

EUVEN 
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European Venom Network **2024**

ABSTRACT BOOK

23th -25th September 2024

Darwin-Dohrn Museum of Stazione Zoologica Anton Dohrn
Villa Comunale - Napoli, Italy

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Program

Monday

23 September

09:00-09:30 Registration

09:30-09:40
Conference Opening:
Greetings from the Organizers

09:40-10:25 Management Session
WG Meeting

10:25-11:00 Coffee Break

11:00-11:30 Management Session
WG Meetings

11:30-12:25 Management Session
Committees

12:25-14:00 Lunch Break

14:00-14:45 Keynote Speaker
Cesare Montecucco

14:45-15:40 Scientific Session
**Venom Diversity in Marine and Terrestrial
Environments**

15:40-16:00 Coffee Break

16:00-17:35 Scientific Session
**Venom Diversity in Marine and Terrestrial
Environments**

Tuesday 24 September

09:00-10:25 Scientific Session
Emerging methodological tools in Venom Research

10:25-11:00 Coffee Break

11:00-11:55 Scientific Session
Emerging methodological tools in Venom Research

11:55-12:40 Keynote Speaker
Mande Holford

12:40-14:00 Lunch Break

14:00-15:35 Scientific Session
Translational Strategies for venom Exploitation

15:35-16:00 Coffee Break

16:00-18:00 Poster Session

18:00-22:00 Welcome Cocktail

Wednesday 25 September

09:00-10:25 Scientific Session
Short-Term Scientific Missions

10:25-11:00 Coffee Break

11:00-12:30 Scientific Session
Novel Targets in Venom Research

12:30-14:00 Lunch Break

14:00-15:20 Scientific Session
Novel Targets in Venom Research

15:20-16:00 Coffee Break

16:00-16:45 Keynote Speaker
Richard Lewis

16:45-17:15 Management Session
MC Meeting

17:15-18:00 Management Session
Future Networking Oppotunities



Abstract



Keynote Speaker: Cesare Montecucco

Degeneration and Regeneration of the Neuromuscular Junction following snakebite Injecting Neurotoxic PLA2s

Cesare Montecucco^{1,2}

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Snake envenomation is a major neglected human disease notwithstanding that millions of people are affected with sequelae and hundreds of thousands die. A major toll is taken by snakes that inject neurotoxic venoms capable of inducing the complete degeneration of axon terminals with ensuing peripheral neuroparalysis caused by presynaptic-specific snake PLA2 neurotoxins. This enzyme hydrolyzes phospholipids to lysophospholipids and fatty acids inducing a large increase of cytosolic [Ca²⁺] that activates endogenous hydrolases causing complete degeneration of nerve terminals. This is followed by a regeneration program defined by a ill-defined intercellular signaling among neurons, Schwann cells and muscle fibers.

Current knowledge on this signaling program will be discussed in the light of the aim of contributing to the treatment of snakebite by discovering small molecules that accelerate the functional recovery of the neuromuscular function and of the respiration which is the critical physiological function to survive the peripheral neuroparalysis induced by the neurotoxic snake PLA2.

We have recently identified two membrane receptors expressed at the neuromuscular junction (NMJ) after degeneration of the motor axon terminals: i) the CXCR4 receptor on the nerve stump and ii) the Melatonin receptor MT1 on the perisynaptic Schwann cell (PSC) surface. The natural ligand of CXCR4 is the chemokine CXCL12 α released by activated PSC. We have identified a specific CXCR4 agonist that shortens the time of recovery from neuroparalysis.

MT1 is activated by melatonin released locally by the muscle facing the degenerating nerve terminal. High affinity MT1 agonists were found to powerfully stimulate recovery of the NMJ function of skeletal and respiratory muscles in envenomed mice. Imaging, and electrophysiological recordings provided recovery-from-paralysis data that paralleled the evaluation of the critical respiratory function.

These agonists employed here are registered therapeutics for other human diseases and can be immediately repurposed to test their efficacy in inducing a rapid recovery of respiration after snakebites by taipans, kraits, coral and viper snakes and other snakes because they are not species specific. These drugs are cheap and stable and are expected to reduce the number of deaths by respiratory blockade and to shorten recovery of respiration thus reducing hospital costs.

Scientific Session

Venom Diversity in Marine and Terrestrial Environments

Exaptation of an evolutionary constraint enables behavioural control over venom composition in giant centipedes

Eivind Undheim

Norway University of Oslo

The evolution of venom is often thought to be primarily shaped by extrinsic ecological factors such as interactions with predators and prey. However, intrinsic physiological processes may also represent similarly important constraints, such as in centipedes, where constraints from effective toxin production by secretory cells has been hypothesized to limit the biochemical complexity of their venom. Here, we tested this hypothesis by examining the correlation between biochemical and morphological complexity of the centipede venom system. We found statistical support for morphology-associated constraints on centipede venom evolution and that complex venom glands have evolved on two independent occasions in Scolopendromorpha, perhaps as an adaptation to feeding on a greater phyletic breadth of prey. We also found that in one of these lineages—giant centipedes in the family Scolopendridae—the adaptations that have resulted in greater venom complexity have also facilitated the evolution of behavioural control over the composition of excreted venom. Taken together, our results suggest physiological constraints are important for the evolution of venom, but also show how exaptation of modifications to circumvent these constraints may drive evolutionary innovation.

Macro-conotoxins of the MLSML and MKAVA superfamilies display structures not previously observed in cone snail venom

Khilji, M.S.¹, Hackney, C.M.¹, Koch, T.L.², Ryding, N.L.¹, Hone, A.³, Rogalski, A.², Watkins, M.³, Olivera, B.³, McIntosh, J.M.³, Safavi-Hemami, H.^{2,3}, Teilum, K.¹ and Ellgaard, L.¹

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Venomous marine cone snails produce a wealth of peptide toxins, known as conotoxins. Given their high target specificity and potency, these biologically active molecules are valuable compounds that can be explored as research tools and potential drug leads. Here, we will present the first structural characterization of members of the MLSML and MKAVA superfamilies of conotoxins. Both proteins were produced recombinantly in *E. coli* and their structures were solved by NMR spectroscopy.

The NMR structure of the MLSML protein from *Conus textile* (124 residues including 12 Cys) revealed a b-barrel structure not previously observed among toxin peptides. A similar fold is predicted for conotoxins of three additional previously uncharacterized superfamilies: E, MMFLM, and Unknown. The MLSML-family toxin shows structural similarity with the fruit fly protein Argos, which harbors three domains that are structurally related to the snake venom three-finger toxins. However, like the individual domains of Argos, the structures of the conotoxins examined here only have two b-stranded loops (“fingers”) in each domain.

The proteins of the MKAVA family are unusually well conserved and likely evolved from schistosomins, proteins of unresolved function found in freshwater snails. In ongoing work, the preliminary NMR structure of an MKAVA protein from *Conus magus* (73 residues including 8 Cys) displays a Kazal-type protease-inhibitor fold and can inhibit serine-protease activity. In the presentation, we will discuss details of the relationship between structure and function in this previously uncharacterized family of conotoxins.

A fresh look into the venomics of genus *Profundiconus*

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The genus *Profundiconus* of cone snails consists of deep-water species, usually found between 100 and 1000 m. Up to date, 33 species are listed in the World Register of Marine Species. Apart from morphological studies based in the shells and, in some cases, the radular teeth, little is known about their ecology and venom. The difficulties in sampling and suitably preserving specimens for study hampers the possibility of analysing their venom composition: up to date, only the transcriptomes of *P. neocaledonicus* Tenorio & Castelin, 2016 and *P. vaubani* (Röckel & Moolenbeek, 1995) have been reported.

We have now carried out a proteomic study on the venom duct extracts of these species. The results of the proteomics study using LC-MS and LC-MS/MS allowed us to detect and validate sequences from both transcriptomes. Based on the hypothesis that the activity depends not only on the peptide sequence itself, but also on the 3D structure that allows it to fit into the pocket of the molecular target, we have built up a pipeline for the *in silico* study of the potential biological activity of peptide toxins. Accordingly, peptides with a similar 3D structure are likely targeting the same molecular target, even though their percentage of identity is low. Under this premise, we have conducted an *in silico* study of the potential molecular target of the sequences of the transcriptome by the tandem use of AlphaFold2 and Rupee software. The results of this study are presented and organized by pharmacological families.

On the other hand, the use of non-tripulated rovers have recently allowed the observation *in situ* of new species of *Profundiconus* in their deep-sea habitat. New live samples have been collected in the course of several research cruises of the Schmidt Ocean Institute along the ridges of Salas y Gomez and Nazca, in the SE Pacific off Chile. These samples will allow for new proteotranscriptomic studies of the venom of *Profundiconus* in the near future.

Venomic studies on West African cone snails of the genus *Kalloconus* (Gastropoda, Conidae)

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The genus *Kalloconus* of cone snails includes several species separated in two groups, one endemic to the Cabo Verde Islands (*K. venulatus* group) and another distributed along the West African coast and the Canary Islands (*K. pulcher* group). The species *K. pulcher* is considered the largest-sized living cone snail, and specimens may reach shell lengths up to 250 mm. All species in genus *Kalloconus* are vermivorous. We have now obtained transcriptomic data for the venom glands of *K. canariensis*, *K. pulcher*, *K. byssinus* and most other *Kalloconus* from the Cabo Verde Islands. We have used these transcriptomic data as reference for a comprehensive proteomic analysis of the venom gland extracts of all species within genus *Kalloconus*. Conotoxin superfamilies T, M, O1 and O2, conkunitzins and con-ikot-ikot account for ca. 50% total diversity. Assorted venom proteins represent ca. 10%. There is little variability in conotoxin superfamily distribution among the different species studied.

Conoporins and novel somatostatin-like peptides (contulakins) are present in the transcriptomes and proteomes of several *Kalloconus* species. The latter are very similar to consomatatin ConSST-Ro1, very recently isolated from the deep-water cone snail *Asprella rolandi*. Our work reveals that non-paralytic conotoxins including conkunitzins, con-ikot-ikot, and very specially the somatostatin-analog contulakin are abundant in the proteome of *Kalloconus* species. Hence, these shallow-water West African cone snails constitute a rich source for the discovery of new conopeptides.

Genome of the green-head ant, *Rhytidoponera metallica*, reveals mechanisms of toxin evolution in a genetically hyperdiverse eusocial species.

