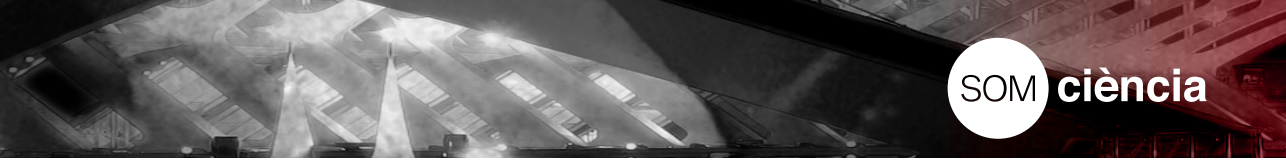

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# 17th Congress of the European Section of the INTERNATIONAL SOCIETY on TOXINOLOGY

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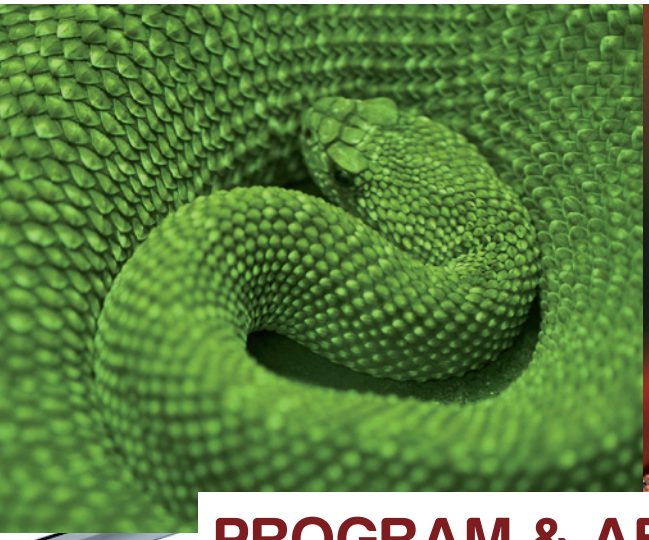
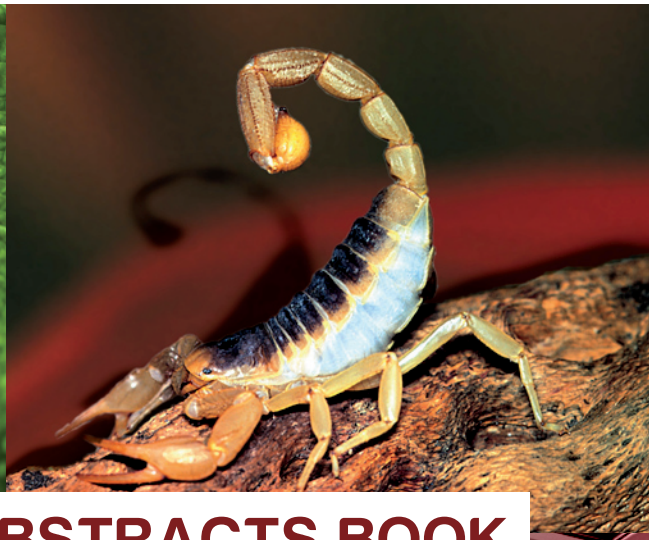
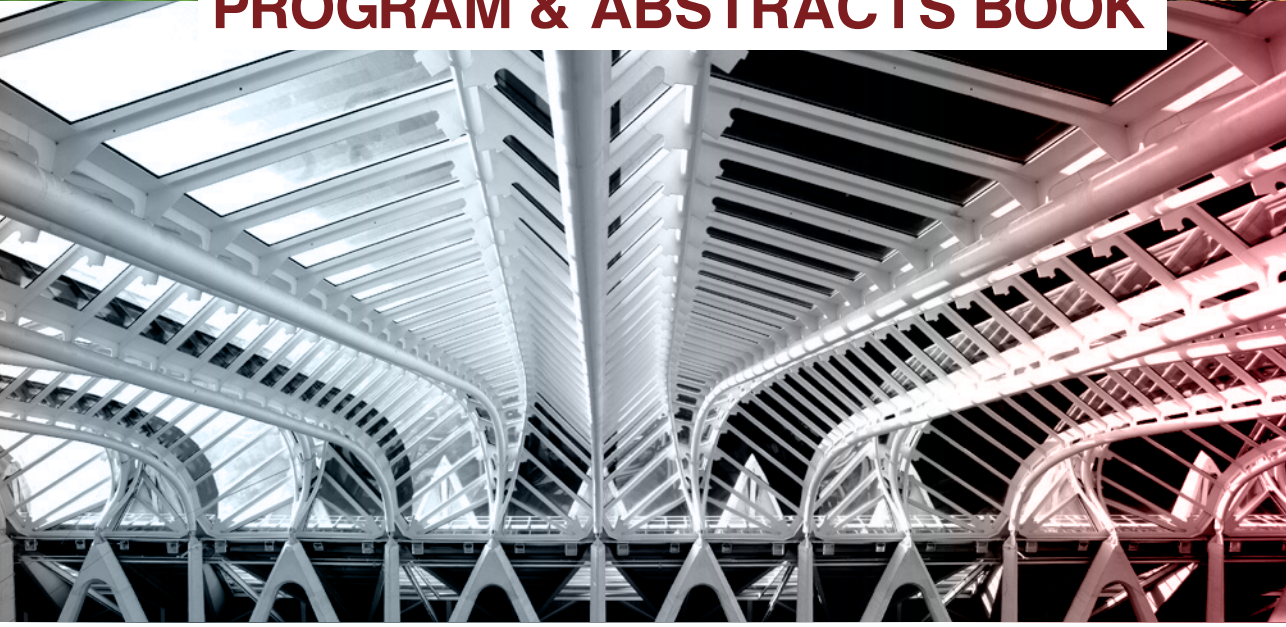




