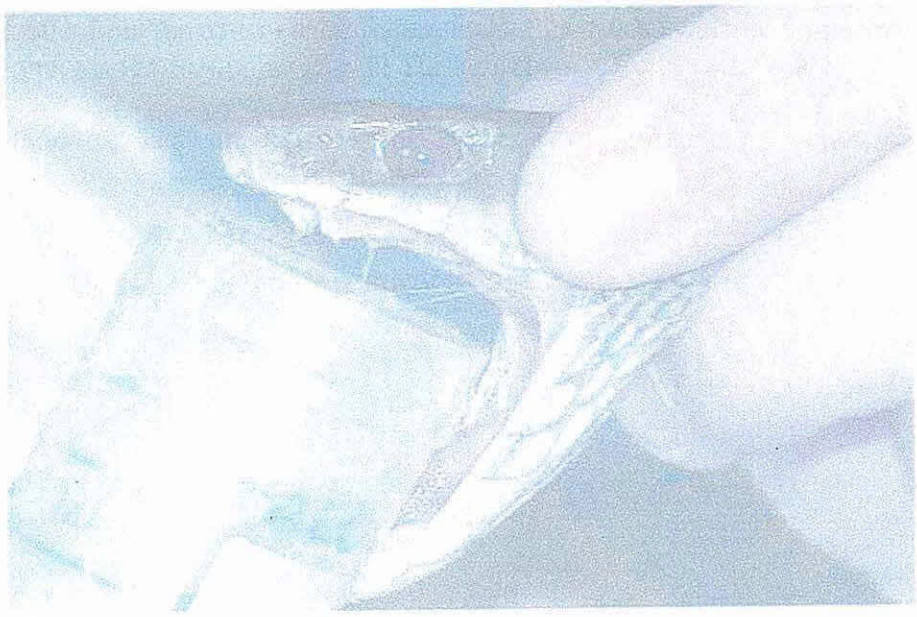
Proteomic and genomic characterisation of venom proteins from *Oxyuranus* species.



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Australia

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Ronelle Welton

June 2005

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Abstract

The genus *Oxyuranus* includes three of the largest and most feared Australasian snakes, and are listed within the top ten of the world's most venomous snakes. This genus includes *Oxyuranus microlepidotus*[1] (inland taipan), *Oxyuranus scutellatus scutellatus* (coastal taipan) and the subspecies *Oxyuranus scutellatus canni* (Papuan taipan). Despite comparative differences in the proportions of venom components the *Oxyuranus* species have been reported to be more closely related to each other than to those of any other large Australian elapid snakes. Nevertheless, differences in the clinical presentation of envenomed patients have been described between species. It has also been shown that the antivenom (produced from *O. s. scutellatus*) may not be as effective in bite victims of *O. microlepidotus* or *O. s. canni* compared to *O. s. scutellatus*.

This project was a comparative study designed to substantially build upon previous research into the venom of *Oxyuranus* species. The objectives of this study were: *firstly*, to conduct a comparative study of the composition of venom proteins from the three *Oxyuranus* species; *secondly* to clone and characterise venom specific proteins. This study was primarily conducted utilising fundamental proteomic tools including chromatography, one- and two-dimensional gel electrophoresis (2DE) mass spectrometry and N-terminal sequence determination. The *third* objective was to use a cDNA expression library, constructed using mRNA from a venom gland of *O. s. scutellatus,* to screen with taipan antivenom in order to isolate nucleotide sequences important within the venom.

The venoms from *Oxyuranus* showed remarkable complexity and stability. It was shown that the three *Oxyuranus* venoms share strong similarities in the protein patterning of major proteins. *O. s. scutellatus* and *O. s. canni* venoms exhibited identical protein patterning throughout electrophoretic, fractionation and immunobinding analysis. The venom similarities, together with previous comparative