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Ant venoms are rich sources of extremely diverse peptide toxins, contrasting the misconceptions that they are primarily acid-based. While this diversity makes ant toxins attractive as pharmacological tools, the social organization of ants also provides opportunities for studying how venom evolution may be affected by selection at different levels of biological complexity. Here, I will present some of our recent work on the genetically hyperdiverse Australian greenhead ant, *Rhytidoponera metallica*, whose colonies contain some of the highest-known intra-colony genetic variance for a eusocial animal. I will also present our most recent findings on the genomic architecture and evolutionary mechanisms that underlie the surprisingly complex toxin arsenal of this species. We generated a high-quality genome assembly from a single worker and identified complete maps of toxin paralogues and their allelic variants from transcriptomic and proteomic data. Contrary to previous suggestions that gene duplication plays a minor role in the evolution of venom in stinging hymenopterans, we find evidence of a large toxin-gene family expansion associated with transposon activity. We have also identified elevated levels of heterozygosity at the toxin regions. Rare alleles are still maintained at the colony-level, suggesting that alleles may diverge into different functions and increase the skill-pool effect of the colony. Although it represents an extreme case of genetically diverse colonies, *R. metallica* provides insights into how selection at both individual and colony levels contribute to driving the evolution of adaptive traits in eusocial animals.

Bee venom genes evolved before the stinger - Illuminating the origin of hymenopteran venom in a holistic approach including genomics & deep learning

Björn Marcus von Reumont¹, Ivan Koludarov¹

¹ *Goethe University Frankfurt*

Venoms, which have evolved numerous times in animals, are ideal models of convergent trait evolution. However, detailed comparative genomic studies of toxin-encoding genes exist for only a few animal groups. The hyper-diverse Hymenoptera are the most speciose venomous clade, but investigation of the origin of their venom genes has been largely neglected.

Utilising a combination of genomic and proteo-transcriptomic data and comparative genomics and large language model based deep learning methods, we investigated the origin of 11 toxin genes in 29 published and 3 new hymenopteran genomes and compiled an up-to-date list of prevalent bee venom proteins.

Observed patterns indicate that bee venom genes predominantly originate through single gene co-option with gene duplication contributing to subsequent diversification. Most venom genes within hymenopterans are shared by all members of the clade and only melittin and the new venom protein family anthophilin1 appear unique to the bee lineage. Our results indicate that prevalent venom proteins thus predate the mega-radiation of hymenopterans and the evolution of the aculeate stinger.

How did EUVEN contribute to expanding our knowledge of venom variation in the Nose-horned viper?

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Snake venom is a highly variable functional trait. Investigating the variation in venom composition of *Vipera ammodytes*, a species of the highest medical importance (WHO) in Europe, can give insights into the ecological drivers shaping it. We studied compositional differences between sexes and age groups from the island and a 2 km-distant mainland population subjected to distinct ecological conditions. The prey availability, an important driver of snake venom diversification, differs greatly between these two populations. Island vipers have an ectotherm-based diet (lizards and centipedes) and are classified as dwarfs, while mainland vipers feed on both ectotherms and endotherms (small mammals). Using the snake venomomics bottom-up approach, we analysed seven venom profiles (three age groups from both populations and adult males and females from the island population). We identified 10 toxin families, dominated by PLA₂, svMP, svSP, and DI, and diverse peptides. Additionally, we analysed 58 individual venom samples using the SDS-PAGE presence/absence matrix of expressed bands. The venom profiles of adult females and males were similar, with minor sex-based variations in toxin abundances in the island population. Analyses of the proteomes and individual venoms revealed a consistent distinction between juvenile venom and the ones of adults and subadults in both populations. Two venom phenotypes were identified: a juvenile svMP-dominated and KUN-lacking phenotype and an adult PLA₂/svMP-balanced and KUN-including phenotype. Despite differences in prey availability between island and mainland populations, no significant differences in venom composition were found, suggesting an insufficient time for natural selection or genetic drift to act on the venom composition of island vipers. Our study highlights the need to consider ecological and evolutionary processes in studies addressing venom variability.

Investigating the genetic basis of venom variation using Australian brown snakes (*Pseudonaja spp.*) as a model

Cassandra Modahl¹, Jory van Thiel², Taline Kazandjian¹, Cara Smith³, Rohit Patel¹, Mark Wilkinson¹, Timothy Jackson⁴

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Australia is known for its large diversity of front-fanged venomous snakes from the family Elapidae, and snakes of the genus *Pseudonaja* are found in almost every part of mainland Australia. The brown snake *P. textilis* is responsible for the majority of snakebite deaths in eastern Australia and has been the focus of intraspecific venom variation studies. These studies have documented geographic, seasonal, and ontogenetic venom variation, one of the few cases of significant ontogenetic venom variation in an elapid. Hatchling *P. textilis* venoms have low amounts of procoagulant toxins and their venoms lack procoagulant activity. This contrasts with the high abundance of these toxins in adult venoms. Ontogenetic shifts in venom phenotype are not present for all snakes of this genus, the ringed brown snake *P. modesta* lacks procoagulant activity for both hatchling and adult venoms. The ontogenetic shift in *P. textilis* venom phenotype has been hypothesized to be linked to feeding ecology, however the exact timing of this venom phenotypic change and the genetic mechanisms regulating this transition are unknown. Using comparative venom proteomics, venom gland transcriptomics, and bioactivity assays for three species in this genus over a developmental time course, we have identified the timing of this ontogenetic venom transition and the influence of gene expression on venom phenotype in this group of snakes.

Scientific Session

Emerging Methodological Tools in Venom Research

Analytical profiling of toxins in snake venoms for evoking zebrafish paralysis with parallel assessment of ion channel modulation

Jeroen Kool

Vrije Universiteit – Netherlands

Snake venoms are complex bioactive mixtures mainly comprising proteins and peptides secreted by cells of venom glands of venomous snakes. The primary purpose of venom is to paralyse or kill the prey. Current pharmacological research on venom is directed towards its use in modulating molecular targets such as ion channels and receptors. Due to their specificity and potency, many venom toxins are invaluable for studying a wide range of these molecular targets (e.g., ligand-activated and voltage-gated ion channels). Studies on ion channels often focus on *in vitro* targeted analysis or *in vivo* behavioural research. In the first case, the molecular target is studied, and its functioning at the molecular level with interacting compounds can be unravelled, but *in vivo* effects cannot be assessed. In the latter case, the opposite is true. In this study, we demonstrate a combined whole organism *in vivo* and ion channel *in vitro* high-throughput screening platform for biological analysis of toxins in venoms. The methodology is based on larval zebrafish locomotor behaviour *in vivo* assessment and calcium flux assays as *in vitro* readouts. A zebrafish model and FLIPR-based ion flux assays were post-column integrated into nanofractionation analytics for which LC-MS was coupled to high-resolution fractionation, high throughput venomics, and bioassaying. Combined *in vivo* behavioural and *in vitro* targeted bioassay results were obtained for all nanofractionated venom toxins in the venoms under study. The results demonstrate that several toxins in the elapid venoms that were analyzed paralyse the zebrafish larvae, for which directly the correlating ion channel targets could be revealed. Using the acquired MS and proteomics data, responsible bioactive toxins were identified. This research leads to discovery platforms to better support the development of novel drugs targeting ion channels and envenomation treatments.

Genetic manipulation of the sea anemone *Nematostella vectensis* unravels the role of venom in interspecific interactions and evolutionary trade-off

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Venom is a complex trait that evolved in numerous animal lineages making it a useful system for understanding the ecology and evolution of interspecific interactions. Yet, our understanding of venom biology is severely limited by the fact that the vast majority of venomous animals cannot be grown under lab conditions throughout their full life cycle and cannot be genetically manipulated. A notable exception is the startlet sea anemone *Nematostella vectensis* that was developed in the last 20 years as a model for evolutionary developmental biology. By developing a novel approach based on stable gene knockdown by incorporating to the anemone genome a synthetic microRNA locus we have blocked the expression of Nv1, a major *Nematostella* neurotoxin encoded by multiple genomic loci. Strikingly, this led to significantly different interactions between the anemone and its predator (grass shrimp) as well as the predator's predator (mummichog fish). Furthermore, anemones lacking the neurotoxin exhibited increased growth rates and improved sexual and asexual reproduction, revealing an evolutionary trade-off between venom production and reproduction. Currently, we harness the CRISPR-Cas9 system to knockout single-locus genes that encode other neurotoxins and assay how these manipulations affect the physiology and interspecific interactions of the anemones. Notably, native *Nematostella* populations exhibit significant variability in their venom-encoding genes and toxin expression levels, including complete loss of Nv1, mirroring our transgenic assays and making them ecologically relevant.

DeTox: A novel pipeline for Detection of Toxins in venomous animal transcriptomes

Allan Ringeval¹, Sarah Farhat¹, Alexander Fedosov^{1,2}, Marco Gerdol^{3,4}, Samuele Greco³, Lou Mary¹, Maria Vittoria Modica⁴, Nicolas Puillandre¹

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Animal toxins serves as a formidable tool for research, both to understand the evolution of venomous organisms and for the pharmaceutical perspectives they offer. Indeed, these deadly compounds are associated to an incredible array of biological properties and have proved efficient in human therapeutics. Nowadays, the study of toxins is primarily conducted through the generation of OMICS data. Among these data, transcriptomes have proven to be the most commonly used due to their low cost. The main challenge is no longer in sequence production but in having the resources and capacity to analyze them through bioinformatics methods. Existing tools typically utilize homology-based methods, but recently, the search for de novo structural characteristics has expanded the research to toxins not present in reference databases. Some pipelines have already been published, but they do not encompass all the steps of both approaches and can be difficult for users to apply. Thus, we have developed DeTox, which implements these two complementary approaches. It is designed to make toxin research in transcriptomics more accessible. It is user-friendly, integrates all analysis steps, can be customized for taxon-specificity by the user, and the steps are parallelized for fast execution. It thus opens the way for toxin research in understudied taxa, which are often underrepresented in databases. DeTox's performance was evaluated on a selection of transcriptomes previously published in studies that conducted toxin research on gastropod mollusks, cnidarians, and snakes. DeTox results were compared to toxins identified in the original publications associated with the transcriptomes. In all cases, DeTox was able to recover most of the initial toxins and to identify a panel of new proteins with toxin-like characteristics. DeTox thus offers a powerful tool capable of easily identifying the different components of venom from transcriptomic data. We also applied this pipeline to detect toxins in two gastropod mollusks, *Stramonita haemastoma* and *Monoplex corrugatus* for which we have whole genome and transcripts data from different tissues. The aim of this study is to first identify novel toxins and also to gain insights into which tissues produce which toxins with the help of differential gene expression analysis.

