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*Proceeding Paper*

# Exploring the Inter- and Intra-Specific Variability of Androctonus Scorpion Venoms †

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**Abstract:** Scorpion venom possesses a lethal sting and potential medicinal properties, making it a captivating natural elixir. Our study aimed to unravel the composition of Androctonus scorpionvenoms in Morocco. Using electrospray mass spectrometry and high-performance liquid chromatography (HPLC), we conducted a thorough analysis to gain detailed insights into venom composition. The data unveiled a wide range of molecular weights, influenced by factors such as species, genus, location, age, sex, and diet. Short toxins (2000–4000 Da) predominated in the venoms, effectively blocking $K^+$ channels, while larger molecular weights (>4000 Da) corresponded to long toxins that modulate $Na^+$ channels. Furthermore, we made intriguing discoveries of previously unidentified peptides (<2000 Da). This study provides valuable knowledge, shedding light on the intricate composition of scorpion venoms.

**Keywords:** scorpion; Androctonus; venom; peptides; LC-MS; peptidic maps

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## 1. Introduction

Scorpions are a fascinating group of arachnids. These venomous creatures pose a significant public health risk, accounting for 30–50% of poisoning cases reported in Morocco, with an alarming number of 30,000 people falling victim to scorpion stings annually, predominantly children under 15 years old [1]. Morocco boasts the highest scorpion diversity in North Africa, with 61 species, including Androctonus scorpions, which are commonly associated with envenomation cases [2,3]. Scorpion venom is a complex mixture of bioactive molecules, particularly neurotoxins that target ion channels. Venom composition varies greatly between species and individuals, influenced by factors such as sex, age, diet, and environmental conditions [4]. Proteomic analysis, specifically mass spectrometry, has revolutionized the study of scorpion venom, enabling the identification of toxins and peptides, aiding in the development of therapeutic agents and antivenoms [5]. Our research focuses on unraveling the mysteries of Androctonus scorpion venoms, utilizing cutting-edge proteomic strategies to understand their composition, variability, and potential applications. Through advanced mass spectrometry techniques, we aim to shed light on these complex venoms, paving the way for scientific breakthroughs and innovative analysis approaches.

## 2. Materials and Methods

### 2.1. Venoms

Scorpions from high-risk areas, known for severe envenomation cases, were studied. Venom milking involved electrical stimulation, and scorpions received weak 12V pulses on

their post-abdomen to extract venom. The collected venom was centrifuged at 10,000 rpm for 10 min, freeze-dried, and stored at −80 °C until needed [6].

### *2.2. Venom Analysis Using Mass Spectrometry Coupled with Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC)*

Moroccan scorpion venoms were analyzed using a Micromass Quattro Microtriple–quadrupole ESI-MS coupled with RP-HPLC [7]. Each venom sample (50 µg of protein) was loaded on to a C18 Zorbax analytical column (with a 150 mm length, a 2.1 mm internal diameter, and a 3 µm particle size). Venom fractionation took place over 100 min with a mobile phase of 0.1%FA (solvent A) and can in 0.1% FA (solvent B). Elution used a linear gradient ranging from 0% to 100% of solution B at a constant flow rate of 0.2 mL/min. The separated peaks were directly analyzed with the Micromass Quattro Micro ESI-MS triple–quadrupole mass spectrometer. MassLynx version 4 software was used to convert the generated MS peaks into molecular masses, analyzing the obtained spectra.

## 3. Results

### *3.1. Fractionation of Venoms Using Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC)*

The venom of *A. mauritanicus* from Tadla exhibited 24 peaks (Figure 1), while *A. mauritanicus* from Oualidia and *A. bicolor* from Draa Valley displayed 37 peaks (Figure 1). The majority of these peaks eluted within a retention time (RT) range of 17 min to 74.45 min (Figure 1). However, a few minority peaks were eluted at an RT of less than 5 min in the venom of *A. maroccanus* from Marrakech, *A. barbouri* from Agadir, and *A. amoreuxi* from Tata, as well as in the venom of all three *A. mauritanicus* specimens (Figure 1). Intraspecific variability is evident in the venoms of scorpions within the same species. This variability is exemplified by the venom profiles of *A. mauritanicus* specimens from Oualidia and Essaouira, which are complex and nearly identical (Figure 1). In contrast, the venom of the Tadla specimen differs and is less complex (Figure 1). This intraspecific variability is likely influenced by their geographical locations.