## PROGRAM & ABSTRACTS BOOK



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## Poster: 152

## Evidences of venom metalloprotease in *Pseudonaja textilis*.

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Snake venom metalloproteases are of extreme relevance in snake envenoming, disrupting the endothelium integrity and affecting platelet aggregation and blood clotting. However, these toxins and their action are frequently neglected in accidents involving Elapidae, since the major symptoms involve mostly neurotoxicity. Preliminary data indicated the presence of a metalloprotease in the venom of *Pseudonaja textilis*, an Australian Elapidae, at the transcript level, but no further characterization has been performed to our knowledge.

*P. textilis* metalloprotease transcripts were cloned from a venom gland cDNA library, built using In-Fusion SMARTer cDNA library construction kit (Clontech). PCR reaction was performed using 20-mers primers, designed based on untranslated regions (UTR) sequences from metalloprotease RNAm from Elapidae, Colubridae and Viperidae species (NCBI). The 5'UTR and 3'UTR sequences were first separated from the open reading frame (ORF) using ORFfinder and the ORFs checked using blastx (NCBI), than aligned independently using the online (EMBL-EBI) Kalign multiple-alignment algorithm and checked using BioEdit software. The specificity of the primers was tested using a cDNA library from *Bothrops erythromelas*, a Brazilian pit viper. Two fragments of approximately 2000 bp and 200 bp were detected from *P. textilis* cDNA, and two fragments of approximately 1000 bp and 600 bp were detected for *B. erythromelas* cDNA.

The presence of metalloprotease in the *P. textilis* venom was also checked by western-blot of crude venom and metal affinity column (Histrap FF, GE Healthcare) purified toxins, using an anti-jarharagin policlonal antibody. Hemorrhagic activity was also assayed *in vivo*, and no hemorrhagy was detected, suggesting that the isolated metalloprotease has no effect on the endothelium integrity. Based on previous data which suggested the presence of metalloprotease in *P. textilis* venom, we designed a set of primers, directed towards highly conserved regions of the 5 ' and 3' UTRs, which enabled the detection of 2 separate bands from the *P. textilis* venom gland cDNA. Noteworthy is the fact that the same set of primers also enabled the amplification of transcripts from cDNA from *Bothrops erythromelas*, a remotely related species, suggesting that these primers might be used as universal probes for the detection of metalloprotease in snake venoms.

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