The potential of vibrational spectroscopy as an emerging tool for the biomolecular characterization and discrimination of crude venoms: A case study on the classification and individual variation of snake venoms as studied by FTIR spectroscopy

¹İğci, N.

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Vibrational spectroscopy is a fundamental method in chemistry and related fields. Mid-infrared (MIR), near-infrared (NIR) and Raman are well-known vibrational spectroscopy methods. Due to their non-invasive and label-free nature, these methods have been applied in many fields. Although peptides and proteins play a major role in the toxicity of most animal venoms, non-polypeptide components are also found and contribute to the pharmacological effects of venom in some groups. In the present proof-of-concept study, lyophilized crude venoms of seven Viperids (from the genera *Macrovipera*, *Montivipera*, and *Vipera*) and one Elapid species (*Walterinnesia morgani*) were analyzed by Fourier transform infrared (FTIR) spectroscopy in the MIR region. Peaks in the FTIR spectra were assigned to various molecules such as proteins, nucleic acids, and carbohydrates. Second derivative spectra in the Amide I region were used to identify and compare the protein secondary structures. Elapid venom showed a very distinct band pattern compared to Viperids. Accordingly, principal component analysis separated Elapid venom from other samples and Viperid venoms were classified at genus level. Individual venoms of *Macrovipera lebetinus*, *Montivipera raddei*, and *M. wagneri* were compared to assess the method's potential for venom variation studies. Based on an FTIR spectrum, both polypeptides and non-proteinaceous components in the venom can be identified, and their amounts can be compared between samples. This method allows using solid and liquid samples in small quantities. The results showed that FTIR spectroscopy can be useful in venom research for general characterization, quality control, and classification of venom samples and to investigate venom variation. Not only FTIR but also NIR and Raman spectroscopy can provide similar information. Vibrational spectroscopy methods deserve further investigation using venoms from different animal groups to reveal their potential in venom research.

Antivenom downstream processing strategy as a platform for a high-quality human immunoglobulin G preparation as an urgent treatment option during emerging virus outbreaks

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Current core human plasma fractionation technology largely relies on a well-established backbone process encompassing cryoprecipitation and cold ethanol precipitation. Over the years its complexity has remarkably increased due to the implementation of additional steps, not only to improve product's purity, enhance its recovery and assure viral inactivation or removal, but also to isolate new clinically useful plasma proteins from the existing fractions. Consequently, the highest possible IgG extraction efficacy might not be achieved in such complex and lengthy fractionation protocols.

However, in specific situations, especially when immunoglobulins are the sole plasma products, current registered technologies are not appropriate for their preparation, but more efficacious, faster and streamlined ones are required. During COVID-19 epidemics, very simple technological platform, originally developed for antivenom downstream processing from horse plasma¹, was adapted for the purification of immunoglobulins G from human convalescent plasma, enriched with SARS-CoV-2-specific antibodies². It consisted of caprylic acid precipitation of the majority of albumin, followed by 100 kDa diafiltration of the IgG-enriched supernatant for the removal of precipitating agent and low Mw proteins, and final AEX chromatography polishing in the flow-through mode which appeared very effective in depletion of unwanted immunoglobulins of other classes from the IgG fraction, as well as aggregates. Overall IgG yield of 75%, with removal of 95% of IgA and 100% of IgM, was achieved.

¹Kurtović *et al.* PLOS Neglected Diseases 2019;13:e0007431;
<https://doi.org/10.1371/journal.pntd.0007431>

²Kurtović *et al.* Frontiers in Immunology 2022;13:889736; <https://doi.org/10.3389/fimmu.2022.889736>

Casting an AI-powered net: Integrating AlphaFold with molecular dynamics to identify the natural target of a cone snail toxin

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Pore modulating toxins block K⁺ channels by inducing asymmetric collapse of the selectivity filter (SF). Conkunitzin-S1 (Cs1), the first identified toxin of this class, was initially studied for its high affinity to the *Drosophila* Shaker channel, but its native targets remained elusive. We leveraged recent advances in protein structure prediction and machine learning to uncover Cs1's natural targets. Our approach combined AlphaFold-based structure prediction with a novel Equivariant Transformer (ET3) model, trained to classify SF-stabilizing water molecules. This pipeline allowed us to model Cs1-channel complexes for the entire space of fish K⁺ channels sharing >50% homology with Shaker. Molecular dynamics simulations highlighted models displaying Cs1-induced asymmetry indicative of pore-collapse. The ET3-based classification method outperformed previous geometric approaches, enabling comprehensive analysis across diverse K⁺ channel families. This computational framework showcases the power of integrating structural prediction and machine learning to decode toxin-channel interactions, opening new possibilities for in silico ecological research and drug interaction studies.

Spider venom gland organogenesis

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In this study we investigated the organogenesis of the venom apparatus in the common house spider, *Parasteatoda tepidariorum*. We performed bulk RNA-seq to identify venom gland-specific markers and assayed their expression using RNA *in situ* hybridisation experiments on whole-mount time-series. These revealed that the gland primordium emerges during embryonic stage 13 at the chelicera tip, progresses proximally by the end of embryonic development and extends into the prosoma post-eclosion. The initiation of expression of an important toxin component in late postembryos marks the activation of venom-secreting cells. Our selected markers also exhibited distinct expression patterns in adult venom glands: *sage* and the toxin marker were expressed in the secretory epithelium, *forkhead* and *sum-1* in the surrounding muscle layer, while *Distal-less* was predominantly expressed at the gland extremities. Our study provides the first comprehensive analysis of venom gland morphogenesis in spiders, offering key insights into their evolution and development. To characterise the function of these genes, we are developing a 3D cell culture model that will allow us to perform knock out and understand the involvement of developmental genes in adult tissues.

Revealing Venom Secrets: Advances in Mass Spectrometry Imaging

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Venoms, honed through millions of years, offer potent adaptations for predation and defense secreted by highly specialized glandular systems. Despite extensive research, there is still limited knowledge of the functional biology of animal toxins, including their venom production and storage. Major aspect is convergent evolution, where disparate species develop analogous venom components, providing insights into selective pressures shaping these biological arsenals.

Venom proteomics, at the nexus of molecular biology and evolution, investigates the intricate compositions of venoms across species and underscores the evolutionary importance of venomous adaptations and highlights mass spectrometry (MS) as a key innovation for unraveling venom complexities. MS imaging (MSI), a cutting-edge technology, stands out for its ability to provide spatially resolved information to map toxin distribution within organisms and reveal nuances in venom production and secretion dynamics. Comparative MSI venom analyses can elucidate evolutionary relationships between species, providing a molecular perspective on phylogenetic connections.

Keynote Speaker: Mandë Holford

A venom trail from snails to cephalopods

Mandë Holford

College, Research Associate, The American Museum of Natural History, CUNY Graduate Center

A jellyfish's sting, the blue-ringed octopus's deadly touch, a cone snail's piercing harpoon, each transforms a physical warfare into a biochemical one that has contributed to breakthroughs in fundamental and translational research. Venoms are prototypical "precision medicines" that form highly specific and potent interactions and are the basis of several FDA-approved drugs for treating chronic pain to diabetes. The evolution of venoms involves the venom genes and their transcriptional control; the cellular adaptations required to secrete it, the organization of glands to store it and release it; and an arms-race of biochemical counter-adaptations. The totality of the system must be considered to push the boundaries of venom knowledge. However, the lack of knowledge about the fundamental biological diversity of venoms and venom systems limits their use to advance drug discovery. As such, we focus on a series of interdisciplinary and complementary questions that investigate how venoms evolve, develop, and function. We want to elucidate how venom genes are expressed in specialized glands, and how we can manipulate them to obtain new therapies. Achieving this goal has been limited in part to lack of genetically tractable model systems. Recent technological advances are making it possible to create these models to interrogate venoms complexity. This seminar will highlight the development of venom model systems and interrogation of their function.

Scientific Session

Translational Strategies for Venom Exploitation

The senolytic properties of animal venoms

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Taking advantage of the extensive and vastly unexplored pharmacological properties of animal venoms, we identified the senolytic properties of a pore-forming toxin that we have named "Senotoxin 1". Senotoxin 1 and an engineered improved form, Senotoxin 1.1, selectively hamper the viability of senescent cells. We encompassed various techniques including, cellular, molecular and metabolic approaches, electrophysiology, RNA sequencing, lipidomics and xenograft animal models (mice and zebrafish). Altogether, demonstrated that mechanistically, these senotoxins cause sodium and calcium influx, and an enduring potassium efflux in senescent cells. This ionic imbalance triggers calcium activated potassium channels, aggravating further the enduring K efflux. Altogether, uncovers a new vulnerability of senescent cells to ionic unbalance. Senotoxins 1 and 1.1 synergized with senescence-inducing chemotherapy for remission of solid tumours *in vivo*. Our findings reveal a new class of senolytics derived from animal venoms with promising potential for cancer therapy.

Paraspecific neutralization capacity of polyvalent snake antivenom against *Montivipera raddei* venom

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Purpose: This study aims to measure the paraspecific neutralization capacity of nationally produced HSGM polyvalent snake antivenom (HSGM-PSAV), produced using *Macrovipera lebetina obtusa*, *Montivipera xanthina*, and *Vipera ammodytes montandoni* venom, against the lethal effect of the venom of *Montivipera raddei*, which is endemic in the Armenia and northeastern Anatolia of Turkey.

Methods: The neutralization capacity of HSGM-PSAV against the lethal effect of *M. raddei* venom was studied using the potency determination testing method specified in the Turkish and European Pharmacopoeia. Lethal dose 50 (LD₅₀) values of the venoms used in immunization, *M. raddei* venom in mice, and effective dose 50 (ED₅₀) values of HSGM-PSAV against four types of venoms were calculated using two-fold dilutions.

Results: HSGM-PSAV neutralized the lethal effect of *M. raddei* venom in mice. The ED₅₀ (95% c.i.) of the HSGM-PSAV against *M. raddei* venom was calculated as 347.84 LD₅₀/ml (266.68 -457.12) LD₅₀/mL.

Conclusion: As a result of this in-vivo study, it was determined that HSGM-PSAV neutralized *M. raddei* venom above the antivenom neutralization capacity threshold values (≥ 50 LD₅₀/mL) specified in the Turkish and European Pharmacopoeia. This result is important preclinical data regarding the usability of HSGM-PSAV in the treatment of poisoning due to *M. raddei* bites.

