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Deep intraspecific divergences in the medically relevant fat-tailed scorpions (*Androctonus*, Scorpiones)

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ABSTRACT

The genus *Androctonus*, commonly known as fat-tailed scorpions, contains 22 species distributed from Togo and Mauritania in the west, North Africa, through the Middle East and to as far east as India. With 13 species, a substantial amount of this genus' diversity occurs in North Africa, which is a major hotspot of scorpion sting incidents. *Androctonus* are among the most medically relevant animals in North Africa. Since venom composition within species is known to vary regionally, the improvement of therapeutic management depends on a correct assessment of the existing regional specific and sub-specific variation. In this study, we assessed the phylogeographical patterns in six species of *Androctonus* scorpions from North Africa using mitochondrial DNA markers. We sequenced COX1, 12S, 16S and ND1 genes from 110 individuals. Despite lacking basal resolution in the tree, we found taxonomical and geographically coherent clades. We discovered deep intraspecific variation in the widespread *Androctonus amoreuxi* and *Androctonus australis*, which consisted of several well-supported clades. Genetic distances between some of these clades are as high as those found between species. North African *A. australis* have a deep split in Tunisia around the *Chott el-Djerid* salt-lake. A novel split between *A. amoreuxi* scorpions was found in Morocco. We also found deep divergences in *Androctonus mauritanicus*, corresponding to areas attributed to invalidated subspecies. In addition we uncovered a clade of specimens from coastal south Morocco, which could not be ascribed to any know species using morphological characters. Based on these findings we recommend a reassessment of venom potency and anti-venom efficacy between these deep intraspecific divergent clades.

1. Introduction

Worldwide, 1.2 million people are stung by scorpions every year. Scorpionism, defined as the severe to lethal incident as a consequence of a scorpion sting (Lourenço and Cuellar, 1995) may be responsible for 3250 global annual mortalities which are mostly concentrated in a few high-risk areas (Chippaux and Goyffon, 2008). North Africa in particular is considered a high-risk area for scorpionism (Chippaux and Goyffon, 2008), with the genera *Leiurus* Ehrenberg, 1828 and *Androctonus* Ehrenberg, 1828 being the foremost cause of serious envenomation in this area (Goyffon and Guette, 2005; Graham, 2011; Habermehl, 1994). Five *Androctonus* species are considered as dangerous to man, particularly *Androctonus mauritanicus* (Pocock, 1902) and thewidespread *Androctonus australis* (Linnaeus, 1758), which are the most dangerous *Androctonus* in the Maghreb region (Morocco, Algeria, Tunisia) (Goyffon and Guette, 2005). *A. australis* is known for envenomating humans and possessing a high toxicity (LD_{50} = 0.32 mg/kg in mice; Watt and Simard, 1984). For this reason, *A. australis* was one of the first species of scorpions to have its venom purified for neurotoxin characterization (Miranda et al., 1966). As in snakes (Daltry et al., 1996; Prasad et al., 1999), scorpion venom is known to have considerable intraspecific regional variation in composition (Devaux et al., 2004; El Ayeb and Rochat, 1985; Newton et al., 2007; Smertenko et al., 2001), and thus a different response to antivenom treatment (Omran and McVean, 2000). Furthermore, other species such as *Androctonus amoreuxi* (Audouin, 1826) may also cause more cases of scorpionism than currently thought (Goyffon et al., 2012). It is therefore important to study the phylogeographical patterns of *Androctonus* over a great part of their distribution as it may have direct applications in therapeutic management.

Androctonus is present in deserts and semi-arid regions from Togo to Morocco in the Atlantic coast of Africa (Lourenço and

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Qi, 2007; Lourenço, 2008) to the Maghreb countries and Egypt where they are also present in relatively elevated areas like the Atlas Mountains and the Sinai Peninsula mountain range (Vachon, 1952), the Middle East (Levy and Amitai, 1980), reaching across Afghanistan (Vachon, 1958) to India (Tikader and Bastawade, 1983). Little is known about the biogeography of *Androctonus*, although the radiation of buthids has been associated with the aridification of the Palearctic Region (Fet et al., 2003, 1998).

In this work, we assess five species of the Maghrebian countries except for Libya, plus Egypt and the Sinai Peninsula. *A. mauritanicus* occurs in Morocco. This country shares a further three species with Algeria, Tunisia and/or Egypt (*Androctonus liouvillei*, *A. australis* and *A. amoreuxi*), while *Androctonus bicolor* occurs in Algeria and Tunisia. *A. amoreuxi* is known to occur mostly in sandy deserts while *A. australis* and *A. mauritanicus* can be found in anthropogenic environments (Stockmann and Ythier, 2010). The country in our area of study with the most species of *Androctonus*, Morocco, is also the most orographically diverse. The mountain chains that subdivide Morocco, North to south the Rif Mountains, Middle Atlas, High Atlas and Anti-Atlas Mountains, are known to be important in the diversification of the scorpion genus *Buthus* (Husemann et al., 2012; Sousa et al., 2012; Pedroso et al., 2013). Similarly, the Tell Atlas Mountains and Aurès Mountains of Algeria and Tunisia are associated with *Buthus* (Pedroso et al., 2013). Although *Androctonus* are generally not as orophilic as *Buthus*, these mountain chains may form a barrier for dispersal of the lowland species. However, at least one Maghreb *Androctonus* species is known to be associated with the Hoggar mountains in Southern Algeria; *Androctonus hoggarensis* (Pallary, 1929)

The scorpion genus *Androctonus* was first described by Ehrenberg in 1828. Vachon (1952) stabilized the genus' taxonomy, transforming it into a morphological and geographical coherent group with seven species known in North Africa. Lourenço (2005) produced an important taxonomical revision of the genus: the subspecies of *A. australis* and *A. mauritanicus* were no longer considered valid, *Androctonus crassicauda gonneti* Vachon, 1948 was raised to the status of species, *Androctonus aeneas* C. L. Koch, 1839 was placed in the synonymy of *A. bicolor* Ehrenberg, 1828 and *A. liouvillei* (Pallary, 1924) was raised to the species level.

