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Review

Pilosulins: A review of the structure and mode of action of venom peptides from an Australian ant *Myrmecia pilosula*

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ABSTRACT

Myrmecia pilosula is an endemic Australian ant whose sting is a frequent cause of insect allergy in southeast Australia, and several deaths due to *M. pilosula* sting envenomation have been documented. In this review, we discuss the composition and bioactivity of *M. pilosula* venom. In addition to various enzymes and pharmacologically active constituents, the venom contains four families of highly basic low molecular weight peptides trivially named Pilosulins. These peptides are unique and have low structural homology to other Hymenoptera venom peptides. Moreover, *M. pilosula* venom is relatively simple in its composition with 5 predominant peptides making up about 90% by weight. These peptides display cytotoxic, hypotensive, histamine-releasing and antimicrobial activities. Within the *M. pilosula* venom, Pilosulin 3 has been classified as a major allergen and [Ile^5]pilosulin 1 and Pilosulin 4.1 are classified as minor allergens. Several uncharacterised higher molecular weight components with allergenic activities have also been identified. The revised naming of *M. pilosula* venom peptides according to the International Union of Immunological Societies (IUIS) criteria for allergen nomenclature is discussed in this review.

1. Introduction

Myrmecia pilosula species complex (F. Smith, 1858), colloquially known as jack jumper ants, is about 10 mm in length with a black body, long yellow mandibles, yellow legs and very large, anteriorly positioned eyes. They are diurnal foragers, well-known for their exceptional aggressiveness, powerful sting and a characteristic jumping movement (Brown, 1953; Ogata, 1991; Ogata and Taylor, 1991; Sutherland and Tibballs, 2001; Taylor, 1987).

M. pilosula is a member of primitive ants of the genus *Myrmecia*, family Formicidae, order Hymenoptera. There are 89 *Myrmecia* species and sub-species which have been identified so far, 88 of which are endemic to Australia and one rare species, *Myrmecia apicalis* (Emery) is found in New Caledonia (Ogata and Taylor, 1991). *M. pilosula* are commonly found in sandy soiled areas from north of Brisbane, south to Tasmania and west to the vicinity of Denmark in Western Australia (Ogata, 1991; Sutherland and Tibballs, 2001).

M. pilosula is a species complex, consisting of several sibling species with almost identical morphological characteristics (Crosland et al., 1988; Crozier et al., 1995; Taylor, 1991). The species is genetically highly heterogenous and contains at least five karyotypically distinct sub-species which have been scientifically named *Myrmecia croslandi*, *Myrmecia imaii*, *Myrmecia banksi*, *Myrmecia hankinsorum* and *M. pilosula* (Imai and Taylor, 1989; Imai et al., 1994). In some instances, these sibling species have been found to co-exist at a single site (Crosland et al., 1988; Taylor, 1987).

A number of *Myrmecia* ant species are a frequent cause of allergic reactions in humans, with *M. pilosula* the most predominant (Brown et al., 2011; Clarke, 1986; Gilhotra and Brown, 2006; Taylor, 1987). In Tasmania where the ants are endemic, 2.7% of the entire population has a history of systemic allergic reactions

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and approximately half of these are life-threatening anaphylaxis. In rural Victoria, 2.4% of the population has a history of systemic allergic reactions to ant stings, most commonly to *M. pilosula* (Brown et al., 2003a; Clarke, 1986; Douglas et al., 1998). Between 2002 and 2005, 60.8% of the 211 hospitalised cases involving ant sting allergy were due to *M. pilosula* and *Myrmecia pyriformis* (bulldog ant) (Bradley, 2008). In addition, Australian coronial records between 1980 and 1999 attributed six deaths due to anaphylaxis to the venom of *M. pilosula*, *M. pyriformis*, or *Myrmecia forficata* (inchman ant) (Brown et al., 2001; Klotz et al., 2005; McGain and Winkel, 2002).

In comparison to other insect venoms, the venom of *M. pilosula* appears to be particularly allergenic (Taylor, 1987). In individuals with a clinically diagnosed history of honey bee and wasp sting allergy, only 25–50% react to subsequent deliberate sting challenges, whereas the re-sting reaction rate in *M. pilosula* allergic individuals is between 70 and 85% for field stings and 72% for deliberate sting challenges (Brown et al., 2003a, 2003b; van Halteren et al., 1995; Weiner et al., 1995).

In *Myrmecia* species, venom is produced in venom glands, which are formed from modified accessory glands of the female reproductive system. Venom secretions are stored in a venom reservoir (venom sac) and are fed by a duct to the sting bulb (Billen, 1990; Cavill et al., 1964; Robertson, 1968). The amount of venom stored in the venom sac varies between different *Myrmecia* species. Larger species such as *Myrmecia gulosa* may hold as much as 300 μg venom (dry weight), or 0.35% of body weight in its venom sac, while smaller species such as *M. pilosula* hold approximately 40 μg venom (Cavill et al., 1964; Sutherland and Tibballs, 2001). In comparison, *Apis mellifera* (honey bee), *Polistes* spp. (paper wasps) and *Vespula* spp. (yellow jackets) hold approximately 60, 20 and 5 μg of protein in their venom sacs respectively (Hoffman and Jacobson, 1984).