Identification of Novel Bioactive Compounds, Using *in vivo* Zebrafish Phenotypic Assays

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Identifying new Bioactive Natural Products (BNPs) that may serve as potential drug lead compounds or cosmeceuticals is a constant challenge. We are performing an extensive high-throughput screening of NPs isolated from various sources including plants, algae and venom extracts aiming to identify novel bioactive molecules with potential antiangiogenesis, anti-aging, wound healing and/or cosmeceutical properties. Venomous organisms produce complex mixtures of bioactive compounds that have evolved through million years of natural selection in evolutionary arms races. Therefore, venoms are highly specialised molecules with many potential applications, especially in pharmaceutical and biotechnological field.

Zebrafish embryos allow *in vivo* monitoring of complex cell behavior and physiological parameters. Transgenic lines, regeneration assays and mutant lines allow for diverse, high-throughput, non-invasive screens to identify bioactive copounds. Abnormal pigmentation correlates with various aesthetic problems, as well as health diseases, including melanoma. We use melanogenesis inhibition during early embryo development to identify natural compounds that block melanogenesis. Based on the responses to the different targets, the extracts are compared, prioritized, and correlated with the extracts' chemical composition.

Pore-forming toxins for advanced sensing technologies

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Pore-forming toxins are an important group of natural toxins that damage cell membranes by forming nanometer-sized pores. This leads to disruption of cell function and can even lead to cell death due to ion imbalance, nutrient loss or disruption of signaling. Pore-forming proteins can be used in translational approaches as part of sensing devices.

We have used an actinoporin-like protein from the coral *Orbicella faveolata* (Fav), a homolog of actinoporins, pore-forming toxins from sea anemones. Fav forms pores in liposomes. Cryo-electron microscopy revealed an octameric pore structure that interacts closely with membrane lipids. Each protomer bound 10 (phospho)lipids and 4 cholesterol molecules with different functions: (i) Receptor lipids initiate monomer-membrane interactions. (ii) Structural lipids facilitate oligomer formation and were an essential component of the final pore. (iii) Bridging lipids stabilize monomer-monomer interactions through lipid acyl chains. The structural models obtained illustrate the multifaceted role of lipids in the assembly and stabilization of membrane proteins.

Through further modifications of Fav, we developed pores that stably insert into the membranes of MinION devices and exhibit improved properties compared to the pores of the wild-type protein. This enabled fast, accurate and high-throughput detection of medically relevant proteins such as histones. For example, the blockades of the currents induced by histone H3.1 were longer and less discrete compared to the blockades of the less positively charged histone H4, while the addition of H3.1 citrullinated at several arginine residues caused discrete blockades with smaller amplitudes. The observed blockades, which differ in amplitude, current noise and dwell times, are the result of electrostatic interactions between the negatively charged surface of the pore lumen and the histones.

The development of the Fav nanopore expands the possibilities of sensor technology for biomedical applications such as DNA/RNA sequencing, protein sensing or protein sequencing.

Design of nature-inspired stabilized peptides for GPCR drug discovery

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Neuropeptides are the most diverse class of signaling molecules with crucial roles for human health and disease. Most of these peptides act as hormones or as neurotransmitters via G protein-coupled receptors (GPCRs). GPCRs represent the largest family of cell surface receptors encoded by the human genome and they have been exploited as drug targets for many years. In fact, drugs that target these receptors account for ~27% of the global market share of therapeutics.

The development of peptide therapeutics targeting GPCRs has been limited by poor pharmacokinetics (metabolic instability, short half-life, and rapid clearance) and lack of oral bioavailability (low gastrointestinal stability and membrane permeability). Furthermore, the pharmacological concepts of biased signaling and receptor selectivity need to be implemented into successful GPCR drug discovery projects to avoid harmful off-target effects.

To tackle these challenges, we explore various bioresources, including venom-derived peptides, genomes/transcriptomes, and plants to identify evolutionary-related human neuropeptides. Via peptidomics and pharmacology-guided screening we discovered novel GPCR ligands. Using state-of-the-art chemical and computational tools such as molecular grafting, backbone cyclization, cysteine stapling and *de novo* design, we have been developing innovative ligands for the κ -opioid receptor that exhibit enhanced stability, improved receptor subtype selectivity and functional bias. As proof-of-concept, our lead candidates, have been analyzed *in vivo* for their analgesic properties, which may be developed in the future as drug candidates for the treatment of chronic abdominal pain or inflammatory conditions.

Development of *Galleria mellonella* (wax moth) larvae as a model for snakebite envenoming

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Snakebite envenoming (SBE) annually kills >100,000 people worldwide, and 4-fold more suffer life-altering morbidity. Antivenom, the only specific treatment, requires preclinical models to assess efficacy and safety as an essential part of the manufacturing and regulatory process. In accordance with the World Health Organization (WHO) ‘Guidelines for the production, control and regulation of antivenom immunoglobulins’, the only validated assays to determine the potential clinical effectiveness of an antivenom are preclinical mouse models. These procedures are rated ‘severe’ in the UK and EU, are low-throughput and require high animal numbers.

Galleria mellonella (wax moth) models have gained momentum as a replacement for vertebrate models in the last decade, mainly focused on infectious diseases. Here we present the development of a *Galleria* model for SBE with the aim of providing a readily accessible (low-cost, low-skill) and standardised non-vertebrate alternative model for both research and commercial use.

Our pilot data from a wide panel of snake venoms demonstrates that *Galleria* envenoming pathology is comparable to mammalian envenoming – i.e. coagulopathic venoms impair the clotting function of both mammalian blood and *Galleria* hemolymph, and neurotoxic venoms cause rapid paralysis in mammals and *Galleria*. Beyond a venom profiling tool inclusive of LD₅₀ assessments, to date we have shown this model can be used to access therapeutic efficacy (ED₅₀s) for *Echis ocellatus* venom neutralisation by both EchiTABG and a small molecule SVMP inhibitor (marimastat).

Scientific Session

Short-Term Scientific Missions

Characterizing toxin diversity across a phylum of predatory marine worms

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Ribbon worms (Nemertea) are a largely understudied group of active predators that utilize an eversible proboscis to deliver toxins into their prey and a toxic epidermal mucus for self-defense. Unlike other organisms, ribbon worms lack differentiated multicellular glands; instead, toxins are produced by secretory cells lining the epithelia of the body wall and proboscis. This unique characteristic is a significant challenge when investigating venom composition in these invertebrates. Furthermore, the three main nemertean lineages, Hoplonemertea, Palaeonemertea, and Pilidiophora, exhibit notable differences in proboscis morphology, hunting strategies, and diets. Recent research from our group has identified numerous lineage-specific toxins, indicating that venom evolution in nemerteans is highly divergent and that toxin mixtures may have evolved to target different prey. However, to confirm this hypothesis, comparative analyses and detailed characterization of toxin diversity across a broader range of taxa within the phylum are necessary.

In our study, we used RNA-Seq differential gene expression analyses to characterize venom composition, toxin gene diversity, and expression patterns in 6 nemertean species, encompassing both marine and terrestrial taxa. We compared expression patterns in the proboscis and the body wall, the two main venom producing tissues, where toxins are expected to be up-regulated, identifying novel nemertean toxins and characterizing the composition of predatory and defensive venoms across the phylum. Our findings can contribute to understand the hidden toxin diversity in nemertean worms, providing new insights into the origin and evolution of venom in this poorly understood group of venomous invertebrates.

The synergistic Anti-Tumoral Effect of OctPep-1 and Rapamycin in a Xenograft *BRAF* Melanoma Mouse Model

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Melanoma is the main cause of skin cancer deaths, with special emphasis in those carrying *BRAF* (serine/threonine-protein kinase B-Raf) mutations that trigger the mitogen-activated protein kinases (MAPK) signalling and unrestraint cell proliferation in the absence of mitogens. Current therapies targeting MAPK are hindered by drug resistance and relapse that rely on metabolic rewiring and Akt activation.

Octpep-1, a venom-derived tachykinin-peptide from the *Octopus kaurna*, impairs melanoma cellular viability in human melanoma *BRAF(V600E)*-mutated, while is innocuous in healthy fibroblasts. Octpep-1 is also effective *in vivo*, reducing the tumor volume in mice and zebrafish melanoma xenograft models. An *in vitro* screening of various FDA-approved inhibitors showed that the combination of Octpep-1 with the mTORC1 inhibitor rapamycin or the ERK/MAPK-inhibitor LY3214996 significantly potentiated the antiproliferative properties of our candidate in melanoma cells as compared to Octpep-1 or the inhibitors alone and with the minimum effect in the viability of healthy fibroblasts.

Therefore, we decided to examine the combination of the peptide with rapamycin *in vivo* for a total of 15 days.

STSM grant presentation: Training in phage-display antibody libraries

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The purpose of this STSM was to gain an understanding and basic skills in using phage display to identify antibodies capable of binding and neutralising snake venom toxins. My home institute at the time of the STSM (CSRI, LSTM UK) was unable to use phage display due to a lack of knowledge of the theory and techniques involved. With reference to the EUVEN objective and deliverables, this STSM enabled me to learn the best practice principles and methodologies for using phage display to identify anti-venom antibodies, and the host lab were able to promote their methods and technologies. The STSM also strengthened the relationship between the host lab (TPL, DTU, Denmark) and my home research group, and has supported me as an early career researcher.

Although my home institute did not have access to a phage-display library to begin implementing these skills immediately, we have begun creating own phage library and this work is in progress. Additionally, I remain working with the host group at DTU on several collaborations, including one that uses phage-display. Subsequently I used the knowledge and skills developed during this STSM to demonstrate important skills and knowledge in Fellowship applications, which focused on using phage-display libraries to discover anti-toxin antibodies for next-generation antivenom therapies, and I will be implementing phage-display in my own research group which I have just started in 2024.

Widow spiders and their Venom toxicity (Araneae: *Steatoda nobilis*)

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The family Theridiidae Sundevall, 1833 (Araneae) comprises 2537 species worldwide, 262 of which are present in Europe. Arguably, the most recognisable clade within the Theridiidae is formed by the “widow spiders” which include the genera *Latrodectus* and *Steatoda*. The venom of all these species comprises the potent vertebrate-specific neurotoxin α -latrotoxin, which has a high affinity for nervous and endocrine receptors, resulting in mild to severe systemic envenoming. While *L. tredecimguttatus* is the only major medically significant spider native to Europe, envenoming by *S. nobilis* have been recently documented, sometimes leading to a systemic neurotoxic syndrome and spider-vectored bacterial infections. *S. nobilis* is also quickly becoming an alien invasive species throughout Europe, Western Asia, and both North and South America. The impact of *S. nobilis* on the native fauna has not been formally assessed yet, but observations in Ireland suggest an important impact on native spider communities.

In September 2023, 150 specimens of *S. nobilis*, both males and females, were collected from Galway City, Ireland and their venom extracted via electrostimulation. After lyophilisation and rehydration, serial dilutions of venom (0.1%-0.0001% of the original venom volume in Phosphate Buffer Saline) were injected in cohorts of 10 crickets (*Gryllus assimilis*) each. Effects (incapacitation and death) were monitored at regular intervals over 24h. LD₅₀ for both males and females were inferred using a ProBit model computed on R. Results show extremely high toxicity for females (LD₅₀= 0.273mg/kg) compared to males (11.81mg/kg). These results may explain the over-representation of females *S. nobilis* in bite reports, and the severity of the symptoms described compared to male-led bites. Results also suggest that females *S. nobilis* have the venom arsenal to be fierce competitors in the habitats they colonise throughout the world.

Keywords: Arachnids, Theridiidae, noble false widow, α -latrotoxin, neurotoxicity, LD₅₀.

Scientific Session

Novel Targets in Venom Research

Venomic adaptations of prey specialised spiders

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Predatory venoms are usually effective against certain prey resulting from evolutionary arms races. Nevertheless, many venomous animals remain understudied, including spiders, one of the most diverse groups of venomous predators. Most spiders are generalists who prey on a wide range of prey. However, a small proportion of spiders are prey specialists that preferentially hunt only one prey type, often represented by dangerous prey, such as termites, ants, or other spiders. They utilise morphological, behavioural and venomic adaptations to subdue such prey. Despite evidence suggesting that prey-specialised spiders immobilise prey with their potent venom, they haven't been thoroughly investigated. Our research discovered that prey specialists have smaller venom glands and less complex venom, but their venom is highly effective towards their focal prey. We are currently investigating their venom composition, aiming to test the prey-specificity of selected toxins.

So far, we have elucidated the venom proteomes of two prey-specialised spiders using a proteo-transcriptomic approach: the araneophagous white-tailed spider (genus *Lampona*) and the termitophagous sand-diving spider (*Ammoxenus amphalodes*). Our analysis revealed 208 putative toxins in the *Lampona* proteome. Most abundant lampotoxins belonged to two families characterised by unique scaffolds containing eight or ten cysteine residues. We also showed that *Lampona* venom is more potent against spider prey than alternative cricket prey. Similarly, we identified 116 putative toxins in the venom proteome of *A. amphalodes*. The most abundant venom components were six cysteine-rich ammotoxins belonging to family 1, comprising over one-third of the venom proteome transcripts. These findings are congruent with the hypothesis that a few structurally similar toxins dominate the venoms of predators with narrow diets. In the future, prey-specific toxins could help develop bioinsecticides targeting only focal pests.

Keywords: adaptation; araneophagy; prey; specialisation; toxins; termitophagy; venom composition; venom potency

What is animal venom? Reassessing a manipulative weapon

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Current definitions of venom emphasize mode of delivery and physiological disruption of victims. While these are legitimate aspects of venom, we argue that a biologically and evolutionarily more fundamental definition should center upon the adaptive purpose of venom. Venoms are adaptations that have evolved to fulfil specific functions, such as predation and defense, which enhance the fitness of venomous animals. The diverse adaptive effects of venoms that serve these functions, such as paralysis and pain, are extended phenotypes that happen outside the bodies of venomous animals. These extended phenotypes are united in attaining their adaptive value by manipulating the physiological functioning of victims, typically to their detriment. By viewing venom as a manipulative weapon that is deployed in arenas of organismal conflict, we highlight two areas of biology that hold great promise for venom researchers: sex and phytophagy. First, we argue for the existence of sexual venoms that are used to manipulate sexual partners against their interests to gain a reproductive benefit. Second, we argue that the sap-sucking and gall-inducing feeding secretions of diverse invertebrates are phytophagous venoms that are striking functional analogues of hematophagous venoms. This new perspective considerably expands the roster of venomous animal species, and urges the conceptual and terminological unification of several previously unconnected fields of research.

Lacewing venom: Detailed insights into the venom system of a neglected group of venomous insects

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Venom has independently evolved across many lineages, but only a few of these have been studied in detail. This is especially true for insects—one of the most species-rich groups of eukaryotes. One of the unexplored venomous lineages is the insect order Neuroptera, which are ubiquitous and include iconic species such as antlions and lacewings. While adults are non-venomous, neuropteran larvae are ferocious predators that have evolved pincer-like mouthparts from which they administer paralyzing and liquefying venom to tackle and ingest prey. Here, we present the first detailed insight into the venom system of Neuroptera through the widely distributed and agriculturally important common green lacewing (*Chrysoperla carnea*; Stephens, 1836). Using high-quality genomes from a male and female, long-read-based reference transcriptomes from their offspring across each life stage, and venom proteomics, we provide a comprehensive description of a neuropteran venom, including toxin gene families, paralogs, alternative splice variants, and allelic variation. While many of the toxin families comprise proteins convergently recruited into the venoms of other venomous lineages, we find few examples of large toxin gene family expansions, which is surprising given their prevalence in other venomous lineages. Further, we show that the only previously characterised toxin described from Neuroptera (a bacterially derived chaperonin) is not a significant venom component. Instead, the most abundant toxin is an insecticidal toxin (Tc) like protein secreted via a non-canonical secretory pathway, obtained by horizontal gene transfer (HGT), and likely facilitated by an endosymbiont—further highlighting horizontal gene transfer as a source of functional innovation in venomous animals. Our results provide new insights into the uncharted arsenals of venomous insect lineages, contrasting the canonical mechanisms of venom evolution and highlight the importance of studying neglected taxa for understanding mechanisms involved in the evolution of molecular novelties.

Purification and partial characterization of a novel Phospholipase A2 enzyme from the Yığılca Honeybee (*Apis mellifera L.*) venom of Türkiye

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Bee venom consists of numerous bioactive molecules, including phospholipase A2 (PLA2), a lipolytic enzyme that hydrolyzes the ester bond at the sn-2 position of membrane phospholipids. Recent studies indicate that bee venom PLA2 stimulates regulatory T-cell growth, making it a promising molecule for various immune system-related disorders.

In this study, crude bee venom was fractionated using High Performance Liquid Chromatography (HPLC) with two different chromatographic mechanisms. Twelve fractions obtained through ion exchange chromatography were further fractionated by reverse-phase HPLC. The phospholipase A2 activity in the crude venom and fractions was determined using the egg yolk-agarose method. The purified fraction with PLA2 activity, having a retention time of 34.4 minutes, showed a single band between 14,2 kDa and 17 kDa in SDS-polyacrylamide gel electrophoresis. The amino acid sequence of the first thirty-four amino acid residues at the N-terminal of the pure PLA2 was determined by Edman degradation.

In conclusion, this study demonstrates that with the use of advanced fractionation techniques such as ion exchange and reverse-phase HPLC, the PLA2 was successfully isolated and characterized. The identification of its molecular weight and N-terminal amino acid sequence underscores its purity and potential therapeutic value. These findings pave the way for further research into the application of bee venom PLA2, highlighting its promising role in medical science.

Keywords: Bee venom, Egg yolk, Phospholipase A2, Purification, Characterization.

The repeated evolution of spider venom double-knot neurotoxins

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Bivalency is a well-known but poorly understood natural mechanism for improving the potency and selectivity of intermolecular interactions. Bivalent peptides are particularly prevalent in the cysteine-rich venoms of spiders, contributing to the high selectivity and potency of various functionally diverse peptides, primarily described in Mygalomorph spiders. However, our understanding of the evolutionary processes that drive and constrain the evolution from single- to multi-domain protein architectures remains limited. Here, we show that bivalent toxins featuring the inhibitor cystine knot (ICK) motif have independently emerged on numerous of occasions across different spider lineages. Leveraging transcriptomic data from the Sequence Read Archive (SRA), we generated *de novo* transcriptome assemblies from all available Mygalomorph spiders, in addition to 118 venom gland transcriptomes from a variety of lineages across the spider tree of life. We identified several novel double ICKs across distantly related spider lineages that exhibit homology with the single ICKs of only one of their two domains, indicating that there has been a diversification of different double ICKs. Our findings suggest that the evolution of bivalent peptides is a recurrent pattern of evolutionary innovation, shedding light on less investigated pathways through which peptides gain new functionalities.

Toxin Repertoire of the False Black Coral *Savalia savaglia* from Mediterranean Mesophotic Zone Revealed by RNAseq Approach

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The zoanthid *Savalia savaglia* is a sessile cnidarian found in the mesophotic zone of the Mediterranean Sea and eastern Atlantic Ocean, typically at depths of 15-90 m, though it can extend to 900 m in the Mediterranean. Also known as the gold coral or false black coral, this species is notable for forming a hard, layered, proteinaceous skeleton on the stems of gorgonians or antipatharians. Despite advances in high-throughput OMICS techniques for characterizing toxins in cnidarians, knowledge about the composition and function of toxins in *S. savaglia* is lacking, like in most zoantharians, which were addressed in a limited number of studies only. Herein, we profiled *S. savaglia* with a total RNAseq approach from colonies collected at 38 m depth in the Banco di Santa Croce, Gulf of Naples, Italy, revealing its toxin repertoire. To maximize the recovery of transcripts, we integrated two assemblies obtained with Trinity v2.15.1: a de novo paired-end (PE) transcriptome, assembled using around 60 million high-quality reads in forward mode, and a single-end (SE) assembly using the unpaired forward reads. Through a preliminary toxin characterization using DeTox, we identified around 800 and 100 putative toxins from the PE and the SE assembly, respectively. These putative toxins were further manually screened, allowing us to recognize several known toxin folds in the repertoire of *S. savaglia*. Our analysis revealed a predominance of well-characterized cnidarian toxin domains, including Astacin, Trypsin, Kunitz, and ShK. Additionally, a collection of cysteine-rich secreted peptides were identified, suggesting the presence of potentially novel toxins. This study represents the first characterization of *S. savaglia* transcriptome, providing insights into its toxin repertoire and constituting a useful resource for further studies on the feeding habits and ecological interactions of this peculiar mesophotic habitat-building species.

Comparison of venom efficacy of euryphagous and ant-eating spiders against different prey

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Predatory venom is an adaptive trait that has evolved independently in many different taxa. It is a mixture of various compounds, such as small molecules, peptides and proteins that allow predator to immobilise different prey species. Recently it was found that venom composition of spiders is adapted to their prey. We tested a hypothesis that the venom of ant-eating spiders is more potent against ants than the venom of euryphagous species and against alternative prey. We used the following species of spiders: *Ariadna* sp., *Eusparassus* sp., *Gnaphosa* sp., *Philaeus* sp., and *Hogna* sp. We extracted crude venom from these spider species by milking. We injected the crude venoms of various concentrations into ants and flies. We recorded the paralysis latency and mortality and compared them among spider species and prey types using logistic regression. Then we performed proteomic profiling using MALDI-TOF and gel electrophoresis. We found that the venom of different species is distinctly effective for different prey species.

From life-threatening poisons to life-saving cardiovascular drugs

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In the Greek world, the God of medicine, Asclepius, and his daughter Hygieia, the goddess of health were depicted with a snake coiled around either a rod or a bowl, still emblems of medicine. The ancient Greeks had, probably, already understood that life-threatening poisons, such as those of snakes, could be exploited for healing purposes.

Several centuries later, some pharmacological tools, cardiovascular therapeutic and diagnostic agents were developed from venom toxins, able to target critical pathways in vessel tone, blood coagulation, and platelet aggregation.

The antihypertensive captopril and enalapril, the antiplatelet tirofiban and eptifibatide, and the defibrinogenating batroxobin are some examples of currently approved toxin-based medicines. Moreover, several promising agents, like the fibrinolytic alfineprase, failed during the late stage of clinical development due to adverse effects, lack of efficacy, and dose-limiting toxicity while others are now under investigation.

Emerging and well established technologies, including high-throughput screening, omics approaches, and computational modeling, are revolutionizing the discovery of novel targets and mechanisms of action for animal toxins. These innovations will not only enhance the efficacy and specificity of existing drugs but also uncover new weapons in the venom arsenal.

Black widow spider envenomation in Albania (*Latrodectus tredecimguttatus*): Hospital Case Studies and Local Knowledge

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Black widow spiders, *Latrodectus* Walckenaer, 1805, comprise 35 species worldwide, nine of which are present in Europe. Only *Latrodectus tredecimguttatus* is found in Albania. These spiders are medically significant due to their potent neurotoxin α -latrotoxin, which can be life-threatening if untreated. Case studies have shown the higher severity of envenomation among local populations, particularly the agricultural workers who are in close contact with black widows. While the public health impact of black widows has not been fully assessed all over Albania, our observations in the Western Lowland provide valuable recommendations for the local community.

An intensive study was conducted from January to April 2024, gathering a decade of clinical case studies from 2013-2023 in the hospitals of the municipalities of Berat, Fier, Lushnje, Shkodër, and Vlorë. We collected 238 hospitalized case studies, with data on gender, age, location, bitten location, time at the emergency, hospitalization, and discharge date, symptoms, and alternative treatment. Furthermore, a comprehensive questionnaire was conducted during May and June 2024, in the same areas to assess the local population's knowledge and precautionary measures regarding black widow spiders. The survey included responses from 532 individuals. Findings indicated varying levels of awareness and preparedness among the population, highlighting gaps in knowledge and preventive practices. This information is crucial for developing targeted educational programs and public health interventions to mitigate the risks associated with black widow envenomation. Additionally, we concluded with extensive field work searching for black widows in late June and early July between 10:00-15:00 and 18:00-20:00 in areas and villages with higher numbers of bitten patients. Positive findings were reported in the villages of Divjakë, where several adult and subadult black widows were identified within lower vegetation like bean and watermelon plants.

The results indicate that the local community of Divjakë municipality is at high risk due to the high abundance of black widows. Conversations with the local community and medical staff revealed some untreated cases of severe envenoming. Therefore, precautions are needed to increase awareness and avoid contact with these spiders.

Keywords: Black Widow Spiders, *Latrodectus tredecimguttatus*, Envenomation, Public Health, Agricultural Workers, Field Survey

The Architecture of Cephalopod Venom Systems: A Histological and Developmental Approach

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Venom systems have independently evolved multiple times throughout the animal kingdom, exhibiting remarkable diversity and complexity across biochemical, physiological, and histological levels. These systems employ various strategies and serve multiple purposes, making them particularly intriguing from a pharmaceutical perspective due to their potential as potent, target-specific drug candidates that elicit rapid and robust physiological responses.

Mollusks possess some of the most ancient marine venom systems, with over half a billion years of evolutionary history characterized by adaptation, selection, and diversification. Cephalopods, a diverse class of mollusks comprising over 900 species, have developed sophisticated venom systems that play critical ecological roles, functioning as both predators and prey. Despite their ecological and pharmaceutical significance, cephalopod venom systems remain underexplored, representing a vast, untapped resource for understanding toxin diversity and potential drug discovery.

Our project focuses on the histological assessment of cephalopod venom systems, examining cell types, distribution, functional relationships, and gene expression patterns with spatial resolution through comprehensive imaging analysis across various developmental stages. By investigating these aspects, we aim to elucidate the architecture, complexity, and diversification of venom secretory tissues and to understand venom production from both histological and physiological perspectives.

These insights will establish a foundation for defining a model of cephalopod venom systems, incorporating venomous, physiological, genetic, and ecological data. Integrating this data will allow us to differentiate between generic structures across the class and identify unique patterns within the orders of cuttlefish, squid, and octopus, as well as potential evolutionary triggers for diversification. Our work will significantly advance marine toxinology by providing a detailed understanding of ancient venom systems optimized over millions of years for function, secretion, and cost-efficiency.

In addition, venomous fishes, comprising nearly 3000 species, have evolved under similar conditions and timeframes, sharing compositional and defensive-purpose-related pharmacological aspects with cephalopod venom. Thus, a comprehensive understanding of marine invertebrate venom systems will enable comparative studies with marine vertebrate systems. This research will offer broader insights into venom evolution and pave the way for the discovery of novel therapeutic agents derived from marine natural resources.

Keynote Speaker: Richard J. Lewis

Developmental shifts in venom composition underlie the evolution of mollusc- and fish-hunting cone snailsAymeric Rogalski¹, Thao N.T. Ho¹, Brett Hamilton², Richard Webb² and Richard J. Lewis¹¹*Institute for Molecular Bioscience, The University of Queensland, Brisbane, 4072, Queensland, Australia.*²*Centre for Microscopy and Microanalysis, The University of Queensland, Brisbane, 4072, Queensland, Australia.*

Marine cone snails have attracted researchers from all disciplines but early life stages have received limited attention due to difficulties accessing or rearing juvenile specimens. Here, we document the culture of *Conus magus* and *C. textile* from eggs through metamorphosis to reveal dramatic shifts in predatory feeding behaviour between post-metamorphic juveniles and adult specimens. Adult *C. magus* capture fish using a set of paralytic venom peptides combined with a hooked radular tooth used to tether envenomed fish, while early juvenile *C. magus* feed exclusively on polychaete worms using a unique “sting-and-stalk” foraging behaviour facilitated by short, unbarbed radular teeth and a distinct venom repertoire that induces hypoactivity in prey. In contrast, early juvenile and adult *C. textile* prey on molluscs. Transcriptomics and mass spectrometry revealed that juvenile and adult VG proteomes were dominated by distinct suites of peptides. To evaluate the pharmacological potential of novel sequences identified, we used sequence homology and AlphaFold to identify likely bioactive peptides to synthesise for evaluation across selected high throughput screens. Our results demonstrate how coordinated morphological, behavioural and molecular changes facilitate the shift from worm- to fish-hunting in *C. magus* and the evolution of juvenile molluscivory. These data showcase juvenile cone snails as a new and unexplored source of novel venom peptides for biodiscovery.

POSTER

Comparative Proteomic Analysis of Venom from Three Snake Species: Evaluating Software-Specific Protein and Peptide Profiles

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Snake venom has gained increased recognition in biomedical research as a potential source of novel and medically relevant proteins, which remain relatively unexplored across different species. In this study, we conducted a proteomic quantification and identification of the venom profiles of three snake species: *Montivipera bulgardaghica* (MB), *Vipera ammodytes montandoni* (VA), and *Vipera kaznakovi* (VK). We compared the performance of three peptide identification software tools: PEAKS, MaxQuant, and Proteome Discoverer. PEAKS identified 19 unique proteins (19 in MB, 11 in VA, and 19 in VK) and 125 unique peptides (55 in MB, 35 in VA, and 63 in VK). MaxQuant identified 577 unique proteins (234 in MB, 275 in VA, and 297 in VK) and 1,233 unique peptides (518 in MB, 647 in VA, and 642 in VK). Proteome Discoverer identified 621 unique proteins (310 in MB, 248 in VA, and 346 in VK) and 1,657 unique peptides (894 in MB, 830 in VA, and 1,041 in VK). The three software tools shared 5 identified proteins and 67 peptides. PEAKS shared 6 proteins and 69 peptides with MaxQuant, and 6 proteins and 79 peptides with Proteome Discoverer. MaxQuant and Proteome Discoverer shared 139 proteins and 781 peptides. All identified proteins were categorized into families for each species and compared to existing literature, revealing significant discrepancies between software outputs and previous studies. Overall, PEAKS performed the least effectively, while MaxQuant and Proteome Discoverer excelled in both protein and peptide identification, with Proteome Discoverer standing out for its highest number of identifications.

What we have discovered about *Naja ashei* venom so far...

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Naja ashei, the largest African spitting cobra, is distributed across Kenya, Ethiopia, Somalia, and Uganda. Initially considered a morphologically distinct variant of *Naja nigricollis*, molecular analyses revealed a closer relationship with *Naja mossambica*, a Mozambican spitting cobra. Since 2007, it has been recognized as a separate species. Our team has been uniquely dedicated to studying the proteomic composition of *Naja ashei* venom for several years. We employed various venom separation methods (2D electrophoresis [1], ultrafiltration [2], ion exchange chromatography [3], and SEC chromatography [4]) and mass spectrometry techniques (MALDI ToF and LC-MS). Early analyses indicated that, similar to other cobras, phospholipases A2 and 3-finger toxins are the major components of *N. ashei* venom. All methods also identified secreted venom metalloproteinases (SVMs), cobra venom factor (CVF), cysteine-rich secretory proteins (CRISPs), and venom nerve growth factor (VNGF). With increasing venom fractionation, we discovered new protein groups, including serine and cysteine proteases, L-amino acid oxidases, and antimicrobial peptides. Notably, our team identified an uncharacterized group with immunoglobulin domains, whose function remains unknown. Conversely, increasing the purity of fractions for abundant protein groups revealed numerous new peptides. This study highlights that for complex proteomes like snake venom, the picture is significantly influenced by both protein fractionation methods and mass spectrometry data analysis, especially when protein groups vary considerably in abundance.