Recently, molecular tools have been used to assess the phylogeny of *Androctonus* in Tunisia. Ben Ali et al. (2000), using nuclear DNA ITS regions (ITS-rDNA), found paraphyletic clades in three well-accepted taxa (*A. bicolor*, *A. australis* and *A. amoreuxi*). Ben Othmen et al. (2004), using allozymic differentiation, found little support for the monophyly of *A. australis* and *A. amoreuxi* individually. However three well-supported monophyletic lineages using 16S-rDNA, each corresponding to a species were recovered in a subsequent study, thus demonstrating the usefulness of this gene as a barcoding marker (Ben Othmen et al., 2009). Furthermore, they found a phylogeographic pattern of *A. australis* in Tunisia, where each of its two lineages are distributed to the north or south of the *Chott el-Djerid* salt lake, situated in central Tunisia. However their molecular dating makes it unlikely that the salt lake formation has generated a vicariant evolution of Tunisian *A. australis*. In recent years, molecular studies using the mitochondrial COX1 gene already uncovered considerable cryptic diversity in other scorpion genera in the Maghreb region (Gantenbein and Largiadèr, 2003; Sousa et al., 2012, 2011, 2010). However, a molecular phylogenetic study of the medically relevant scorpions of the genus *Androctonus* across the Maghreb region has thus far not been performed.

In this study, we assess the patterns of diversity estimated from COX1, 16S and 12S sequence data across the Maghreb region and Egypt. A separate reduced dataset was also made from ND1 sequences. We here provide the first molecular phylogeny for *Androctonus* scorpions in Morocco, Algeria and Egypt.

2. Materials and methods

2.1. Taxon sampling

We collected samples in the field in three countries: Morocco, Tunisia and Egypt (Fig. 1). Algerian samples were donated by Dr. Said Larbes. Additional specimens were purchased through the pet-trade for which no locality data were available other than the country of origin. Sampling sites are illustrated in Fig. 1 and further details are provided in Table 1. Scorpions were captured with long forceps and preserved in 96% ethanol. To minimize the impact on scorpion populations, non-lethal sampling methods were used when possible, and consisted of removing the distal part of one of the second leg pairs. The scorpion was subsequently placed in the sand, facilitating the clotting process and thus minimizing haemolymph loss. Scorpions can recover partially amputated appendages after a few molts and it is common for scorpions to be active and functional even when missing appendages (pers. obs.).

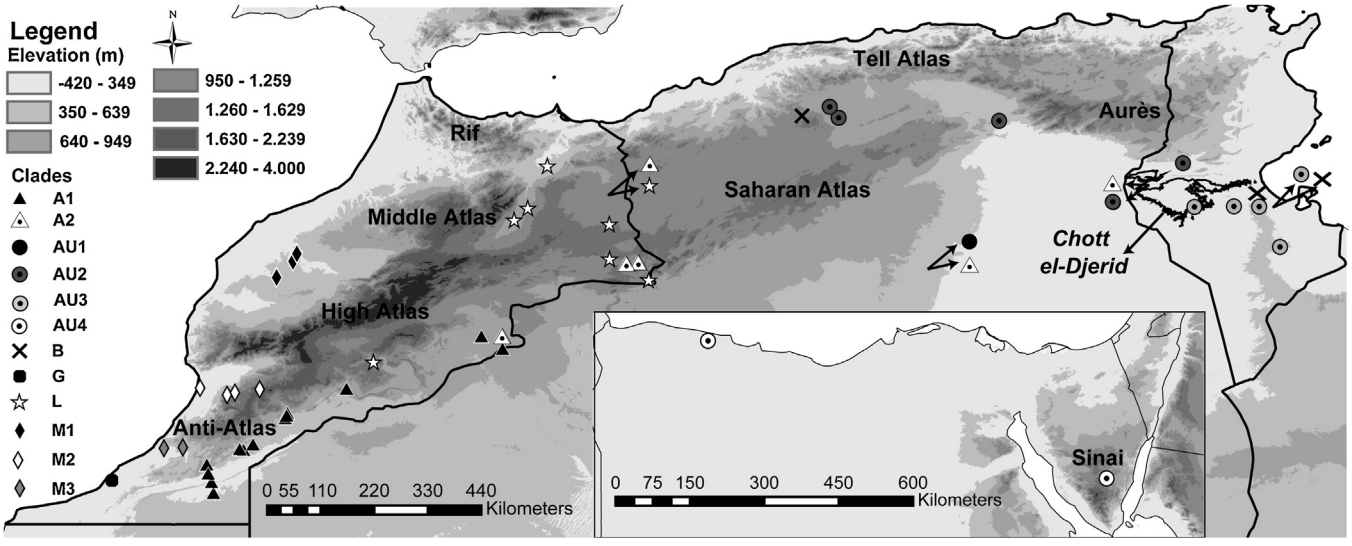

Fig. 1. Map representing the sampling locations across North Africa of *Androctonus* scorpions. Inset shows Egyptian samples. Pet trade acquired samples were without locality data, and are not shown. Symbols correspond to the phylogenetic clades (see Fig. 2).