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[1] (Note for future annotation: *O. s. scutellatus* may be taken to mean the Australian species, the coastal taipan, *Oxyuranus scutellatus scutellatus* (OS in some figures), *O. microlepidotus* to mean the inland taipan or *Oxyuranus microlepidotus* (OM in some figures), and *O. s. canni* to mean the Papuan subspecies *Oxyuranus scutellatus canni* (OSC in some figures))

research using mitochondrial DNA sequencing and morphology studies indicated *O. canni* is not a subspecies but the same species of *O. s. scutellatus*. *O. microlepidotus* on the other hand, though sharing major proteins, revealed patterns distinctly different to *O. s. scutellatus* through electrophoretic and fractionation examinations. Immunobinding studies showed the major banding differences in *O. microlepidotus* were antigenic against taipan antivenom (CSL). These results also indicated that diet, feeding and habitat (captive/wild) do not play a major role in contributing to the venom compositions of the taipan, opposing literature stating the contrary in other species. Variation within the venom composition between the species may be due, instead, to the geographic distances and habitat pressures between the species.

From these comparisons, an antigenic 300 kDa glycoprotein from *O. s. scutellatus* was identified using 15% SDS-PAGE and was isolated using size exclusion and affinity chromatography. This protein was comprised of a homo trimer, three subunits of 100 kDa joined by disulphide bonds. N-terminal sequence shared homology to a previously identified serine protease. This protein cleaved chromogenic substrate S-2288 and Michaelis-Menten kinetics revealed this protein to undergo allosteric interactions. Results of inhibition profiles, pH optimum and kinetic studies confirmed this to be a trypsin-like serine protease. It was highly sensitive to benzamidine and PMSF and was inhibited only by high concentrations of alkylating and reducing agents NEM and iodoacetamide. Metal chelators 1,10-phenanthroline, EGTA and EDTA showed little inhibition; whereas SDS was the only reagent to completely prevent any activity. Based on this evidence it is proposed all three subunits are required for activity. This protease appeared to be present in the venoms of both *O. microlepidotus* and *O. s. canni* and may represent another coagulative enzyme within the venom.

Sequencing studies elucidated the nucleotide sequences of a variety of clones from an *O. s. scutellatus* cDNA library. These clones, translated into full-length protein sequences, included homologous matches to characterised proteins including polymerases, carboxylases, dehydrogenases, Protein disulphide isomerases (PDI's), Heat shock proteins (HSP's) and myosin. Based on the sequences obtained, six PLA_2 toxins, two post-synaptic toxins and the complete factor V component of a prothrombin activator (scutelarin) were proposed. This study represents the first report of full-length amino acid sequences of taipan venom proteins; previous studies have been conducted

using isolated venom proteins that lack the nucleotide and transit peptide sequences. cDNA and deduced amino acid sequences were compared with those of other snake species. These comparisons conformed to the established primary structures found within the differing peptide classes and, within the putative pre-synaptic neurotoxins, conformed to the evolutionary outline suggested for these sequences.

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Abbreviations

Ach	acetylcholine
bp	base pairs
BSA	bovine serum albumin
Da	daltons
DAB	diaminobenzidine
DNA	deoxyribonucleic acid
ECL	enhanced chemiluminescence
EDTA	ethylene diamine tetraacetic acid
ELISA	enzyme linked immunosorbant assay
FV	Factor V (coagulation co-factor)
g	gram
HCl	hydrochloric acid
His	histidine
hr	hour
HRP	horse radish peroxidase
hrs	hours
IPTG	isopropyl β-D-thiogalactopyranoside
kDa	kilodaltons
LB	Luria Bertani
LD_{50}	lethal dose, 50%
M	molarity
µg	microgram
µl	microlitre
mA	milliamps
ml/min	milliliters per minute
min	minute/s
ng	nanograms
nm	nanometers
Ntx	neurotoxin
OD	optical density (at a given wavelength)
OM	*Oxyuranus microlepidotus*, *O. microlepidotus*
ORF	open reading frame
OS	*Oxyuranus scutellatus scutellatus, O. s. scutellatus*
OSC	*Oxyuranus scutellatus canni, O. s. canni*
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PBST	phosphate buffered saline containing 0.05% Tween 20
PCR	polymerase chain reaction
PLA_2	phospholipase A2
RNA	ribonucleic acid
sec	second/s
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
TRIS	tris(hydroxymethyl)aminomethane
V	volts
Xgal	5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside