Scorpions, Science and Folklore in Durango City

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Abstract: Scorpions are incredible venomous animals found on almost every continent. According to fossil data, these animals have been able to adapt to the different environments from the Cambrian period until today with minimal anatomical changes. Scorpions are mostly nocturnal animals, and their ability to detect and tolerate light stimuli seems to be an essential tool for their subsistence, homing and mating. Centruroides suffusus is the most predominant specie of scorpions in Durango City, Mexico. Interestingly, and despite their life-threatening venom, these predatory arthropod animals have been adopted by locals as part of the landscape and daily life, by including them as part of their folklore and their economic resources, and learning how to take advantage of their abundance. In addition, the venom of scorpions possesses potential for therapeutic uses, while the scorpions themselves represent a nutritional food resource rich in protein, which has been poorly explored so far. Therefore, they are an excellent model for exploring the interplay between light sensibilities, survival and therapeutic-medicinal uses. Here, we review some of the potential benefits of scorpions and share the ways people in Durango City, Mexico, use UV light devices to detect and avoid or catch them for business and research purposes.

Keywords: Centruroides suffusus; Durango scorpion; venom; ion channels

1. Introduction

Fossil remains suggest that scorpions were among the first aquatic animals which adapted to be land-dwelling [1]. It is estimated that they have existed on our planet for more than 400 million years, and despite the multiple geographic and ecological modifications that have occurred since the Cambrian period, scorpion morphology has exhibited no major changes apart from size reduction. These kinds of arthropods have developed many mechanisms to defend themselves against predators, improved their skills to capture prey, and adapted to most ecosystems on Earth. Morphologically, scorpions possess an exoskeleton, which is an external structure that covers and protects their bodies, and it is fundamental for reducing water loss and sensing light stimuli [1,2]. These characteristics help them to camouflage themselves within the environment through exoskeleton ecomorphotype adaptations to mimic the color of the substrate on which they live [3,4]. However, the most well-known representative characteristic for which scorpions are so famous is probably their sting, which is the most common defense mechanism used when they feel threatened, and can sometimes be deadly. Their poisonous stinger is located in the tail.

The global distribution and diversity patterns of scorpions have been research topics for many scientific groups. To date, more than 2200 scorpion species, classified into 208 genera and 20 families, have been reported [5–7]. Scorpions are mainly distributed in tropical and subtropical regions around the world; however, they thrive in extreme climates,
such as arid and semi-arid ecoregions. Mexico is a country with exquisite characteristics for scorpion proliferation, and therefore their diversity is continually increasing, as new phylogenetic and phylogenomic approaches allow changes in their classification. For instance, more than 281 species have been found in Mexico, representing more than 12% of the global diversity [7–12].

1.1. Durango Society Coexists with Scorpions

In the city of Victoria de Durango, commonly known as “the scorpion city” by locals, in the state of Durango, in the northwest of Mexico (Figure 1), scorpions represent an emblematic symbol because of their abundance. Finding a scorpion in Durango is not an exceptional event, and even less so is encountering Centruroides suffusus, the most abundant species of scorpions in the state and one of the most poisonous species in Mexico [7,13,14]. While scorpions can inflict a poisonous and painful sting, no deaths have been reported across health centers and hospitals [15]. This is due to the necessity that has emerged of the population managing and living with them, as well as the access to an antidote that counteracts the effects produced by their sting.

The folklore of Durango has been remarkably influenced by scorpions, better known locally as “alacranes”. From myths and legends to the local soccer team named “Los Alacranes”, scorpions represent an insignia for the city of Durango, as well as a significant economic resource. For instance, scorpions are marketed as souvenirs in keyrings, ashtrays, and napkin holders; suspended in alcoholic drinks such as mezcal; or used as ingredients in tacos, tostadas, desserts, sweet or spicy lollipops, and ice cream (Figure 2). Exotic delicatessens with scorpions have become prevalent tourist attractions, even when, considering all species on earth, scorpions might not be considered the most edible because of their poisonous tail sting. However, if the venom gland is removed, or the animal is cooked well, there appears to be no side-effects from eating scorpions. Although the nutritional value of scorpions has not yet been fully documented, one report from Androctonus australis indicated that, among other nutrients, scorpions contain approximately 50% protein, and 100 g of shredded product contains ~300 kilocalories [16]. Therefore, scorpions may be considered as a food resource in the future, given that they can be properly prepared to preserve the nutritional value without risk of poisoning or foodborne illness.
Additionally, because of their medical importance, mostly related to venom compounds, scorpions are also bought for research purposes [7, 17-18].