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SF-mi2 conotoxins: Diving into the world of EGF-like toxins

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EGF-EGFR (Epidermal growth factor – EGF receptor) signaling pathways are highly conserved and present in both vertebrates and invertebrates. There is great medical value in finding high-affinity EGFR ligands, since EGF signaling is involved in many important processes, including neuronal development, pain, hair growth, tumor progression. Conotoxins of the gene superfamily mi2 (SF-mi2) comprise a signal sequence starting with MMSTT and a predicted EGF-like domain. To resolve the structure of an SF-mi2 conotoxin and find a target, SF-mi2 EGF-like conotoxins have been recombinantly produced as fusion proteins with a His₁₀ (10 poly-histidine) tag using a cytosolic *E. coli* expression system and purified using immobilized metal affinity chromatography, tobacco etch virus protease cleavage and gel filtration. SF-mi2 EGF-like toxins from vermivores cone snails, such as *Conus litteratus*, have high sequence identity to a hypothetical endogenous protein in the marine annelid *Capitella teleta*. The predicted AlphaFold structures of the EGF-like conotoxins also show structural homology with the predicted structure of this endogenous EGF-like protein in *C. teleta*. The poster will visualize the results from experimental work in producing pure SF-mi2 EGF-like conotoxins, as well as the data from the bioinformatic search for a doppelganger-related peptide (DREP) in the annelid prey and the AlphaFold structures of the SF-mi2 conotoxins and endogenous DREP EGF-like protein. Understanding the SF-mi2 EGF-like conotoxins and their target receptor may reveal its function in marine annelids. Finding a target would tell a story of the evolution of the SF-mi2 EGF-like conotoxins and provide insight into its potential application in research and medicine.

In silico synthesis of melittin determined by proteomic sequencing from *Apis mellifera anatoliaca* venom, which has lipotoxic potential in skin cancer cells

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Bee venom has traditionally been used for various medical applications. Specifically, melittin is an important invaluable medium-length peptide that occurs in bee venom that displays great pharmaceutical potential and presents several structural differences depending on the genus and flora. Türkiye has a rich diversity of honeybee species, which could pioneer the development of melittin-derived drug candidate peptides with pharmaceutical potential including against cancer. In this study, bee venoms were collected from the apiary located entirely in an eucalyptus forest. Crude bee venoms containing melittin were tested on healthy human neonatal foreskin fibroblast (NFF) and melanoma SK-MEL-103 and MM96L human cells by MTT assay for cytotoxic potential. Additionally, the cell line was stained with oil red and imaged with a fluorescence microscope to investigate lipid accumulation. The results showed that crude bee venom has significantly higher cytotoxic potential on skin cancer cells than healthy cells at a concentration of 5 µg/mL and increased lipid accumulation at a concentration of 3 µg/mL. Based on the results, the primary structure of the venom melittin was determined by proteomic analysis and reproduced *in silico*. Future experiments will confirm whether melittin was responsible for the observed cytotoxicity in melanoma cell lines as well as its underlying molecular mechanism.

Key words: bee venom, proteomic, melittin, in silico, skin cells, lipotoxic

Genomic and proteomic analysis of melittin structure of different honeybee species from Türkiye and Cyprus; Venom and venom gland samples

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Western honeybees (*Apis mellifera mellifera*) are social insects belonging to the family of Hymenoptera. Türkiye has a rich diversity in terms of speciation, that exhibits 5 pure honeybee species (*Apis mellifera anatoliaca*, *Apis mellifera carnica*, *Apis mellifera caucasia*, *Apis mellifera syriaca* and *Apis mellifera meda*) and dozens of ecotypes. Besides, Cyprus island has the endemic species *Apis mellifera cypria*. In the present study, venom and venom gland samples of different honeybee species were collected from different territories, genomic and proteomic sequences of melittin as well as the major proteins of the venom were studied comparatively. The results revealed that melittin was O-glycosylated and appeared in varying abundances across species, which is significant for pharmaceutical applications.

In conclusion, the discovery of the structural variations of melittin conducts high potential for pharmaceutical use and might lead to development of new drug candidate proteins.

Key words: bee venom, venom gland, melittin, genomic, proteomic

Bioinformatic Analysis and Experimental Characterization of Novel Pore-Forming Proteins from Mollusca with Potential for Nanopore Sensing

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Pore-forming proteins (PFPs) are abundant in all kingdoms of life. Despite their structural and functional diversity, they share one key feature: they are initially synthesized as water-soluble monomers, which undergo conformational rearrangement and oligomerization upon binding to lipid membranes, leading to pore formation. The assembled pores vary both in size and selectivity, which makes PFPs a valuable tool in the field of nanopore sensing. As the demand for the detection of clinically important molecules is increasing, new protein nanopores with unique characteristics are needed to offer sensing with high sensitivity and specificity.

We have performed a thorough bioinformatic analysis of PFPs from mollusks, which are a rich source of PFPs. Using the BLAST suite, we found 1340 PFP-like sequences from genomic and transcriptomic databases and aligned them with the sequences of known PFPs. Based on the alignments, we chose sequences with interesting features (e.g. insertions, gaps or extensions at either termini) and modeled them with AlphaFold3 to compare the predicted structures with the ones of known PFPs. Using this approach, we obtained 36 interesting PFP sequences, the majority of which showed similarity to the actinoporin and aerolysin families.

For the experimental characterization, we have selected 15 proteins similar to actinoporins, which are a well-known family of PFPs where the membrane-spanning region is an α -helix. We have expressed all 15 actinoporin-like proteins (ALPs) in *Escherichia coli* and tested the hemolytic activity of the obtained cell lysates on bovine red blood cells (RBCs). Our preliminary results show 6 lysates containing the expressed ALPs to be hemolytic within the first 20 minutes of red blood cell addition, while an additional 6 show mild to moderate hemolytic activity when incubated with bovine RBCs overnight.

Characterization and design of actinoporin pores for the detection of small proteins

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Actinoporins are a family of α -pore-forming toxins from sea anemones. They bind specifically to lipid membranes containing sphingomyelin, where they oligomerize and form oligomeric pores. The pores have a unique funnel shape with a constriction in the range of 1-2 nm. They are suitable for nanopore sensing, an emerging technology that can be used to detect and characterize individual molecules in real time. Potential applications of this method include DNA/RNA/protein sequencing, protein detection and direct or indirect detection of small molecules, such as metabolites, biomarkers, drugs etc. The detection of each analyte requires a nanopore whose biophysical properties such as charge or size are complementary to their own. We are interested in protein nanopores formed by Fav, a newly discovered actinoporin-like protein from the coral *Orbicella faveolata*. We used cryo-EM for high-resolution structural characterization of the octameric Fav pore and designed Fav variants that form pores with greatly improved biotechnological potential. By mutating the outside of the pore, we were able to produce a stable nonameric pore with a larger diameter. By altering the length of the transmembrane helices, we designed pores that stably insert into lipid and polymer membranes, making them suitable for use in high-throughput devices such as Oxford Nanopore Technologies' MinION. The electrostatic potential of the Fav pore channel is highly negative, making these pores perfect for the uptake of positively charged analytes. As proof of principle, we have detected the medically relevant full-length histone proteins H4, H3.1 and their post-translationally modified variants. Furthermore, using machine learning, we were able to quantify and distinguish two important extracellular histones H4 and H3Cit in mixtures. As histone proteins are increasingly emerging as potential biomarkers, our results represent a new step towards the development of a rapid, accurate single-molecule method for real-time monitoring of histone proteins in human body samples.

Human stem cell-derived hepatocyte-like cells in a microfluidic device: an improved platform for assessing venom toxicology

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Human-based hepatic in vitro models are fundamental for studying venom pharmacology and toxicology. Human stem cell-derived hepatocyte-like cells (HLCs) represent an alternative tool to address the limitations posed by human primary hepatocytes. Still, differentiating HLCs in 2D systems results in an immature phenotype. Microphysiological systems (MPS) are described to better mimic liver microenvironment, namely hepatocyte organization and fluid flow, improving the hepatic cell functionality. The aim of this work was to create a MPS suitable to maintain functional HLCs employing microfluidic technology. Yet, moving to microfluidic devices (MD) is challenging. Here, we reported the optimisation of the MD design, cell inoculation procedure and coating. The MD was fabricated by photolithography and soft lithography techniques, based on polydimethylsiloxane (PDMS) molding. 3 MD designs were tested: 2 square-shaped MDs, with 3 or 7 inlets, and a rectangular MD with 2 triangular segments and 3 inlets. As such, at day 17 of differentiation, cells were transferred to the MD (1×10^6 cells/mL) sealed against polystyrene dishes, operated under perfusion (0.2 μ l/min) or maintained in traditional static 2D culture plates (2×10^4 cells/cm²), as control. The optimised square-shaped MD design with 7 inlets sealed against a collagen-coated polystyrene surface allowed for a homogeneous cell distribution with an hepatic like morphology. HLCs could be maintained up to 10 days under perfusion, presenting the hepatic markers HNF-4a, CK-18, OATP-C and MRP2, as well as increased ammonia detoxification ability. Importantly, HLCs displayed enhanced phase II biotransformation competence in the MD, as shown by the increased formation of diclofenac glucuronidation products, associated with UGT2B7 activity. Thus, this work highlights the potential of HLCs-derived MPS for venom toxicology and pharmacology studies.

Venom Proteins in Robber Flies: Determination of Electrophoretic Profile of *Machimus setibarbus* Loew, 1849 (Diptera: Asilidae) Venom and Its Enzymatic Activity

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Machimus setibarbus Loew, 1849 (Diptera: Asilidae), a member of the robber flies, is a strong, aggressive predator fly from 18-27 mm in length and colored grey. In species identification, key distinguishing characteristics include the width of the basal part of the male genitalia's dystistylus, the concave notch on its ventral edge, and its pointed apex. The type locality is Asia Minor, from Scandinavia to the Mediterranean and east to Central Asia. In Anatolia, it is seasonally active in July. It prefers habitats such as forest edges, meadows, shrublands, and rocky areas.

In this work, *Machimus setibarbus* species of robber flies were collected in Eskişehir, Türkiye. Crude venom was extracted using electrostimulation at 12V. The soluble portion of the venom was subjected to electrophoresis to obtain an electrophoretic profile. The crude venom was tested for phospholipase A2 activity using the egg yolk-agarose method. In SDS-polyacrylamide gel electrophoresis, several protein bands were observed such as a broad band from 130 to 175 kDa, and a thin band from 95 to 130 kDa. Also, three protein bands from 60 to 95 kDa including a large one, and another cluster with four protein bands from 52 to 66 kDa with a significant protein band were noticed. Additionally, a group of five bands from 37 to 57 kDa with a broad protein band, a quartette of thin protein bands from 30 to 37 kDa, and finally, numerous bands below 30 kDa forming a rough cloud-like appearance were also seen. Among the observed bands, proteins with phospholipase activity have been identified in this crude venom.

