Time to speak about scorpionism, a “forgotten” tropical disease

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The occurrence of medically significant scorpion stings is a major public concern in Central America, South America, North Africa, the Middle East and in India. On average, 3000 children below the age of 15 die from scorpion stings every year globally. In North Africa alone, scorpionism kills approximately 500 to 600 children per year, with up to a 14+ fold variation (0.4% - 5.9%) in lethality rate between adjacent provinces. Limb amputations, permanent nerve damages, nephropathies and pancreatitis are some of the life-changing conditions experienced by survivors but the factors influencing these outcomes are not yet understood. While serotherapy has been a precious tool to diminish the impact of scorpionism in North and central America, it has shown limited efficacy in other parts of the world, especially in the Maghreb and West Asia.

Overall, little is known about the drivers of scorpion sting-related morbidity, and scorpionism remains a “forgotten” tropical disease targeting the poorest, most vulnerable communities worldwide. In 2020, the University of Galway Venom Systems Lab (Ireland), in collaboration with the Technical University of the Shannon (Ireland), the University of Liège (Belgium) and the University Ibn Zohr of Agadir (Morocco) started to investigate the ecological, social, and epidemiological factors influencing the likelihood and the outcome of severe scorpionism. Our ongoing research suggest that scorpionism is a polymorphic syndrome with significant differences in the progression of symptoms and envenomation outcomes, depending on patient profile, geographic location and the scorpion species / population involved. Here, we present our research to date, and discuss some leads to address global scorpionism.

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Spatial Venomics: On-tissue imaging of toxin genes and peptides in venomous marine invertebrates

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Our knowledge about the venom systems and toxins of many animal lineages, particularly venomous marine invertebrates, is rather limited. This is partly due to the absence of a centralized venom system with distinct multicellular venom glands in several taxa, which makes it challenging to use techniques that are commonly applied in venomics research. However, recent advancements in spatial methods, including Spatial Transcriptomics (ST) and mass spectrometry imaging (MSI) can help circumvent these obstacles. ST in particular, is a novel technology that allows the visualization and quantitative analysis of whole transcriptomes, generating gene expression maps within individual histological sections. MSI on the other hand, allows to examine peptide distribution on tissue, without prior knowledge of their identities. These novel non-targeted methods add a spatial dimension to venomics, linking specific toxins with particular morphological features, therefore providing essential functional information about venom systems. We are combining these novel technologies, with classical approaches like RNAseq DGE and proteo-transcriptomics, to characterize spatial gene expression and protein distribution patterns in poorly studied marine invertebrates. The combination of these methods allows us to recover distinct gene expression clusters linked to specific histological features and particular cellular types. Some of these clusters are characterized by an upregulation of known and putative novel toxins, which map to previously known parts of the venom system, while others correspond to previously uncharacterized venom producing tissues. Our findings suggest there is a hidden diversity of marine invertebrate toxins and illustrate the great potential of spatial methods to investigate challenging venomous organisms, such as those without distinct venom glands or where venom cannot be milked. They are excellent tools that facilitate the identification of toxins and their spatial distribution within the tissue, potentially revealing venom composition and the producing tissue simultaneously.

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From Venoms to Drugs

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A broad range of drugs used in modern era are derived from venoms. A real treasure from snakes, lizards, scorpions, spiders, and predatory marine snails comprises millions of unique peptides proteins and countless possibilities for developing life-changing therapies uncovering new targets in drug discovery. Venoms have not evolved for the purpose of treating disease in Homo sapiens, nor have the plant compounds. There are about 14 venom-derived drugs on the market starting from Captopril in 1960s, the first ever antihypertensive, to the modern Byetta and Prialt. Especially Byetta, a glucagon like peptide from the venom of the Gila Lizard, used for the treatment of Type II Diabetes, was the ancestor of a series of Glp1 agonists like Liraglutide and Semaglutide which are used nowadays not only for Type II Diabetes but also for obesity while more clinical trials are underway for other diseases.

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Enhancing Venom Research Experience at the Liverpool School of Tropical Medicine

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As a part of the Short-Term-Scientific Mission (STSM) program by European Venom Network Cost Action, I recently spent two weeks at the Liverpool School of Tropical Medicine (LSTM), where I had the opportunity to gain valuable hands-on experience in various venom research techniques. I conducted immunochemical analyses such as affinity, avidity ELISA, and Li-Cor Western Blot on different snake venoms, observed high-throughput functional analyses of venom proteins, and assisted with techniques aimed at toxin purification and protein activity analyses. Consultations with individual team members enabled expanding my knowledge and establish closer cooperation. The visit to LSTM organized within the STSM program broadened my abilities and provided insight into technical improvements that I have already been able to implement in my research. Overall, this experience was invaluable and has already positively influenced my work and career. I highly recommend the STSM program to any researcher seeking to broaden their horizons and gain immersive experience in their field.

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Venomic studies on cone snail and spiders - from venom extraction to peptide synthesis

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We transported about one dozen of live specimens of the largest europoean spider, *Macrothele calpeiana*, from southern Spain (with the necessary permits) to the laboratory of Dr. Sebastien Dutertre in the Institute of Biomolecules of the University of Montpellier. The spiders will be kept alive in Montpellier, in suitable containers. The venom of the spiders would be periodically extracted on the anesthetyzed specimens by electrode stimulation. One of the goals was to learn about the details of the experimental procedure for venom extraction, and the materials needed for carrying it out in an efficient manner. The crude venom will be used for further proteomic studies by chromatographic techniques (LC/MS and MS/MS).