As a consequence of the increasing popularity of scorpions, jobs have been generated for scorpion hunters (better known in Spanish as “alacraneros”), who catch thousands of scorpions each summer to sell in local markets as souvenirs and satisfy tourist curiosity. Additionally, because of their medical importance, mostly related to venom compounds, scorpions are also bought for research purposes [7,17,18].

Scorpions are highly abundant in the Sierra Madre Occidental, situated to the west of Durango city, where they are predominantly collected by “alacraneros”. Using a hook, the “alacraneros” move stones and wood to find scorpions hiding from the sun during the daytime. However, as scorpions prefer hunting at night, some hunters prefer to catch them in the dark. Night-time hunters use common devices that produce green-blue lights instead of white lights, as scorpions are fluorescent under ultraviolet light, as well as to avoid scaring them away. This enables “alacraneros” to identify and catch scorpions easily, keeping themselves safe from being stung. Scorpions can crawl on most surfaces; thus, when caught, they are kept in a glass container with a pierced lid to allow the scorpion to breathe. Once scorpions are captured, buyers can then decide how they will be used: dead or alive. For instance, most of the scorpions used to make handcrafted souvenirs are killed by alcohol immersion, whereas it is better to keep them alive if used for gastronomic consumption or in biomedical research.

1.2. Scorpions’ Responses to Light

Scorpions usually build a burrow which serves as a shelter from predators and protection from adverse environmental conditions. From there, they navigate around, hunting, and then subsequently returning to their shelter [19,20]. This action may seem very simple; however, it is not when considering that scorpions have a very small brain, called a protocerebrum, which, working in tandem with a basic nervous system, can sense the surrounding environment and detect whether there are any predator or prey animals nearby [21,22]. Scorpions are nocturnal animals which exhibit negative phototaxis [23]; thus, scorpions prefer hiding or building their burrows under the cover of wood or stones.

Even when scorpions come out of their shelter during the day, they prefer to stay in darker areas away from bright light sources, as they have several light detection systems. The Durango scorpion has a pair of median eyes called ocelli, positioned in the top of the head, and three pairs of lateral eyes. These scorpions also have at least one other optical structure, called an eyespot, which is a very primitive visual organ, mainly composed of light-sensitive cells called photoreceptors. The eyespot can be classified as a simple eye [24] which enables some organisms to detect light, locate shadows, and identify colors.
Furthermore, studies have shown that scorpions may have 360° vision, giving them a complete panoramic perspective [23,25]. The median ocelli can detect images at low resolution, whereas the lateral ocelli are highly sensitive to light and cannot detect images [25]. Lateral and median ocelli are sensitive to green light (~500 nm), whereas lateral eyes have dichromatic vision with peaks of sensitivity corresponding to green light (~509 nm) and ultraviolet light (~371 nm) [26]. When scorpions are exposed to UV or green light, they move quickly and sporadically to escape to shelter. Apparently, scorpions perceive light at these wavelengths as danger signs [27]. On the other hand, it is unsurprising that infrared wavelengths do not bother them, because some animals, such as vipers and bats, are known to use this wavelength as a kind of thermal vision [28]. These differences between median and lateral eyes could be related to structural changes in the photoreceptor cells; in the median ocelli, these are discrete units, while cell clusters integrating a continuous cellular network are present in the lateral ocellus, suggesting a faster and direct signal transmission [24].