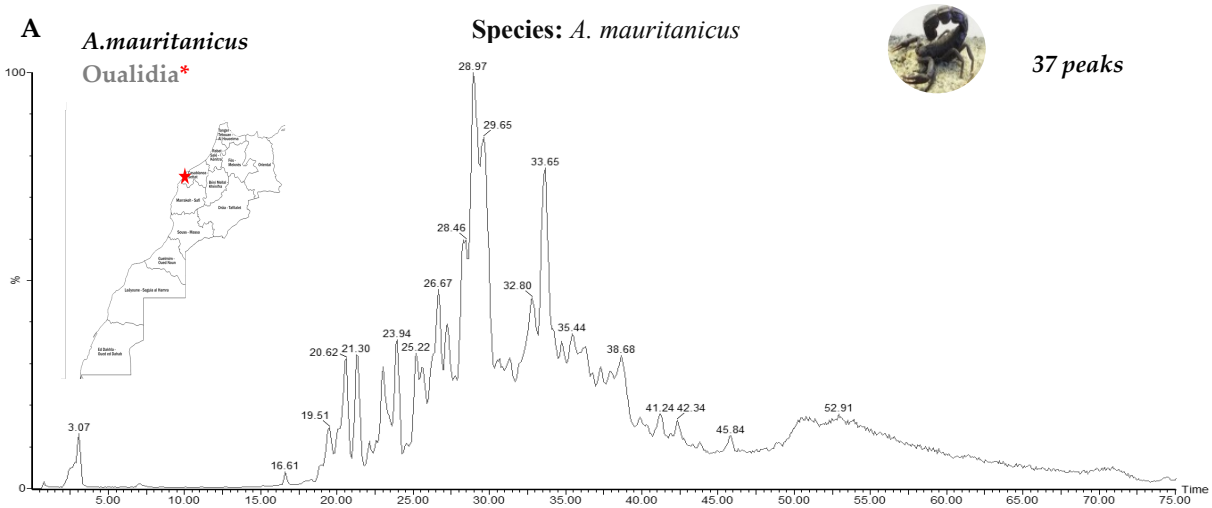
