

Characterisation of Novel African Diplopod Defensive Fluids

Carol M Trim¹ & Steven A Trim²

1) University of Kent, Canterbury, UK. 2) Venomtech Limited, Canterbury, UK.

Abstract

Millipedes (Myriapoda, Diplopoda) produce defensive fluids which are secreted through multiple ozopores, arranged laterally along the body. The fluids are largely believed to act as a deterrent to predators, but may also act as antimicrobials protecting the hosts from many pathogens within the detritus in which they feed (including carrion). Black lemurs use these millipede defensive fluids as potential antiparasitics, biting the millipede to induce toxin secretion then rubbing these toxins into their fur. This study aims to characterise such fluids from several African and Madagascan millipede species (including *Dendrostreptus macracanthus* and members of the *Aphistogoniulus* genus) using a number of proteomic and biochemical approaches. Many millipede species secrete a complex mixture of organic compounds including benzoquinones, a class of organic molecule that may have use as novel anticancer agents. This is the first time defensive fluids from these species have been studied, to the best of the authors knowledge, and it demonstrates a potential new source of bioactive compounds.



A. corralipes

Defensive fluids from *Aphistogoniulus corralipes*, *Aphistogoniulus* sp. *Black*, *Dendrostreptus Macracanthus* and *Aphistogoniulus* sp. *yellow* form were collected onto cotton buds, in ddH₂O or parafilm and stored at -20°C until required for experiments.

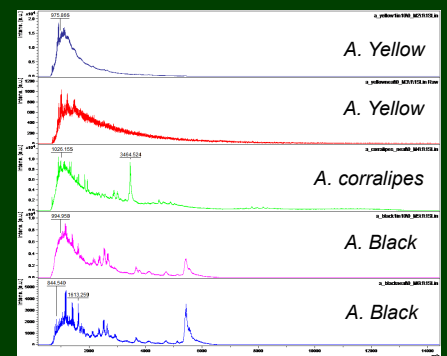
MALDI –TOF Mass spectrometry

Defensive fluids were mixed with a saturated solution of sinapinic acid (Sigma D7927) in matrix buffer (40% acetonitrile, Fisher HPLC grade A/0627/17, 60% 0.1% TFA, Acros Organics 293) and 1 µl of this mixture was loaded onto a 384 MTP ground steel MALDI target (Bruker) and allowed to air dry. 1 µl of the Bruker Calibration Standard I was also loaded onto the target. A MALDI TOF instrument (Bruker Ultraflex) was utilised to analyse the samples. 120 shots were collected for each sample using a laser power of 65%.

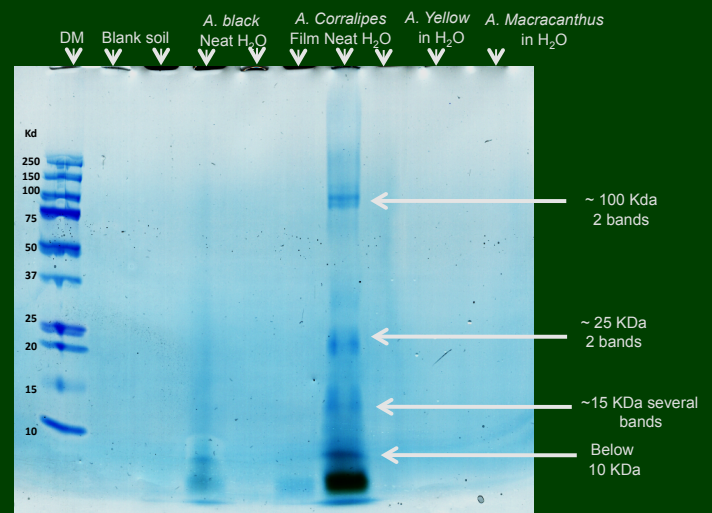
Reducing SDS PAGE

Defensive fluids were mixed 1:1 with reducing Laemmli sample buffer (Biorad), heated for 5 min. at 100°C and loaded onto 4-20% tris-glycine gradient gels or a 15% tris-glycine single percentage gels with a 3.75% stacking gel. Gels were run at 60V constant through the stacking gel and 125V after the stack. Gels were then washed 3 times with ddH₂O and stained with Colloidal Coomassie blue (Biorad) and destained with ddH₂O.

MALDI TOF mass spectrometry profiles display marked differences in the composition of defensive fluids from different Diplopods.



Reducing SDS PAGE reveals a protein component to the defensive fluids



D. macracanthus



A. black

VENOMTECH

www.venomtech.co.uk. e-mail s.trim@venomtech.co.uk. Tel +44(0)1227 827114

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