The phototransduction mechanisms by which light signals detected by the ocellus are converted into information in the scorpion protobrain are unknown. Similarly to other animals, the internal anatomy of scorpions shows interconnected ocular and nervous systems [29]. Figure 3 depicts a simplified view of the scorpion nervous system (SNC), where the cephalothoracic mass (protobrain) is connected to a ventral nerve cord (analogous to the spinal cord in vertebrates), nerves, and ganglia. The SNC also processes signals from hair-like structures called trichobothria, located mostly in the tail, pedipalps, and legs. Trichobothria detect air vibrations and other environmental factors, enabling scorpions to catch aerial prey, detect predators, navigate, and improve their homing abilities [30,31].

![Figure 3. Scorpion nervous system. Graphic representation of a scorpion, where the silhouette is marked in gray and the protobrain connected to a ventral nerve cord, which runs along the length of the body, is highlighted in green. The nerves and ganglia that extend from this cord to the different bodily segments are also shown.](image)

In addition to their ocular photosensitivity, scorpions may have non-retinal photoreceptors in their tails [32]. Moreover, all scorpions emit a unique glow in response to UV light irradiation [33–35]. This peculiar phenomenon is called scorpion fluorescence, and suggests that the exoskeleton is a photon collector which absorbs energy in the UV light range and emits part of this energy in the form of blue-green light.

Other wavelengths can be used to excite the exoskeleton. Figure 4 shows the representative fluorescence pattern of a Durango scorpion exposed to light at 475 nm and the detection of emissions above 505 nm. The image reveals some non-fluorescent body features. The stinger, median and lateral ocelli, eye spots, denticles either from mandible (located at chelicerae), or the fixed fingers (movable and immovable) on pedipalps did not
flouresce under 475 nm excitation. Interestingly, fixed fingers are an excellent source of
taxonomic data, with size and dentition patterns used to distinguish animals [35–37]. Non-
fluorescent denticles (or light-guiding denticles) have also been reported in shark species
expressing regional skin biofluorescence and visually detectable biofluorescence [38].

Figure 4. Scorpion body fluorescence. A 16-megapixel camera was used to photograph a Durango
scorpion’s full body, exposed to a blacklight blue fluorescent lamp (Wildfire SableLux® BLB lamp
with a peak wavelength of 368 nm). To obtain a better resolution of nonresponsive body parts, the
animal was exposed to a light beam of 475/505 nm (λex/λem), and multiple sequential images were
taken, using the 5 × objective of an epifluorescent-inverted microscope (Nikon ECLIPSE TS2) coupled
with a digital camera (Infinity 3 Lumenera); then, a panorama collection was assembled with PTGui
12.13 software. Circles show: (A) the stinger at the end of tail; (B) median ocelli on the middle
of the head; (C) lateral ocelli and eye spot (shown as a little black spot above the left ocellus); (D,E) the
fingers on the pedipalps, and (F) mandibular teeth. Scorpion body segments are indicated as follows:
prosoma (head), mesosoma (abdomen) and metasoma (tail).

Although autofluorescence is an intrinsic property of many animals, plants, and minerals, its biological function is not clear [39]; this characteristic may favor UV camouflage,
protection, and animal communication [40–42]. Particularly in fish, biofluorescence is phy-
logenetically widespread and a phenotypically variable phenomenon [42], which clearly
affects animal behavior, prey selection, mating, and other physical abilities [33, 43].

Even though the molecular mechanisms which induce scorpion fluorescence remain
unknown, it is clear that it depends on cuticle hardening [39]. This is evidenced by the fact
that after ecdysis (molting), the remaining exoskeleton is still fluorescent, but the emerging
exoskeleton only emits light 48 hours after molting [44,45], when the cuticle becomes
thicker. The aromatic molecules beta carboline and 7-hydroxy-4-methylcoumarin [39,46]
are involved in the hardening and tanning process of the scorpion exoskeleton, and are also
experimentally associated with fluorescence [46]. Recently, a new fluorescent component
from Liocheles australasiae was identified and classified as a macrocyclic diphthalate ester, which seems to be present in the scorpion cuticle [47].

