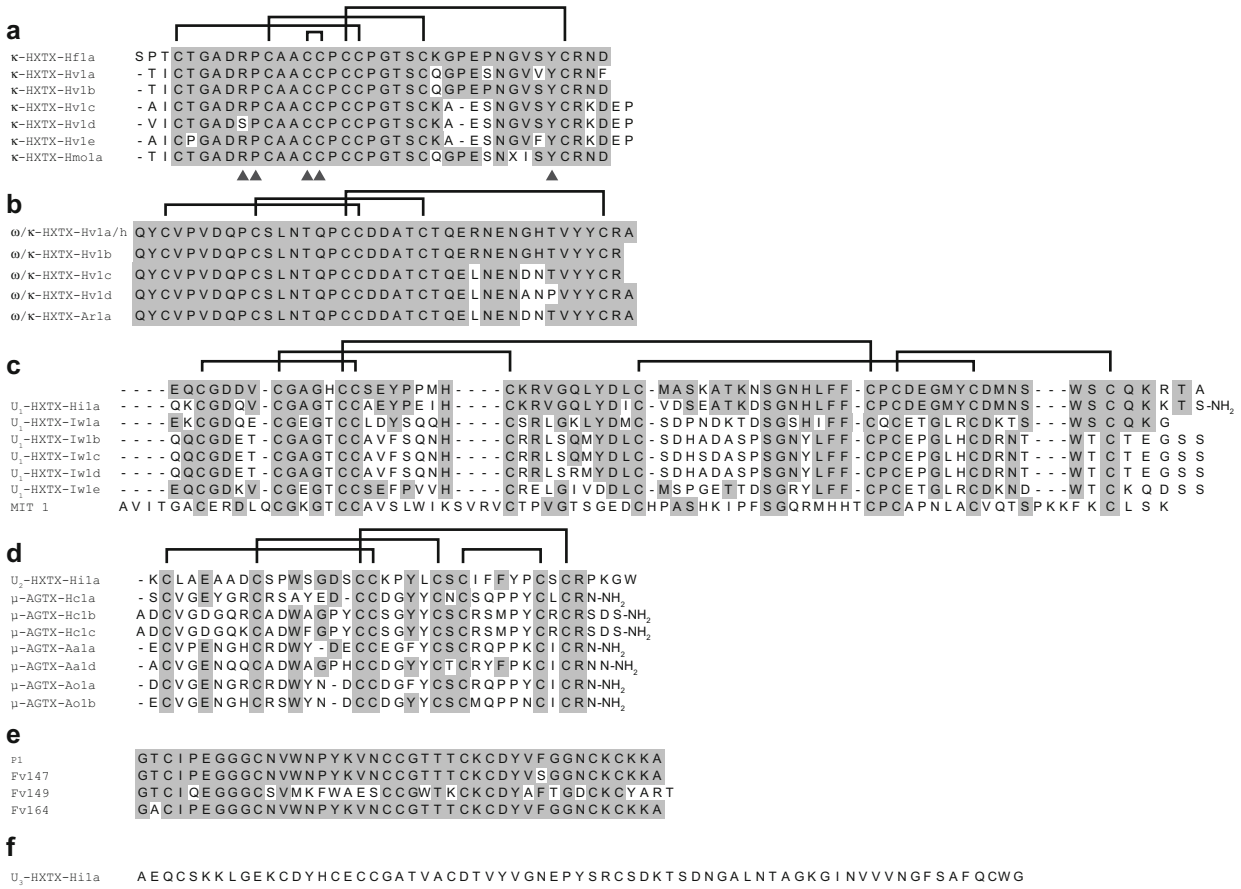

Fig. 6 Amino acid sequences from Australian funnel-web spiders. (**a**) κ -HXTX-1 family, (**b**) ω/κ -HXTX-1 family, (**c**) U_1-HXTX-1 family, (**d**) U_2-HXTX-1 family, (**e**) P1 family, and (**f**) U_3-HXTX-1 family. Included in (**c**) is MIT1 (mamba intestinal toxin 1) from the black mamba, *Dendroaspis p. polylepis*. Included in (**d**) are μ-AGTX-1 sequences from the completely unrelated American funnel-web spiders (Agelenidae) *Agelenopsis aperta*, *Hololena curta*, and *Agelena orientalis*. Identical residues are boxed in *gray*, and the disulfide bonds are shown, where known or predicted. The key functional residues, where known, are highlighted by *triangles* below the sequences

ω/κ-HXTX-Hv1a (where the first two amino acids at the N-terminus, GlnTyr, were replaced by GlySer) was determined by NMR and found to adopt the ICK motif (see Fig. 5f). The recombinant version was shown to possess dual activity, targeting both insect voltage-gated calcium channels (Ca_v) and high-conductance calcium-activated potassium channels (K_{Ca}), and appears to act as a pore blocker, rather than a gating modifier. It is postulated that ω/κ-HXTX-Hv1a imparts a synergistic effect by blocking the inward flow of calcium through Ca_v channels, decreasing the local pool of intracellular calcium available, and potentiating the block of K_{Ca} channels (King and Sollod 2006; Pineda et al. 2014a).

The U_1-HXTX-1 Family

One of the major components of the venom of *H. versuta* was determined to be a 68-residue peptide containing five disulfide bonds, U_1-HXTX-Hv1a (see Fig. 6c). This peptide has homology with two "nontoxic" peptides isolated from the venom of *Phoneutria* spp. spiders for which the target is unknown, U_9-CNTX-Pr1a and U_{19}-CNTX-Pn1a. The U_1-HXTXs also shows some sequence homology to a number of colipases and a variety of AVIT family proteins that target prokineticin receptors involved in gastrointestinal smooth muscle activation. The AVIT family peptides include mamba intestinal toxin 1 (MIT1) from the black mamba *Dendroaspis p. polylepis*, Bv8 and Bm8 orthologs isolated from skin

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secretions of the toads *Bombina* spp., prokineticin 1 (also known as endocrine-gland vascular endothelial growth factor or EG-VEGF), and prokineticin 2. There is also limited homology to the C-terminal cysteine-rich domain of the embryonic head inducer Dickkopf-1 protein family. Szeto et al. (2000a) showed that U_1-HXTX-Hv1a does not possess insecticidal activity in crickets (*A. domesticus*) and does not affect vertebrate smooth or skeletal muscle contractility in rat vas deferens and chick biventer cervicis nerve-muscle preparations. Furthermore, it was shown that U_1-HXTX-Hv1a does not have colipase activity (Szeto et al. 2000a). Wen et al. (2005) went further to show that U_1-HXTX-Hv1a does not stimulate smooth muscle contractility or inhibit contractions induced by human prokineticin 1 in isolated rat stomach fundus and guinea-pig ileum organ bath preparations. Additionally, U_1-HXTX-Hv1a lacked activity on rat aorta smooth muscle preparations and did not activate or block human prokineticin 1 or 2 receptors in a FLIPR Ca^{2+} flux assay using HEK293 cells expressing the prokineticin receptors. Modeling of the three-dimensional structures of the U_1-HXTXs on MIT1 illustrated that the peptides appear to adopt the ancestral disulfide-directed β-hairpin protein fold; however, variations in the amino acid sequence and surface charge support the assay data and suggest a different biological target (Wen et al. 2005).

The U_2-HXTX-1 Family

The sequence of a 38-residue peptide, U_2-HXTX-Hv1a, was elucidated from the venom of *H. infensa* (Orchid Beach). The peptide was found to contain eight cysteine residues, forming four disulfide bonds, in a framework homologous to the insect sodium channel active μ-agatoxin family from the completely unrelated funnel-web spiders, *Agelenopsis aperta, Hololena curta*, and *Agelena orientalis* (see Fig. 6d). Despite the conserved cysteine framework, the remainder of the sequence displayed very limited homology to the μ-agatoxins. Determination of the three-dimensional structure revealed that U_2-HXTX-Hv1a adopts a triple-stranded antiparallel β-sheet consistent with the ICK motif. Interestingly, U_2-HXTX-Hv1a possesses two equally populated conformations in solution due to *cis*/*trans* isomerization of the peptide bond preceding Pro^{30} (see Fig. 5g). No activity data have been reported for this peptide (Rosengren et al. 2002).

A further two families of peptide from Australian funnel-web spider venom are reported in the literature and are currently not represented in the ArachnoServer 2.0 database; the P1 family from female *H. versuta* (Escoubas et al. 2006) and the U_3-HXTX-1 family from *H. infensa* (Pineda et al. 2012) (see Fig. 6e, f). However, limited information beyond the sequences and the fact that U_3-HXTX-Hi1a is encoded by an intronless gene are available.

The Venom of Redback Spiders (*Latrodectus hasselti*)

The toxin in redback spider (*L. hasselti*) venom responsible for the clinical symptoms associated with latrodectism has been identified as an α-latrotoxin (α-LTX), the vertebrate-specific toxins found in potentially all *Latrodectus* species and also in other theridiid spiders. The α-LTXs are large ~130 kDa hydrophilic proteins, initially isolated from the European widow spider (*L. tredecimguttatus*), that exist as a homodimer under nonreducing conditions, with each monomer composed of four domains: domain I, a signal peptide; domain II, a conserved N-terminal domain with two hydrophobic segments; domain III, a domain containing 20 ankyrin repeats (22 if two imperfect repeats are included); and domain IV, a C-terminal propeptide domain. α-LTXs are synthesized as a large protoxin (~157 kDa) that is believed to be processed via enzymatic cleavage of domains I and IV by the endopeptidase furin, resulting in the mature toxin composed of domains II and III. Study of the three-dimensional structure using electron cryo-microscopy showed the mature monomer of α-LTX contains three regions: the wing (composed of the majority of domain II), the body (comprising a portion of domain II and most of the ankyrin repeats), and the head (composed of the C-terminal ankyrin repeats).

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It is currently believed that α-LTX binds to extracellular cell surface membrane proteins in an initial step in α-LTX-induced neurotransmitter exocytosis. Three structurally unrelated cell adhesion receptor classes have been identified: (1) neurexin 1α, a neuronal protein containing a single transmembrane domain; (2) latrophilin 1 (CL1 or lectomedin), a member of the CL family of G-protein-coupled receptors, also known as calcium-independent receptor of α-LTX (CIRL); and (3) receptor-like protein tyrosine phosphatase σ. These receptors are thought to serve to target α-LTX to an appropriate location on the cell surface, such as nerve terminals at the neuromuscular junction. Pore formation then occurs via α-LTX oligomerization into amphipathic cyclical tetramers, membrane insertion, and nonselective cation channel formation. The pore causes an osmotic-mediated increase in vesicular exocytosis and non-vesicular neurotransmitter leakage. In addition, Ca^{2+} influx through the α-LTX pore causes vesicle exocytosis and receptor-mediated vesicle exocytosis (Graudins et al. 2012). A recent study determined the amino acid sequence of α-LTX-Lh1a from the Australian redback spider (*L. hasselti*) and showed that the protein comprises 1180 residues (~132 kDa) and has 93 % sequence identity with α-LTX-Lt1a from the European widow spider (*L. tredecimguttatus*). The results revealed a number of key residue substitutions in the 4C4.1 epitope, the region of binding of the 4C4.1 monoclonal antibody raised against α-LTX-Lt1a, and support the finding that the 4C4.1 monoclonal antibody does not neutralize *L. hasselti* venom (Graudins et al. 2012).

The Venom of Mouse Spiders (*Missulena* spp.)

Based on the reported clinical cases of envenomation by *Missulena* spp., the similarities of the envenomation syndrome to that observed for *A. robustus*, and the reversal of the envenomation syndrome by Australian funnel-web spider antivenom, studies ensued to identify the toxins responsible. Two studies concentrated solely on the venom of *M. bradleyi*, and one study focused on both *M. bradleyi* and *M. pruinosa*. In a comparative study of the activity of crude venom of male and female *M. bradleyi*, Rash et al. (2000) showed that the venom of the male specimens only facilitates neurotransmitter release by modifying tetrodotoxin-sensitive sodium channel gating and has no effect on tetrodotoxin-resistant sodium currents. This activity is similar to that of the δ-HXTXs from the Australian funnel-web spiders, and the authors confirmed the activity is blocked by Australian funnel-web spider antivenom (Rash et al. 2000).

The intersexual differences observed in venom activity for *M. bradleyi* were confirmed and expanded to *M. pruinosa* (Herzig et al. 2008). This study showed little intersexual differences in a cricket (*A. domestica*) acute toxicity assay but demonstrated that *M. bradleyi* venom is considerably more potent than *M. pruinosa*. In contrast, reversed-phase HPLC analysis illustrated a substantial degree of intersexual variation in venom composition. Mass spectrometry analysis of crude venom showed significant intersexual differences in venom composition for *M. bradleyi*, but less so for *M. pruinosa*. Male, but not female, *M. bradleyi* venom induced large and sustained muscle contractions with fasciculation and decreased twitch height in the chick isolated biventer cervicis nerve-muscle preparation bioassay, and these effects were reversed by Australian funnel-web spider antivenom. Interestingly, both male and female *M. pruinosa* venom failed to induce significant effects in this test (Herzig et al. 2008).