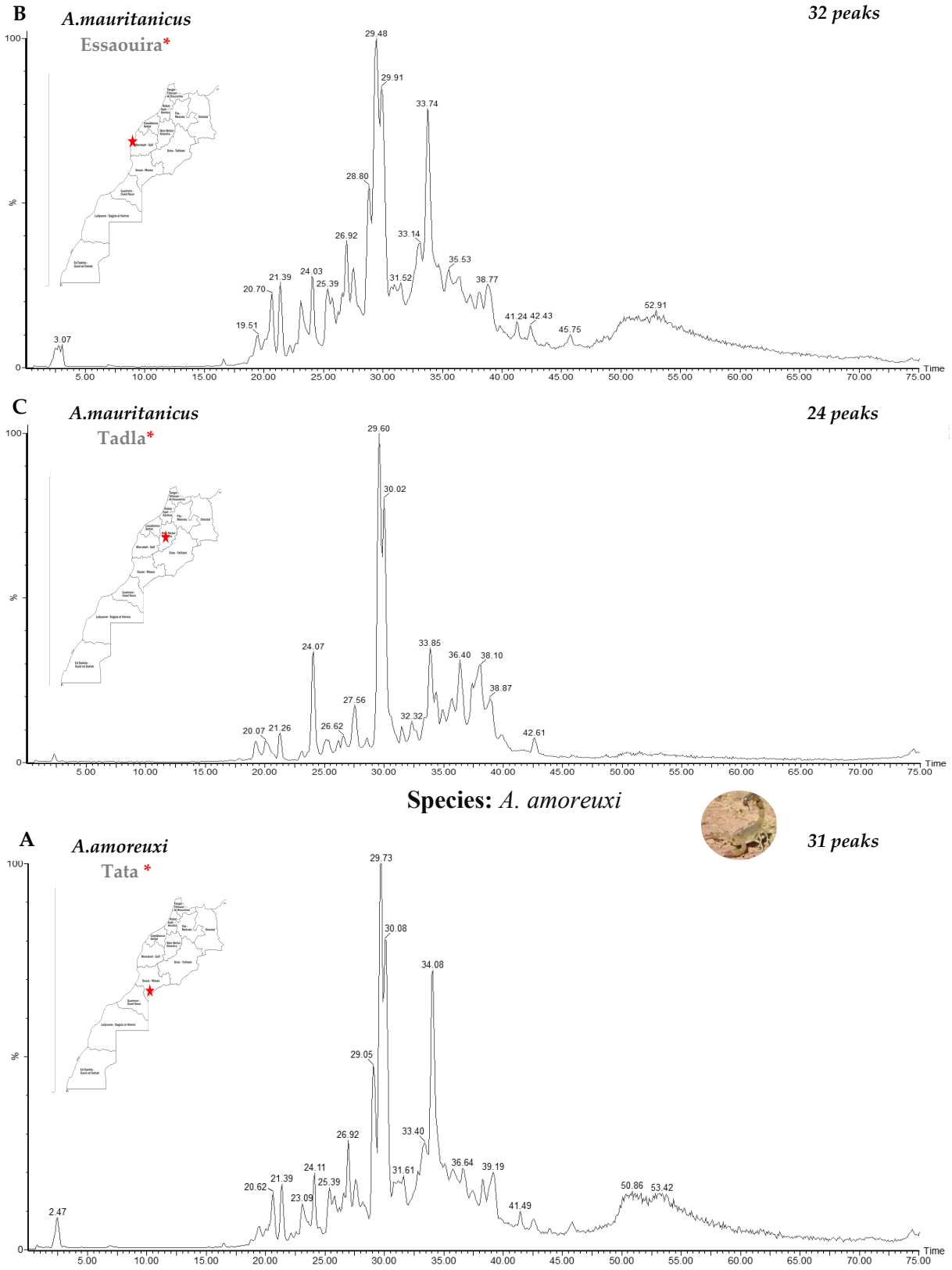