In summary, some evidence suggests that scorpion fluorescence could have played an
important role in their survival through the several environmental and biological changes
that occurred over the past 400 million years. The physiological function of scorpion
fluorescence deserves more in-depth examination. The molecular factors inducing bioflu-
orescent targeting in specific scorpion body parts have been explored, as has the possibility
of using this fluorescent pattern to improve scorpion typification; indeed, in Durango city,
biofluorescence is suitable for identifying scorpions at night using a fluorescent blacklight UV light bulb.

1.3. Venom Extraction and Preparation

Scorpion venom is used for multiple research purposes. Venom varies from species to species, and may differ in intensity due to the changes in composition according to environmental and genetic variations [48, 49]. The process of obtaining venom can be a time-consuming and dangerous task, as the scorpions must be alive. For this process, commonly known as “scorpion milking”, different methods have been developed, such as manual extraction, electric stimulation, puncturing the abdominal gland of the scorpion, or telson maceration [50].

In Durango, as in many places around the world, milking scorpion venom via electrical stimulation is the preferred method. This procedure requires the following implements: (1) an electrical source (to induce a shock of ~5 to 7 volts) with two electrodes—the negative electrode clamped to long iron–steel forceps (partially covered with rubber for safety) and the positive electrode clamped to a metal plate or a metal wire; (2) long iron–steel forceps; (3) gloves to avoid electrical shocks and cross-contamination; (4) a micropipette to collect venom; and (5) a tube to store the venom.

With the scorpion resting on a metal plate, with one hand, one person holds the forceps connected to a negative electrode to clamp the tail of the scorpion between the mesosoma and metasoma; with the other hand, a second pair of forceps is used to clamp the end of the tail, very close to the telson gland. The second person can then collect venom with a micropipette and drop it into a tube (Figure 5). After receiving an electrical shock, the scorpion is stunned, but is still alive, and it will recover after a while. Extracted venom can be stored frozen (−20 °C) or lyophilized until use; however, diluting it in 0.1% BSA has been suggested to improve storage [51]. Approximately 2 μL of venom per animal can be collected with this method [52]; depending on the desired use, hundreds of scorpions may need to be processed.

![Figure 5. Electrical extraction of venom. Two people work together to milk fresh venom. Collecting the droplets that dribble from the venom gland is a dangerous task, as the scorpion is alive.](image)

Although milking scorpion venom via electrical stimulation is a widely used method, there is a risk of death or injury from scorpion stings, as well as suffering from electric shocks. To eliminate the chances of such accidents, recently, a robot designed to milk scorpions was patented by Moroccan scientists. Following a similar procedure, this machine holds down the tail of the animal and then, after electrical stimulation, droplets of venom are collected in a tube. This automated collection method makes the process easier and
increases the efficiency of recollection [53]. However, this novel technology is not yet widely available.

Scorpion venom is a fascinating study object since it contains many chemical molecules (such as water, peptides, enzymes, amino acids, amines, mucopolysaccharides, and mucoproteins) which guarantee that venom will induce potent synergistic effects when it is injected into the prey. Thus, elucidating the biophysical, biochemical, and pharmacological properties and characteristics of scorpion venom represents a scientific challenge due to their complexity [54,55].

To preserve wildlife, many countries around the world have implemented species conservation programs. Mexico is no exception: The Ministry for the Environment and Natural Resources, SEMARNAT (Spanish abbreviation for “Secretaría de Medio Ambiente y Recursos Naturales”), in agreement with research institutions, civil societies, and native communities, protects scorpions by law. For research purposes, synthesizing the metabolite (or peptide) of interest has been suggested instead of using, and later killing, specimens [56].

1.4. Biophysical, Biochemical, and Pharmacological Importance of the Centruroides suffusus Venom for Voltage-Gated Ion Channels

In many cultures, scorpion body parts and venoms have been used by practitioners of medicine since ancient times [44,57–59]; in addition to using scorpions for mystical or pathological issues, crushed or fried scorpions have been applied as topical remedies to cure scorpion stings [60]. Antivenom therapies made from immunized animals are available around the world [55].