2. *Myrmecia* ant venom composition

The earliest work on *Myrmecia* ant venoms described chemical characteristics of *M. gulosa* venom and showed that the venom contains histamine (2% of dry venom weight) and proteinaceous components that separated electrophoretically into eight bands (Cavill et al., 1964). Venom was shown to contain strong hyaluronidase, heat-labile haemolytic and kinin-like activity (Blum, 1992; Cavill et al., 1964), and was able to inhibit mitochondrial respiration (Ewen and Ilse, 1970).

Analysis of *M. pyriformis* venom also identified a broad range of enzymatic activities including hyaluronidase, phospholipase A_2, phospholipase B, acid phosphatase, and alkaline phosphatase (Lewis et al., 1968; Lewis and de la Lande, 1967; Matuszek et al., 1994; Wanstall and De la Lande, 1974). Furthermore, using isolated rat peritoneal mast cells, potent histamine releasing (Lewis and de la Lande, 1967), and haemolytic and smooth muscle stimulating activity were detected (Wanstall and De la Lande, 1974). Like *M. gulosa*, the venom of *M. pyriformis* also contains histamine (2% of venom dry weight) and a kinin-like substance(s) (Blum, 1992; Cavagnol, 1977; Lewis et al., 1968; Lewis and de la Lande, 1967; Matuszek et al., 1994; Wanstall and De la Lande, 1974).

Analysis of *M. pilosula* venom began in the early 1990s, and it was observed to have very similar pharmacological properties to *M. pyriformis* venom, but there was significantly less enzymatic (i.e. phospholipase B, acid phosphatase, and alkaline phosphatase) activity (Matuszek et al., 1994) and no kinins or acetylcholine content (Matuszek et al., 1992). Analysis using casein-zymography assay did not find proteinase (endopeptidase) activity (Unpublished results, T Wanandy). Histamine accounts for 0.9% of dried venom weight and the venom stimulates inflammatory reactions by releasing cyclooxygenase products (Hodgson, 1997; Matuszek et al., 1992). *M. pilosula* venom also possesses heat-sensitive haemolytic activity, indicating that the component responsible for this property is likely proteinaceous in nature (Matuszek et al., 1992).

The enzymatic and pharmacological components of the three *Myrmecia* venoms are summarised in Table 1.

Contemporary studies on *M. pilosula* venom were started in the early-1990s by Baldo, Donovan et al. and involved electrophoretic separation of the native venom proteins and identification of the IgE-binding components (Ford et al., 1991; Street et al., 1994). The studies led to the cloning, synthesis and characterisation of two venom allergens and their peptide sub-sequences (Donovan et al., 1996; King et al., 1998; Street et al., 1996; Wu et al., 1998).

Table 1
Enzymatic and pharmacological components of *Myrmecia* venoms.

	M. gulosa	*M. pyriformis*	*M. pilosula*	References
Enzymatic components				
Acid phosphatase	n.i.	+	+	(Matuszek et al., 1994)
Alkaline phosphatase	n.i	+	+	(Matuszek et al., 1994)
Cholinesterase	0	n.i.	n.i.	(Cavill et al., 1964)
Esterase	n.i.	0	0	(Matuszek et al., 1994)
Hyaluronidase	+	+	+	(Cavill et al., 1964; Lewis and de la Lande, 1967; Matuszek et al., 1994; Wanstall and De la Lande, 1974)
5-nucleotidase	0	n.i.	n.i.	(Cavill et al., 1964)
Phosphodiesterase	n.i.	0	0	(Matuszek et al., 1994)
Phospholipase A_2	n.i.	+	+	(Lewis et al., 1968; Matuszek et al., 1994; Wanstall and De la Lande, 1974)
Phospholipase B	n.i.	+	+	(Matuszek et al., 1994)
Phospholipase C	n.i.	0	0	(Matuszek et al., 1994)
Proteinase	0	n.i.	0	(Cavill et al., 1964)
Pharmacological components				
Acetylcholine	n.i.	n.i.	0	(Matuszek et al., 1992)
Eicosanoid-releasing factors	n.i.	n.i.	+	(Matuszek et al., 1992)
Haemolysins	+	+	+	(Cavill et al., 1964; Lewis and de la Lande, 1967; Matuszek et al., 1992; Wanstall and De la Lande, 1974)
Histamine	+	+	+	(Cavill et al., 1964; Lewis and de la Lande, 1967; Matuszek et al., 1992; Wanstall and De la Lande, 1974)
Histamine-releasing activity	n.i.	+	n.i.	(Lewis and de la Lande, 1967; Wanstall and De la Lande, 1974)
Kinins or kinin-like activity	+	0	0	(Cavill et al., 1964; Lewis and de la Lande, 1967; Matuszek et al., 1992)
Smooth muscle stimulant activity	n.i.	+	n.i.	(Lewis and de la Lande, 1967; Wanstall and De la Lande, 1974)
Mitochondrial respiration inhibitor	+	n.i.	n.i.	(Ewen and Ilse, 1970)

Symbols: + Present; 0 Absent; n.i. Not investigated.