**Figure 1.** *Cont.*

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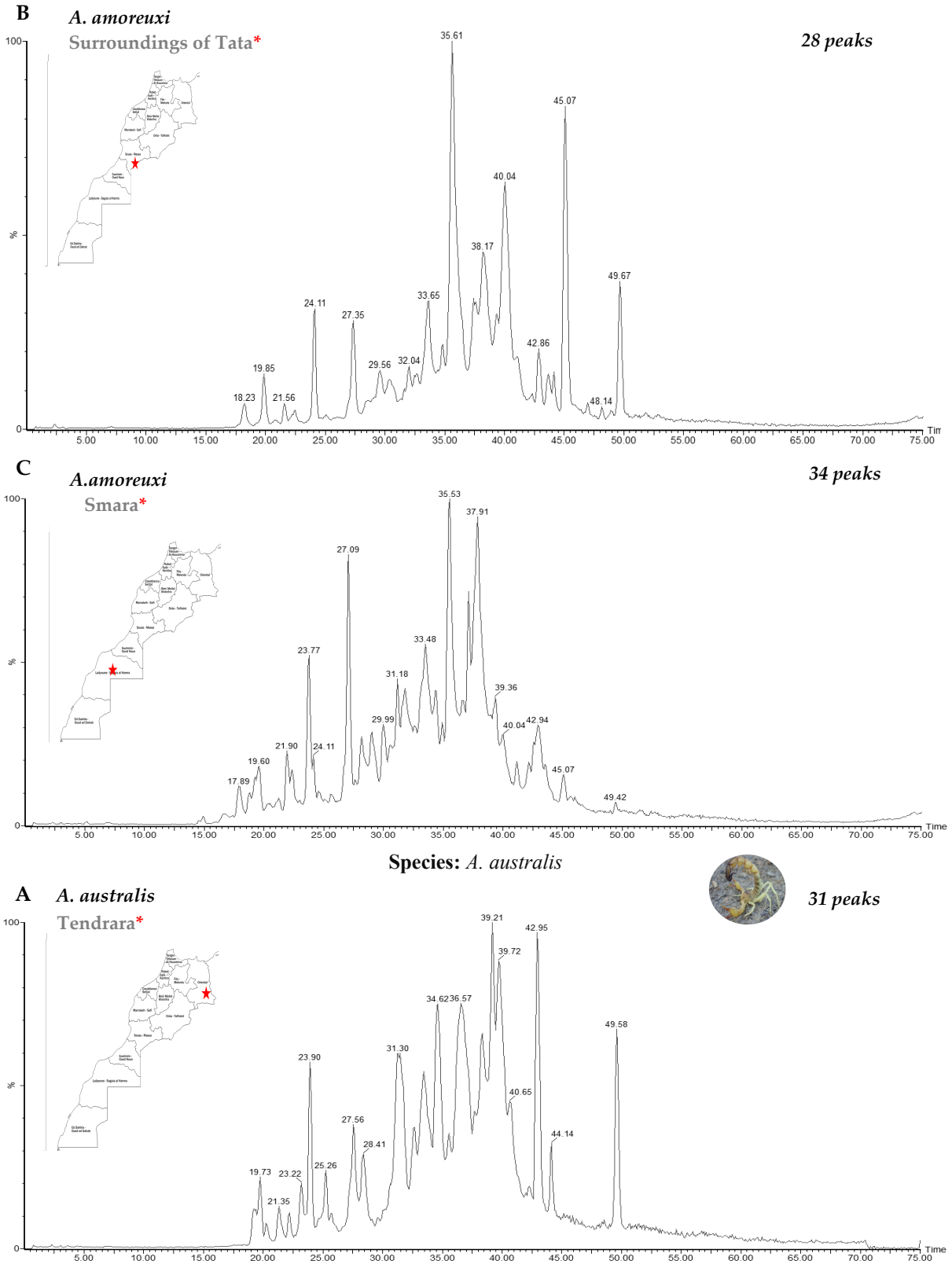