A 42-residue peptide, δ-actinopoditoxin-Mb1a (δ-AOTX-Mb1a), isolated and identified from the venom of male *M. bradleyi* showed 81 % identity to δ-HXTX-Ar1a (see Fig. 3a) from the venom of the male Sydney funnel-web spider, *A. robustus*. Purified δ-AOTX-Mb1a was concluded to be equipotent with δ-HXTX-Hv1a and δ-HXTX-Ar1a on TTX-sensitive sodium channels and caused the same slowing of channel inactivation, reduction in peak current amplitude, and shifts in the voltage dependence of activation. Similarly, δ-AOTX-Mb1a is inactive on TTX-resistant sodium channels. These actions support binding of δ-AOTX-Mb1a to site 3 of the voltage-gated sodium channel and support the findings of crude venom studies. Similar to δ-HXTX-Hv1a, δ-AOTX-Mb1a was also shown to possess

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insecticidal activity, however is approximately twofold less toxic to crickets (*A. domesticus*) (Gunning et al. 2003).

One other peptide is reported from the venom of *M. bradleyi* in the ArachnoServer 2.0 database, but details have not been formally published. The 39-residue peptide, ω-AOTX-Mb1a, is highly homologous to the ω-HXTX-1 family from the venom of Australian funnel-web spider species (see Fig. 3b) and includes identical residues at the pharmacophore sites. By homology the peptide is predicted to target insect, but not vertebrate, voltage-gated calcium channels.

The Venom of Australian Tarantulas (Theraphosidae)

Despite demonstrating minor clinical relevance, but significant activity in other mammals, the venoms of the Australian Theraphosidae are largely unstudied. More recently the venom of these spiders has become a point of interest as a source of insect active toxins and therapeutic leads, particularly in relation to targets for pain. The peptide composition and insecticidal activity of crude venoms from four Australian theraphosids (*Coremiocnemis tropix*, *Phlogius (Selenocosmia) crassipes*, *Selenotypus plumipes*, and *Selenotholus foelschei*) were compared in a 2009 study. The study determined that the venom composition is dominated by peptides in the mass range 4–10 kDa but is different between the four species. Despite the compositional differences, the insecticidal potency of the crude venoms was determined to be similar, with LD_{50} values ranging from 69 to 126 μg/g in crickets (*A. domesticus*) and 0.46–4.0 μg/g in mealworms (*Tenebrio molitor*) (Gentz et al. 2009).

The venom gland transcriptome of *S. plumipes* was sequenced and used in a study to develop an algorithm (SpiderP) as a precursor prediction tool in the ArachnoServer 2.0 database for the prediction of propeptide sequences in spider toxins. As part of this study, five novel venom peptide sequences were reported (OAIP1–5) (see Fig. 7a) (Wong et al. 2013). A further study by the same group on the 34-residue peptide, orally active insecticidal peptide 1 (OAIP1), determined that the peptide has orally active insecticidal properties. The oral insecticidal activity against the agronomically important pest, the cotton bollworm (*H. armigera*), was found to have an LD_{50} of 104.2 ± 0.6 pmol/g and is reported as the highest per os activity currently known for an insecticidal venom peptide. Furthermore, OAIP1 was found to be equipotent with synthetic pyrethroids and acts synergistically with the neonicotinoid insecticide, imidacloprid. The three-dimensional structure of OAIP1 was determined by NMR spectroscopy and was found to adopt a classic ICK motif (see Fig. 5h). The molecular target of OAIP remains unknown and is still to be determined (Hardy et al. 2013).

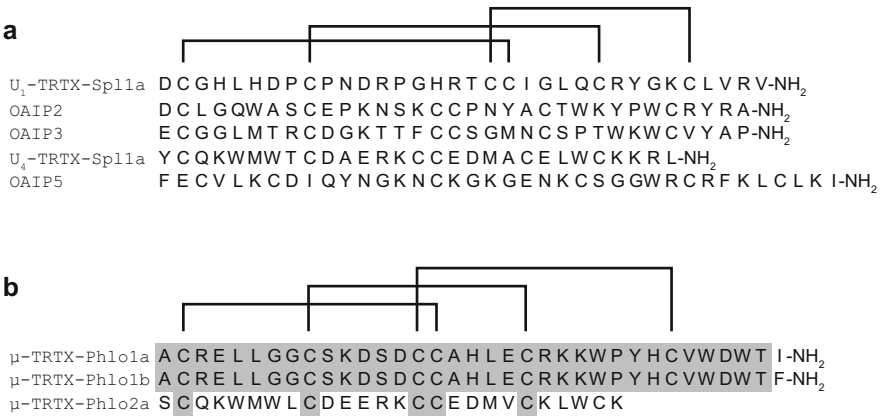

Fig. 7 Amino acid sequences from Australian tarantulas. (**a**) U_1-TRTX-Spl1a (OAIP1), OAIP2, OAIP3, U_4-TRTX-Spl1a (OAIP4), and OAIP5 families from *Selenotypus plumipes* and (**b**) μ-TRTX-1 and μ-TRTX-2 families from *Phlogius* spp. Identical residues are boxed in *gray*, and the disulfide bonds for U_1-TRTX-Spl1a and μ-TRTX-1 are shown