Another research activity was to gain the necessary knowledge about the chemicals and hardware needed to perform the preparation by solid-phase synthesis of small peptides mimicking conotoxins identified by previous transcriptomic and proteomic studies in cone snails. This technique involves the use of a suitable resin to bind the peptide, and protection of the reacting groups by fluorenylmethyloxycarbonyl (Fmoc) in order to achieve the desired aminoacid sequence. Strategies for producing the proper folding (formation of disulfide Cysteine bridges) were addressed.

Finally, we exchanged information about the current state of ongoing joint research activities. There are preliminary data on the transcriptomes of the venom gland of the cone snail *Kalloconus pulcher* from Senegal, and the spider *Macrothele calpeiana*, which will adequately complemented by proteomic studies and modecular modeling of the peptides *in silico*.

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Testing physiological and behavioural responses of mollusc venoms in the invertebrate model *Caenorhabditis elegans*

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Venoms evolved in marine molluscs as a defence or predatory strategy to survive in the aquatic environment. However, depending on the species-specific composition and dosage, these compounds can also affect terrestrial species in a toxic or even beneficial way. In fact, the conotoxin Ziconotide allowed the development of the FDA-approved drug Prialt®, utilized for the treatment of chronic pain. The main goal of this STSM was therefore to evaluate and compare the effect of two marine mollusc venoms, the conotoxin ziconotide and the alike α-cephalotoxin, on the physiological and behavioural responses of the well-characterised nematode *C. elegans* at different time points. A further aim was to test any inhibitory effect on nociception in the worm, thus suggesting a potential role as analgesics. Due to unexpected supply issues the investigation focused on the ziconotide-like conopeptide ω-conotoxin GVIA instead. When 10µM conotoxin was administered in the food, pumping rate was not affected at any time point analysed and the animals exhibited a typical wild-type locomotion and the same was true when the stock concentration (100µM) was directly microinjected into the worms. In the latter experiment however, increased egg-laying and decreased pumping rate were observed at the early and 24h time points respectively. The effect of conotoxin on mortality was not significant even though 60% of worms died. Finally, bacterial lawn supplemented with 10mM conotoxin did not cause the worms to leave the food except for a trend at 24h. This suggests conotoxin is not an aversive sensory cue – an important consideration when assessing its potential analgesic properties.

Altogether, this data proved *C. elegans* as a valid platform for the pharmacological screening of marine venoms with key factor being the route of administration. However, future extensive experiments are needed to explore the optimal dose and incubation time at which the conotoxin can exert its potential dual action.

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Determination of the chemical content of Anatolian Bee venom samples collected from different geographical regions in Turkey

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Apitherapy is a medical complementary practice that is scientifically accepted as all the applied methods of the protection and therapeutic properties of bee products in human, animal and plant health. Bee venom is one of the bee products used in apitherapy. The use of bee venom dates to before more than 6000 years (ancient Egypt). Greeks and Romans also used bee products for medical purposes. Recent studies draw attention to the variance in the quantity of bee venom that is collected in different seasons from different regions. Considering the current and ongoing studies in our country, more research and data are needed in this field. For this reason, determination of the biochemical contents and quality characteristics of bee venom obtained from bee races and ecotypes of different geographical regions of Turkey was aimed to study.

During the STSM process, we carried out our work in coordination with Benjamin F-Hempel at his laboratory at TZR (Veterinary Centre For Resistance Research) at Freie Universitat Berlin. Within the scope of the project that we took part in, we carried out analyses of bee venom samples collected from different geographical regions of Turkey.

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Proteomics and histological assessment of an organotypic model of human skin following exposure to *Naja nigricollis* venom

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In 2017, the World Health Organization (WHO) reclassified snakebite envenoming as a Category A Neglected Tropical Disease, drawing increased attention to the affliction and prompting efforts to better understand how snake venoms exert toxic effects and how antivenoms can be used to counter them. However, most of our knowledge on this topic comes from *in vivo* animal models, which do not always accurately reflect how the pathogenic effects of snake venoms manifest in humans. Moreover, animal experiments are associated with pain, distress, and eventual sacrifice due to the toxicity of snake venoms. To address this issue, the WHO recommends implementing the 3Rs principle (Replacement, Reduction, and Refinement) in snakebite envenoming research. As such, there is a need for more humane experimental designs and alternative *in vitro*/*ex vivo* models to reduce the use of experimental animals.

Here, an organotypic model of human skin is presented that was used for investigating the dermonecrotic effects caused by the venom of the black-necked spitting cobra, *Naja nigricollis*, in humans. In summary, the organotypic model was able to recapitulate certain effects that *N. nigricollis* venom exerts in mouse model, both from a histological and a proteomic perspective.

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Identification of fluorescent compounds in the shed exoskeleton of *Mesobuthus Cyprius*

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Scorpion *Mesobuthus Cyprius* is one of the two endemic species that inhabit in Cyprus with no data available on its venom nor structural characterisation. Scorpions belong to venomous organisms which produce complex mixtures of bioactive compounds within their venom. Bioactive compounds are also expected to be found in their exoskeleton. A rare *Mesobuthus Cyprius* shed exoskeleton has been discovered in Cyprus. It was found that both scorpion and scorpion shed exoskeleton emit fluorescence under UV light. During the STSM the structural and optical properties of the scorpion shed exoskeleton were investigated, the fluorescent compounds present in the autochthon were isolated, and identified, as well as other biomolecules. The presence and distribution of fluorescent compounds in this exoskeleton was imaged and revealed essential information for the understanding of the functional morphology of these systems. Having a deeper insight of these rare species enables us to discover future applications (e.g., therapeutic, tissue regeneration) of the hidden compounds of their exoskeleton.

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