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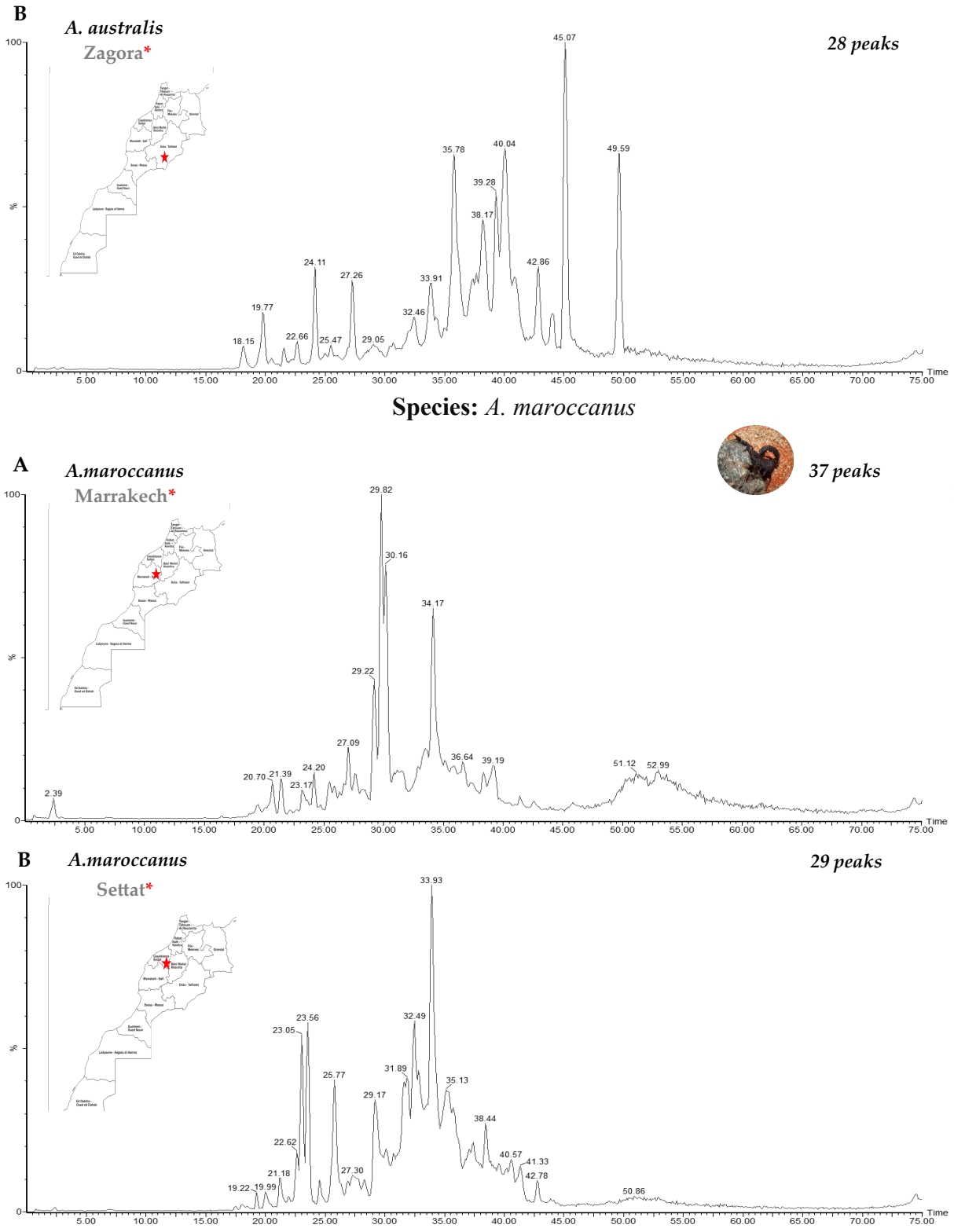


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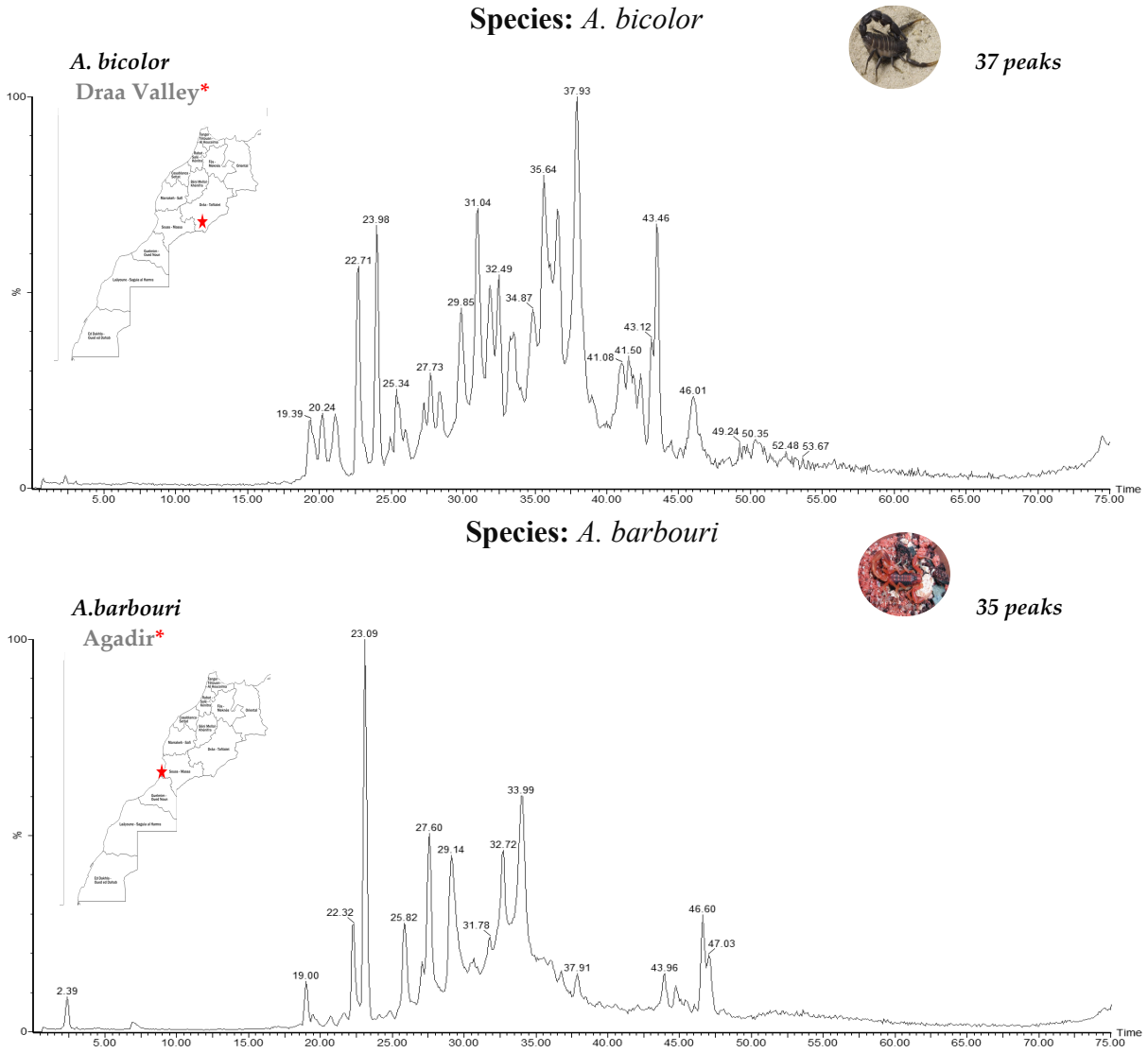


**Figure 1.** RP-HPLC profiles of venom from Androctonus specimens collected from different regions in Morocco. Species: *A. mauritanicus* scorpions, collected in Oualidia (**A**), Essaouira (**B**) and Tadla (**C**); Species: *A. amoreuxi* scorpions, collected in Tata (**A**), Tata surroundings (**B**) and Smara (**C**); Species: *A. australis* scorpions, collected in Tendrara (**A**) and Zagora (**B**); Species: *A. maroccanus* specimens collected in Marrakech (**A**) and Settat (**B**); Species: *A. bicolor* specimen, collected in the Draa Valley; Species: *A. barbouri* specimen, collected in Agadir.

Additionally, intraspecific variability is observed in the venom of *A. amoreuxi*, where the three venom profiles demonstrate differences in appearance and complexity (Figure 1). This difference persists even among scorpions collected from two adjacent regions, Tata and its surroundings.

Similarly, intraspecific variability is noted in the venom of the two specimens of *A. australis* and the two specimens of *A. maroccanus* (Figure 1).

### *3.2. Analysis by Mass Spectrometry*

The RP-HPLC- separated peaks were subjected to analysis using triple–quadrupole ESI-MS mass spectrometry. The resulting data were then processed using Mass Lynx 4 software to identify the molecular masses associated with each peak. Table 1 presents the number of molecular masses identified in the venoms of Androctonus scorpions.

