

**Table 1** Full sequence of ponericin W5 (P82427) and its fragments. The full peptide corresponding to ponericin W5 and its fragments were identified in the venom of *N. villosa*. Isoforms of the full peptide and its fragments were also identified

Sequence	Condition	Mw (Da)	Peptide processing/Mutations
FWGALIKGAALKIPSWGLFKKKQ	Ponericin W5	2598.57	–
FWGALIKGAALKIPSWGLFKKKQ	Nv1; Nv2; Nv3; Nv4	2598.57	–
FWGALIKGAALKIPSWGLFKKK	Nv1;Nv3; Nv4	2470.51	Carboxypeptidase
FWGALIKGAALKIPSWGLFKK	Nv1	2342.42	Carboxypeptidase
FWGALIKGAALKIPSWGLF	Nv4	2086.23	Carboxypeptidase
FWGALIKGAALKIPSWGL	Nv2;Nv4	1939.16	Carboxypeptidase
FWGALIKGAALKIPSWG	Nv1; Nv3; Nv4	1826.08	Carboxypeptidase
FWGALIKGAALKIPSW	Nv1	1769.06	Carboxypeptidase
ALIKGAALKIPSWGLFKK	Nv4	1952.25	Endopeptidase
ALIKGAALKIPSWGLF	Nv1	1696.06	Endopeptidase
ALIKGAALKIPSWG	Nv4	1548.99	Endopeptidase
GAALKIPSWGLFKK	Nv1	1526.95	Endopeptidase
GAALKIPSWGLF	Nv4	1270.76	Endopeptidase
AAKIPSWGLF	Nv1;	1213.74	Endopeptidase
IPSWGLFKK	Nv4	1342.83	Endopeptidase
PSWGLFKKK	Nv3;	1101.69	Endopeptidase
IPSWGLFK	Nv4	958.58	Endopeptidase
WGALIKGAALKIPSWGLFKKKQ	Nv1;	2451.50	Aminopeptidase
GALIKGAALKIPSWGLFKKKQ	Nv4	2265.42	Aminopeptidase
ALIKGAALKIPSWGLFKKKQ	Nv1;Nv4	2208.40	Aminopeptidase
IKGAALKIPSWGLFKKKQ	Nv1; Nv4	2024.28	Aminopeptidase
GAALKIPSWGLFKKKQ	Nv1; Nv2;Nv4	1783.10	Aminopeptidase
AAKIPSWGLFKKKQ	Nv1;Nv2; Nv4	1726.08	Aminopeptidase
PSWGLFKKKQ	Nv1; Nv2; Nv4	1229.74	Aminopeptidase
FWGALIKGAALKIPSWG <b>M</b> FKKKQ	Nv1; Nv3; Nv4	2616.53	(Leu19Met)
FWGALIKGAALKIPSWG <b>M</b>	Nv3	1957.12	(Leu19Met)/carboxypeptidase
GALIKGAALKIPSWG <b>M</b> FKKKQ	Nv3	2283.38	(Leu19Met)/aminopeptidase
ALIKGAALKIPSWG <b>M</b> FKKKQ	Nv3	2226.36	(Leu19Met)/aminopeptidase
ALIKGAALKIPSWG <b>M</b>	Nv3	1566.95	(Leu19Met)/endopeptidase
GAALKIPSWG <b>M</b> FKKKQ	Nv1;Nv3	1801.06	(Leu19Met)/endopeptidase
FW*GALIKGAALKIPSWGLFKKKQ	Nv2; Nv4	2614.57	*Oxidation
FW*GALIKGAALKIPSWGL	Nv2	1955.16	*Oxidation/carboxypeptidase
FW*GALIKGAALK	Nv3	1176.66	*Oxidation/carboxypeptidase

Nv1: winter; Nv2: summer; Nv3 ground-dwelling ants; Nv4: arboreal ants

Bold letter indicates a mutation

\* Indicates an oxidation

of the samples, or that this class of antimicrobial peptides might undergo enzymatic cleavages. This peptide processing seems to occur at both extremities of the peptide, suggesting the action of carboxypeptidases, aminopeptidases and/or endopeptidases (Table 1). This extensive proteolysis was observed only in ponericins and not in the other peptides identified in our work, suggesting an enzymatic preference to this peptide subfamily. In this way, it appears to be implausible that the peptide proteolysis observed is

caused by sample degradation but it is, indeed, the result of post-translational modifications.

Toxin proteolysis was previously described and was related to the increase of the structural and molecular diversity of the venom protein repertoire [32]. Thus, with a single gene product cleaved in different positions, several other peptides with different targets and modes of action are produced, therefore generating an immense molecular repertoire with low energy costs [32].