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Subtypes of the voltage-gated sodium (Na_v) channels have numerous vital roles in the human body. In particular, $Na_v1.7$ has a crucial role in the pain signaling pathway and is a therapeutic target of significant interest for the treatment of chronic pain. Chow et al. (2015) conducted a transcriptomic and assay-guided analysis of the venom of an Australian *Phlogius* sp. and identified three novel peptides that inhibit human $Na_v1.7$ channels. The sequences of two 35-residue peptides were determined (μ-theraphotoxin-Phlo1a, μ-theraphotoxin-Phlo1b (μ-TRTX-Phlo1a, μ-TRTX-Phlo1b)) (see Fig. 7b) and showed sequence similarity to peptides identified from *Chilobrachys guangxiensis* (e.g., μ-TRTX-Cg1a) and *Grammostola rosea* (e.g., U_3-TRTX-Gr1c). A 26-residue partial sequence was determined for the third peptide (μ-TRTX-Phlo1a) that illustrated sequence similarity to peptides from *Grammostola rosea* (κ-TRTX-Gr2b) and *Paraphysa scrofa* (κ-TRTX-Ps1b). The three peptides were established to inhibit human $Na_v1.7$ with similar IC_{50} values in the range 330–470 nM. All three peptides shifted the voltage for activation of the human $Na_v1.7$ to more positive potentials in a concentration-dependent manner and are proposed to be gating modifiers that inhibit channel activation via interaction with one or more voltage-sensor domains. In addition, μ-TRTX-Phlo1a showed a high level of subtype selectivity for $Na_v1.7$ over the $Na_v1.2$ and $Na_v1.5$ channel subtypes and offers the most promising starting point of the three peptides for the development of a human $Na_v1.7$ therapeutic (Chow et al. 2015).

The Venom of Other Australian Spider Species

The venom of one other Australian spider, *Trittame loki* (Araneae: Mygalomorphae: Barychelidae), has been studied at the molecular level in a proteomic and transcriptomic study. In this study, the authors identified 42 diverse isoforms of classic ICK/knottin spider venom peptides, in addition to variants of the prokineticin family, CAP (cysteine-rich secretory proteins, antigen 5, and pathogenesis-related 1 proteins) and Kunitz domain proteins, and the enzymes acetylcholinesterase and neprilysin (Undheim et al. 2013). The biological activity of the identified components was not investigated and remains to be determined; however, the activity and targets of some of the components were inferred by homology.

Conclusions and Future Directions

Spider bite in Australia has been shown to be a relatively common occurrence, in excess of 5,000 suspected cases annually. From a medical perspective, the majority of these bites are not significant, with the most common bites from the Sparassidae family. Redback spider bites are the most common clinically relevant bites, and antivenom is available, although there is ongoing debate over the effectiveness of redback spider antivenom use. Bites by the Australian funnel-web spiders, while potentially posing the greatest health risk, are rare, and envenomation requiring clinical intervention and administration of the effective antivenom is rarer.

Despite significant work and increasing interest in spider toxins for different applications (e.g., therapeutic leads, bioinsecticides), only a very small proportion of the total predicted number of spider toxins have been studied. With the continued advance in rapid and high-throughput technologies available at more affordable prices, particularly next-generation DNA sequencing providing genome and transcriptome data, it is predicted that the rate of discovery of new spider toxins will rapidly accelerate. The transcriptome of at least two species of Australian funnel-web spider (*H. infensa* and *H. modesta*) and one barychelid spider has been reported (Pineda et al. 2014a; Undheim et al. 2013, 2015). One of the limitations remaining, despite these advances in technologies to identify venom components, is the ability to characterize the biological target of activity of these molecules.

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Cross-References

- ▶ Pain Modulating Peptides in Spider Venoms: Good and Evil
- ▶ Recent Insights in *Latrodectus* ("Black Widow" Spider) Envenomation: Toxins and Their Mechanisms of Action
- ▶ Structural Diversity and Basic/Acidic Residue Balance of Active Cysteine-Rich Insecticidal Peptides from Spiders

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