**Table 1.** Molecular masses generated after the analysis of different peaks using MassLynx 4 software.

| Genus | Species | Number | Region | Molecular Masses |
|---|---|---|---|---|
| Androctonus | *Androctonus mauritanicus* | 3 | Oualidia | 469 |
| | | | Essaouira | 410 |
| | | | Tadla | 328 |
| | *Androctonus amoreuxi* | 3 | Tata | 374 |
| | | | Tata surroundings | 452 |
| | | | Smara | 309 |
| | *Androctonus australis* | 2 | Zagora | 336 |
| | | | Tendrara | 359 |
| | *Androctonus bicolor* | 1 | Draa Valley | 578 |
| | *Androctonus maroccanus* | 2 | Marrakech | 312 |
| | | | Settat | 338 |
| | *Androctonus barbouri* | 1 | Agadir | 236 |

The total number of molecular masses observed ranged from 236 to 578. *A. bicolor* venom exhibited the highest number of different masses (578), followed by *A. mauritanicus* from Oualidia with 469 masses. The least complex venoms were found in *A. australis* from Zagora (336 different masses) and *A. barbouri* from Agadir with 236 molecular masses. The mass spectrometry results validated the RP-HPLC fractionation data and demonstrated both inter-and intraspecific variability in the venom of Moroccan scorpions. Regarding the distribution of molecular weights in the venoms, masses between 2001 and 5000 Da (corresponding to neurotoxins targeting $K^+$, $Cl^-$, and $Ca^{2+}$ channels) were the most abundant across all species analyzed (Figure 2). The venom of *A. maroccanus* from Settat exhibited the highest percentage at 58.28%. On the other hand, masses between 5001 and 10,000 Da (corresponding to neurotoxins targeting $Na^+$ channels) were more prevalent in the venom of the three specimens of *A. mauritanicus*, with the highest percentage in the Essaouira specimen (36.42%). Similarly, *A. amoreuxi* showed a significant percentage in the venom of all three specimens, with the highest in the Tata surroundings specimen (34.71%). In *A. australis*, the Tendrara specimen had a percentage of 25.86%, while *A. bicolor* from Draa Valley exhibited 21.33%. However, these molecular masses were less abundant in the venom of *A. barbouri* from Agadir (3.39%).

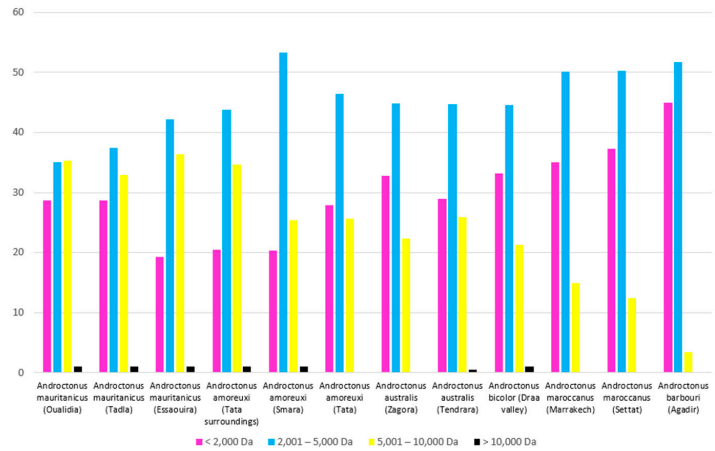


**Figure 2.** Molecular weight distribution of venoms from Androctonus species.

## 4. Discussion

The aim of this study was to characterize the venoms of scorpions belonging to the Androctonus genus collected from various regions in Morocco. The venoms were analyzed using MS mass spectrometry coupled with RP-HPLC, revealing differences in chromatographic profiles, including peak number, intensity, and retention time, both within and between species. For example, in *A. mauritanicus*, the venom profiles of specimens from Oualidia and Essaouira were highly similar and complex, while the venom from the Tadla specimen was less complex and distinct. The RP-HPLC profiles obtained provide a partial representation of the venom composition for each species, with characteristic peaks that can aid in taxonomic identification and differentiation.