In brief, this work investigates the venom proteins of the robber fly *Machimus setibarbus* (Loew, 1849) focusing on its electrophoretic profile and enzymatic activities. Throughout electrophoresis, a profile of the protein size of the venom was obtained, revealing numerous proteins across various molecular weights. Notably, the venom exhibited phospholipase A2 activity, with expected specific peptides responsible for this activity identified among the protein bands. These findings provide valuable insights into the biochemical properties of *Machimus setibarbus* venom, highlighting its potential for further biochemical and pharmacological investigations.

Keywords: *Machimus setibarbus*, venom, phospholipase A2, electrophoretic profile

Combating Multidrug Resistance of *Pseudomonas aeruginosa* with a *Naja ashei* Venom-Derived Antimicrobial Peptide

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Pseudomonas aeruginosa (PA) is a Gram-negative bacterium considered an opportunistic pathogen, meaning it primarily causes infections in individuals with compromised immune systems or underlying health conditions. Growth inhibition of PA is extremely difficult due to rapid mutations and its adaptation to gain resistance to antibiotics. Importantly, of all bacterial biofilm aggregates that infect chronic wounds, those of PA are the largest, since PA in biofilm states may survive in a hypoxic atmosphere or other extremely harsh environment. This study explores the antibacterial, including antibiofilm, efficacy of a novel peptide designed based on a tryptic peptide derived from the CRISP protein from *Naja ashei* venom. The AMPAB5 peptide was synthesized by the SPPS method obtaining 24 amino acid peptide with a mass of 2660.16 Da. The obtained peptide shows a hydrophobicity of 42% and a net charge + 4, GRAVY (the Grand Average hydropathy value of the peptide) = 0.121; the Wimley-White whole-residue hydrophobicity of the peptide = 2.96, and Protein-binding Potential (Boman index) = 1.7 kcal/mol. The AMPAB5 peptide was evaluated through a series of *in vitro* experiments, focusing on its antibacterial properties against certified and clinical strains of PA. The serial dilution method enabled the determination of minimum inhibitory concentrations (MIC) against certified PA strain allowing us to determine the MIC value of 93.75 µg/mL, while against PA clinical strains it was between 46.87 and 187.50 µg/mL. The combinational effect of AMPAB5 and chloramphenicol (CHL) on the growth of PA strains was assessed by checkerboard assay and revealed that simultaneous use of AMPAB5 and CHL reduces the concentration of antibiotic necessary to inhibit PA growth 8-fold. Additionally, with the use of MTT, the inhibition of bacterial biofilm formation by AMPAB5 was assessed. It showed that the presence of tested peptide in a bacteria environment reduces the level of the formed biofilm depending on the AMPAB5 concentration. No toxicity of novel peptide on normal human fibroblasts was evaluated by neutral red assay. In conclusion, the antimicrobial peptide from *Naja ashei* venom presents a promising therapeutic candidate for enhancing the sensitivity of PA with its potential for safe application in clinical settings.

Development of a custom-made bioinformatics tool for the automatic analysis of cone snails' transcriptomes

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Transcriptomics is a major discipline within Venomics. However, there is no scientific consensus on the automatized bioinformatics process of filtering the results obtained from the sequencing. Therefore, we have developed a custom-made bioinformatics methodology which we have named as Transcripto-filter that allows the automatization of the pipeline from the assembly of the raw reads obtained from the sequencing platform to the end. Transcripto-filter consists of the following tools publicly available at <https://github.com/schuman94/transcripto-filter>: Curation-filter consists of a sliding window algorithm that allows the filtration of the candidate sequences; Methionine-filter classifies the candidate sequences depending on the coincidence of the first methionine with respect to the reference sequence; SF-filter carries out a BlastX against a custom-made database containing a representative selection of the conotoxins organised by superfamilies.

This novel tool has been successfully applied to the study of two closely-related cone snail species: *Rhombiconus imperialis* (Linnaeus, 1758) and *Rhombiconus fuscatus* (Born, 1778). We have been able to identify 164 conotoxins using Transcripto-filter, 131 of them have been also identified by proteomics studies. We have compared our results with the two published transcriptomes of *R. imperialis*: 56 previously published sequences were detected and 108 new conotoxins have been described for the first time, which validates the pipeline developed in this work.

This bioinformatics tool greatly reduces the time used in the annotation of the transcriptomes. Curation-filter has discarded more than 80% of the initial alignments. Moreover, the classification performed by Methionine-filter and SF-filter reduced the manual revision of the initial alignments to only 5% of them. Even though Transcripto-filter has been originally developed for the study of cone snails it could be adapted to the study of other venomous organisms.

***Macrovipera lebetina lebetina*'s venom
Exploring its cytotoxic and antibacterial effects**

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Venoms are a rich source of compounds ranging from small organic molecules and peptides to proteins which have attracted the researchers' interest to find new chemical compounds with therapeutic applications. *Macrovipera lebetina lebetina* is an endemic Cypriot subspecies possesses a venom that has not been widely studied, and which precise composition remains to be determined. The crude venom is tested on several cancel cell lines.

Here we present the current bioassays performed with the crude venom on HEK293T and MDA-MB231 cell lines which already highlight promising results regarding the cytotoxicity of the venom and its anticancer potential. Furthermore, the crude venom is tested against *Escherichia coli*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae* and *Neisseria subflava* with significant results which demand further investigation. These results could not only deepen our understanding of the composition of *M. lebetina lebetina*'s venom, but also pave new pathways for the development of innovative therapeutic treatments. In addition to our studies with the crude venom, we further identified several peptides. Among them, a characteristic tripeptide pGlu-Lys-Trp occurring in most of the *Macrovipera* species venoms. This peptide has been further synthesized in both its native and amide form and is currently studied for its physicochemical properties, exploring its role in the venom.

Is there a difference in the clinical aspects of scorpionism between regions? A systematic literature review and meta-analysis

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Envenoming by medically important scorpions (scorpionism) represents a major public health issue in North Africa and globally. It is often assumed that scorpion envenoming cases produce a consistent suite of largely autonomic symptoms, ranging from frequent, mild, local symptoms to rare, severe, life-threatening pathology. However, the assumption that scorpionism is a homogenous disease, with a consistent clinical presentation, is probably due to the absence of a global evaluation of scorpionism symptomology and assumptions relating to the venoms of Buthid scorpions. This lack of understanding regarding the diversity (or not) of the scorpionism pathology in the clinic could have significant implications for the effective treatment of scorpionism, as well as the priorities of preclinical research. Here we conduct a global systematic literature review to capture the range, incidence, and severity of the symptoms exhibited by patients presenting to a health care facility following a confirmed scorpion sting. Using logistic regressions, we test if scorpionism is a polymorphic syndrome and demonstrate how clinical presentations of scorpionism varies globally. This work will contribute to the development of more effective, specific treatments for the diverse aspects of scorpionism globally, as it facilitates a more thorough understanding of the diverse clinical aspects of scorpionism.

Investigating Animal Venom Peptides for Therapeutic Potential using Xenograft Melanoma Zebrafish Embryos

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The zebrafish (*Danio Rerio*) is a model organism that has become a valuable tool for cancer research. Although the cancer research community has mainly used mouse xenografts for decades, zebrafish provides an alternative animal model with numerous advantages. It is affordable, easy to work, allows highthroughput screening as well as real time cellular and molecular observations. Therefore, it is an attractive *in vivo* model for the xenotransplantation of human cancer cells and toxicity studies for the identification of drug candidates.

Small peptides are t main components of the animal venom and have many promising therapeutic assets. Small venom-peptides are highly stable and resistant to proteolytic degradation, with high specificity and potency towards molecular targets of therapeutic importance. Many preclinical and clinical studies have highlighted their potential for the treatment of cancer.

The Australian octopus Kaurna peptide (Octpep-1) is a small neuropeptide that inhibits tumor progression in xenograft *BRAF* mutated melanoma animal models.

In this study, we inoculated melanoma (MM96L) cells in zebrafish embryos of 48 hours after fertilization (hpf). The objective was to analyze the impact of Octpep-1 (alone or in combination with other FDA approved inhibitors) on the proliferation of melanoma cells injected into the caudal hematopoietic region. We observed a decrease of 55% in the proliferation of MM96L cells in the Octpep-1 treated animals compared to the control group (n=30; p<0.0001). In combination assays of the Octpep-1 with ERK1/2 inhibitor (Ly32), we reported a synergistic decrease in cell proliferation in comparison to the group treated with Octpep-1 alone or with the ERK1/2 inhibitor.

Yeast surface display technology for the discovery of monoclonal antibodies against snake venom toxins

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Snakebite envenoming is a neglected tropical disease, causing high rates of mortality and morbidity worldwide. Currently, antivenom is the standard treatment, however there is a pressing need for next generation antivenoms that offer improved therapeutic efficacy and safety. Here, we present early-stage data demonstrating the use of yeast surface display (YSD) technology to generate new monoclonal antibodies (mAbs) against snake venom toxins. This approach utilizes a nonimmune human library in which each yeast cell typically displays up to 1×10^5 copies of a unique single-chain antibody fragment (scFv) fused to a surface protein. ScFv are isolated through rounds of magnetic-activated cell sorting (MACS) followed by rounds of fluorescence-activated cell sorting (FACS), a high throughput screening technique. YSD is an advantageous platform utilizing eukaryotic protein-directed evolution, it is highly versatile, cheap and screening strategies are based on quantitative methods. Moreover, scFv candidates can be improved through iterative maturation processes, enriching biochemical properties such as affinity and stability. We initially targeted alpha-bungarotoxin (α -Bgtx) to validate our approach and successfully isolated diverse antibody clones. Neutralizing assays and cross-reactivity tests for these candidates are ongoing. Building on this initial selection success, we expanded our pipeline to develop antibodies targeting Ecarin – a key pathogenic toxin, belonging to the snake venom metalloproteinase family, that degrades prothrombin. Functional assays are underway to identify antibodies that can neutralise the effects of Ecarin, to potentially offer therapeutic benefits to address the global health challenge presented by snakebite.

Preclinical testing service

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The Czech Centre for Phenogenomics offers a broad portfolio of highly standardized test assays required for preclinical studies on rodents including established disease models. The experiences from standardized phenotyping enable us to run comprehensive experiments and answer key questions for drug development. Furthermore, participating in the services of the Centre for Preclinical Testing (CPT), we offer established preclinical tests under GLP regulations. Other testing is provided according to the strict internal guidelines with controlled quality.

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