The first observations of interactions between scorpion venom and electrical signals triggered by nerve tissue were reported toward the end of the 1960s [61,62]. Since then, laboratories around the world have conducted exhaustive scientific research to understand their mechanisms of action [63–67]. These findings demonstrate that venom is composed of polypeptides as active components (toxins) that target voltage-gated ion channels such as sodium (Na\(^+\)), potassium (K\(^+\)), calcium (Ca\(^+\)), and chloride (CLC) channels [68–70]. These unique properties of toxins have been implemented as an important tool for structure–function relationship studies of ion channels [71–74]. The blocking effect of the crude venom of *Centruroides suffusus* (Durango scorpion) on Na\(^{+}\) 1.4, Shaker K\(^{+}\) from the larva of the fruit fly *Drosophila melanogaster*, expressed in oocytes of *Xenopus laevis*, was evidenced using the cut-open voltage clamp (COVC) technique [75,76]. The main effect of blocking venom from *Centruroides* scorpion species occurs mainly on the Na\(^{+}\) and K\(^{+}\) ion channels, supporting previous reports [77–79] (Figure 6).

![Figure 6](image_url)

**Figure 6.** Venom from *Centruroides suffusus* scorpions interacts with sodium and potassium voltage–gated ion channels. (A) Cartoon of the COVC electrophysiology technique setup. An oocyte (green-brown sphere) that heterologously expresses a voltage-gated ion channel is placed into the recording chamber, where it is electrically separated into three regions, the top, middle and bottom, leaving exposed a fraction of the membrane where the voltage stimulus is imposed and controlled (top chamber) by the feedback of the capacitance and resistance process. In response to the stimulus, the ion channels produce a macroscopic current due the passive flux of ions through its aqueous...
pores. Then, the ion current is amplified and recorded by an acquisition system controlled by a computer (see Stefani and Bezanilla (1998) [76] for more details). Because this methodology provides access to the interior (and exterior) of the cell, it is possible to perform studies of ion channels under physiological environment conditions. Representative records elicited from two different oocytes expressing (B) Mouse Na\textsubscript{V} 1.4 sodium ion channel and (C) Shaker K\textsubscript{V} ion channel from Drosophila melanogaster. Ion currents were elicited via a voltage pulse stimulus, shown on the top of each recording first in the absence of venom (black) and then in the presence of a 10^3 (a thousand)-fold dilution of venom (red). Dashed lines represent a zero level of current. Vertical and horizontal bars represent the current amplitude and temporal pulse duration scale for each recording. The chemical reagents used for these representative recordings were acquired from Sigma-Aldrich (Sigma–Aldrich Co., St. Louis, MO, USA). Each electrophysiological experiment was repeated at least three times in order to verify the trend observed. More detailed information regarding the electrophysiology methods and heterologous expression of ion channels in Xenopus oocytes can be found in Rodriguez-Rangel et al., (2020) [80].

At least nine peptides with toxic properties have been identified from Centruroides suffuses venom (CssI to CssIX) [81–83]. Several research groups around the world have reported that CssI, CssIV, CssVI, CssVIII, and CssIX bind Na\textsubscript{V} channels with high affinity. Na\textsubscript{V} channels are macro-molecules which are essential during the generation and propagation of electrical signals (action potentials) triggered by excitable cells, such as neurons [84–87]. The study of interactions between Na\textsubscript{V} channels and Centruroides suffuses toxins is of physiological relevance. Interestingly, the bioactivity of these neurotoxins exhibits a high level of specificity, which means that only some ion channels integrating the Na\textsubscript{V} family (Na\textsubscript{V} 1.1 to Na\textsubscript{V} 1.9) will be affected in a specific way by scorpion toxins; the CssII toxin is one of the most potent blocker toxins on Na\textsubscript{V} channels. Life-threatening conditions caused by scorpion stings are mostly related to the impairment of neurotransmission, which affects some vital functions and causes a wide range of conditions, including pain, anaphylactic reactions, severe local skin reactions, and neurologic, respiratory, and cardiovascular collapse, and can result in death as a consequence of venom toxins. In addition to the effect of CssII on the Na\textsubscript{V} ion channels for the CssII peptide, the inhibitory effect of \gamma-amino butyric acid (GABA) uptake in neuronal cells has been reported [84].