Analyzing the peaks using MassLynx 4 software showed that species from the Androctonus genus exhibited a high number of molecular masses, ranging from 236 (*A. barbouri*) to 578 (*A. bicolor*). A similar number of molecular masses was detected in the venoms of *Tityus metuendus* and *Rhophalurus junceus* (200 masses); *Serradigitus gertschi* (204 masses); *Tityus discrepans* (205 masses); *Paravaejovis schwenkmeyeri* (212 masses); *Leiurusquinquestriatus quinquestriatus* (380 masses); *Tityus serrulatus* (382 masses); *Pandinus cavimanus* (390 masses); *Centruroides limpidus* (395 masses); *Tityus bahiensis* (464 masses); and *Leiurus quinquestriatus hebraeus* (554 masses) and *Tityus stigmurus* (632 masses) [8–15]. Meanwhile, a low number of masses was identified in the venoms of some scorpions, namely *Leiurus Abdullah bayrami* (45 masses); *Buthacus macrocentrus* (60 masses); *Scorpio maurus palmatus* (73 masses); *Andrcotonus mauretanicus mauretanicus* (74 masses); *Androctonus crassicauda* (80 masses); *Tityus stigmurus* (100 masses); and *Opisthacanthus elatus* (106 masses) [16–21].

The molecular weight distribution of Androctonus venoms revealed a predominance of molecular masses between 2001 and 5000 Da, corresponding to neurotoxins that target $K^+$, $Cl^-$,and $Ca^{2+}$ channels. A high percentage of these masses was found in the venom of *A. maroccanus*, followed by masses between 5001 and 10,000 Da, representing neurotoxins that act on $Na^+$ channels, with a significant presence in the venom of *A. mauritanicus* from Essaouira. These findings align with previous studies demonstrating the prevalence of molecular masses between 2001 and 5000 Da in the venom of *Scorpio maurus palmatus* and *Buthus occitanus* [19,22].

Neurotoxins targeting $Na^+$ ion channels, which are responsible for envenomation symptoms, were prominently represented in the venom of Androctonus scorpions. In particular, the three specimens of *A. mauritanicus* exhibited percentages of 32.95% (Tadla), 35.27% (Oualidia), and 36.42% (Essaouira), while the three specimens of *A. amoreuxi* showed percentages of 25.4% (Tata specimen), 25.4% (surroundings of Tata), and 34.71% (Smara). The two specimens of *A. australis* displayed percentages ranging from 22.31% (Zagora) to 25.86% (Tendrara), and *A. bicolor* from Agadir had a percentage of 21.33%.

These results support the literature's depiction of the Androctonus genus as the most dangerous worldwide, particularly in North Africa, the Middle East, and Asia. The findings also provide insights into the areas at high risk of envenomation, mainly concentrated in the center of the kingdom. This correlation aligns with the distribution of severe envenomation cases according to the Center for Antipoison and Pharmacovigilance of Morocco (CAPM) [1].

Thus, the mass peptide maps generated from analyzing different venoms can be similar to an overview of the genome of the species studied, which then facilitates the genetic matching of specimens, thus demonstrating the use of venom profiles for taxonomic purposes.

## 5. Conclusions

This study significantly enhances our understanding of Androctonus scorpion venom. By employing proteomic techniques, we successfully characterized the venom proteome of scorpions from the Androctonus genus across different regions of Morocco. The findings highlight the remarkable variability of scorpion venoms, influenced by factors such as scorpion biology and ecological conditions. Additionally, the study identifies the most potent

venom sources and high-risk regions for envenomation. This knowledge is invaluable for developing effective antivenoms and potential drugs.

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