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A first exploration of the venom of the *Buthus occitanus* scorpion found in southern France

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ABSTRACT

Even though *Buthus occitanus* scorpions are found throughout the Mediterranean region, a lack of distinctive characteristics has hampered their classification into different subspecies. Yet, stings from this particular scorpion family are reported each year to result in pain followed by various toxic symptoms. In order to determine the toxicity origin of the rare French *B. occitanus* Amoreux scorpion, we collected several specimens and studied their venom composition using a nano ultra high performance liquid chromatography and matrix assisted laser desorption/ionisation time-of-flight mass spectrometry (nano UHPLC/MALDI-TOF-MS) automated workflow combined with an enzyme-linked immunosorbent assay (ELISA) approach. Moreover, we compared this dataset to that obtained from highly lethal *Androctonus australis* and *Androctonus mauretanicus* scorpions collected in North Africa. As a result, we found that the *B. occitanus* Amoreux venom is toxic to mice, an observation that is most likely caused by venom components that inhibit voltage-gated sodium channel inactivation. Moreover, we identified similarities in venom composition between *B. occitanus* scorpions living in the South of France and other Buthidae collected in Morocco and Algeria. As such, the results of this study should be taken into consideration when treating stings from the *B. occitanus* species living in the South of France.

1. Introduction

Of the 1900 different scorpion species identified worldwide so far, less than 20 of them constitute a health hazard to humans. Strikingly, almost all of the dangerous specimens belong to the Buthidae, a scorpion family stemming from the 'Old World'. For example, the genus *Androctonus* is highly lethal for mammals and is responsible for roughly 100 000 stings each year in North Africa and the Middle East, 1% of which results in death of the victim (Benguedda et al., 2002). Scorpion venoms are complex mixtures containing numerous neurotoxic polypeptides, which typically enhance nerve and muscle excitability. Although various families of scorpion toxins have been reported to target voltage-gated potassium (Kv) channels, voltage-gated calcium (Ca_v) channels, and chloride channels (Martin-Eauclaire and Couraud, 1995; Possani et al., 1999), a large majority of peptide toxins is active on voltage-gated sodium (Na_v) channels, a family of transmembrane proteins that regulate cellular excitability (Catterall, 2012).

The venoms from North African *Androctonus* scorpions primarily contain classical α-toxins, which target the domain IV voltage sensor within mammalian neuronal and muscle Na_v channel subtypes (Bosmans et al., 2008;

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Campos et al., 2008). Upon binding to their receptor site, these α-toxins are thought to prevent the domain IV voltage sensor from moving upward, thereby slowing down the fast inactivation process and provoke a persistent depolarization of cell membrane (Bosmans et al., 2008; Campos et al., 2008; Capes et al., 2013; Catterall, 2012; Rogers et al., 1996; Sheets et al., 1999). In contrast, scorpions from the *Buthus* genus, such as *Buthus occitanus* in North Africa and the Middle East, *Buthus eupeus* in Russia, and *Buthus martensii* in China, are considered to be less dangerous for humans. The difference in overall toxicity may be related to the low amount of classical α-toxins in their venoms. Indeed, the majority of peptide toxins identified in *Buthus* venoms belong to the so-called α-like toxins, a group of peptides that target both mammals and insects and have been shown to be less lethal to mammals as opposed to classical α-toxins (Gordon et al., 1996). Moreover, their venoms contain a large amount of toxins that selectively influence ion channel function in insects, the principal prey of scorpions.

The habitat of *B. occitanus* scorpions spreads across North-Africa, Egypt, Ethiopia, and Somalia (Lourenço, 2003), to the Southern part of Europe (Amoreux, 1789). Even though toxic scorpions are a rare sight in Spain and the French Mediterranean coast, *B. occitanus* Amoreux is of medical importance since painful stings attributed to this scorpion are described each year. A previous investigation using nuclear 18S/ITS-1 DNA sequences and mitochondrial 16S and COI DNA sequences revealed a phylogenetic relationship between the *B. occitanus* populations across the Strait of Gibraltar (Gantenbein and Largiadèr, 2003). Based on their results, the authors of this work concluded that the European clade was highly separated from North African clades and even split into three divergent subclades. Moreover, they reported that most of the detected mitochondrial DNA lineages, including the European lineages, were about three times older than the reopening of the Gibraltar Strait. As a result, they estimated an older separation time between the *B. occitanus* living in France and Spain. Despite their medical relevance, the biochemical, pharmacological, and immunological properties of the European *B. occitanus* venom have not been investigated; as for any European scorpion. Here, we analyse the venom composition of the rare French *B. occitanus* Amoreux. To this end, we collected several specimens in two different parts of the South of France (Provence-Alpes-Côte-d'Azur area): in the mounts around Orange city (Vaucluse County) and in Massif des Maures (Var County) and examined their individual venoms using nano ultra high performance liquid chromatography (UHPLC), matrix assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS), and enzyme-linked immunosorbent assay (ELISA) tests using specific antibodies raised against other Buthidae toxins (Martin-Eauclaire et al., 2013). Moreover, we compared venom components to those characterized using the same analytical methods in the venoms from different highly lethal Buthidae collected in Morocco (*Androctonus mauretanicus*) and in Algeria (*Androctonus australis*). Finally, we explored the biological activity of the *B. occitanus* venom in mice and on a neuronal Na_v channel isoform expressed in a heterologous system. As a result, we found that the venom from the *B. occitanus* scorpions collected in the Provence-Alpes-Côte-d'Azur area is unexpectedly similar to those from the lethal North African Buthidae. Even though we identified several K_v channel toxins, most of the proteins found belong to α-like toxins targeting Na_v channels. Surprisingly however, we uncovered the presence of a range of classical α-toxins as well, which may explain the toxicity of this venom towards humans.

2. Material and methods

2.1. Materials

Four specimens of *B. occitanus* were collected in different biotopes of the Provence-Alpes-Côte-d'Azur area: three in the Western part (Orange city, Vaucluse County) (Fig. 1A) and one other in the Easter part (Massif des Maures, Var County) (Fig. 1B), two places distant from each

Fig. 1. Pictures of the specimens of *Buthus occitanus* studied. A) Animal collected near Orange city (Vaucluse County; Photo credit: S. Diochot) and B) in Massif des Maures (Var County; Photo credit: Thierry Hupin).