Structurally, the CssII toxin comprises 66 amino acids, forming four disulfide bridges; it is the most abundant toxin present in the venom gland. CssII is also the most studied toxin from Centruroides suffuses; its three-dimensional structure was predicted by nuclear magnetic resonance spectroscopy [88]. Non-toxic and recombinant peptides of CssII have been used to produce antivenom against the Durango scorpion. Furthermore, several research groups around the world consider CssII as a “classical” \beta-type toxin affecting Na\textsubscript{V} channel activation [84–87]. The recombinant protein can be acquired commercially for scientific laboratory research purposes.

Css54 is a peptide also found in venom from Centruroides suffuses. It consists of 25 amino acid peptide residues, and has been predicted to form an alpha helix without disulfide bonds. Css54 was identified by testing the antibiotic activity of reverse-phase high-performance liquid chromatography (HPLC) fractions [83]. This product inhibits the active growth of Gram-positive and Gram-negative bacteria such as Staphylococcus aureus, Escherichia coli, Listeria monocytogenes, Streptococcus suis, Campylobacter jejuni, and Salmonella typhimurium, which frequently cause infections through contaminated water or food [89,90]. Therefore, Css54 peptides have been proposed to act as ‘host defense peptides’ [91], and may form part of the innate scorpion immune system to protect against bacteria and other pathogens [83]. Although antimicrobial activity has been evidenced in vitro and is not truly clear in mammalian models, we hope that it might be useful by itself or in combination with other molecules as an alternative to health treatment.

Despite the enormous progress in our understanding of the structure and function relationships of voltage-gated ion channels and the inhibitory properties of toxins from scorpion venoms, there are still many open questions that need to be addressed in this field.
For instance, to gain further insight into the biophysical, biochemical, and pharmacological importance of *Centruroides suffusus* venom, it is vital to perform more exhaustive research with chromatography and functional methodologies in order to separate and identify new active components, peptides, and toxins that interact with ion channels to determine possible therapeutic and medical uses.

Multiple studies suggest that scorpions and their venom might have therapeutic applications (Table 1); however, thus far, chlorotoxin is the only scorpion toxin which has been evaluated in clinical medical trials [92]. Although various toxins and peptides from different scorpion species represent promising tools for scientific and biotechnological approaches, scorpions remain a key study topic, as they are fascinating survivors of multiple evolutionary events.

### Table 1. Insight into the potential application of scorpion venom.

<table>
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<tr>
<th>Uses</th>
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<tbody>
<tr>
<td>Analgesic</td>
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<td>Antibacterial</td>
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<td>Antifungal</td>
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<td>Antiviral</td>
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<td>Insect pests</td>
<td>[137,138]</td>
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<tr>
<td>Treatment for autoimmune diseases</td>
<td>[139–144]</td>
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<tr>
<td>Treatment for cardiovascular diseases</td>
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<tr>
<td>Treatment for epilepsy</td>
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### 2. Conclusions

Scorpions may seem threatening to some, but this is not the case for the citizens of Durango, who have adopted them into their nature, folklore, and economy. The multiple uses for scorpions include basic scientific, clinical, and biological applications and beyond. Venoms are deadly; however, for some conditions, the cure might be found within them. In addition to the therapeutic potential of scorpions, many biotechnological applications may emerge by taking advantage of the biodiversity of venoms and their intrinsic nutritional factors, or by understanding the physiological relevance of exoskeleton fluorescence and their ability to survive, among other unexplored qualities. Thus, sustainable production systems, including agriculture, animal breeding, organic food production, and many other bio-based economical resources, can be positively influenced by increasing our knowledge of scorpions.


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