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Scorpion speciation in the Holy Land: Multilocus phylogeography corroborates diagnostic differences in morphology and burrowing behavior among *Scorpio* subspecies and justifies recognition as phylogenetic, ecological and biological species ☆

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ABSTRACT

Scorpio Linnaeus, 1758 (family Scorpionidae Latreille, 1802) was considered monotypic for over a century, and comprised a single species, *Scorpio maurus* Linnaeus, 1758, with 19 subspecies, distributed from West Africa, throughout the Maghreb and the Middle East, to Iran. Two parapatric subspecies, *Scorpio maurus fuscus* (Ehrenberg, 1829) and *Scorpio maurus palmatus* (Ehrenberg, 1828), have long been recognized in the eastern Mediterranean region. We examined morphological variation, burrow architecture and genetic divergence among 39 populations across the distribution of the two subspecies to assess whether they are conspecific and, if not, how many species might be involved. Cuticle coloration, pedipalp chela digital carina condition, and selected measurements were recorded. Sixty burrows were excavated and examined for burrow structure and depth. A multilocus dataset comprising concatenated fragments of one nuclear (28S rDNA) and three mitochondrial (12S rDNA, 16S rDNA, Cytochrome c Oxidase Subunit I) loci, totaling ca. 2400 base-pairs, was produced for 41 individuals, and a single-locus dataset comprising 658 base-pairs of the COI locus for 156 individuals. Despite overlapping ranges in morphometric characters of pedipalp chela shape, the putative subspecies were easily distinguished by cuticle coloration and condition of the pedipalp chela digital carina, and were also found to differ significantly in burrow architecture and depth. Phylogeographical analyses of the COI and multilocus datasets recovered seven distinct clades. Separate analyses of mitochondrial sequences, and combined analyses of mitochondrial and nuclear sequences support most clades. The two major clades corresponded with the geographical distributions of *S. m. fuscus* and *S. m. palmatus* in the region. Specimens from these clades were genetically distinct, and exhibited different burrow structure in geographically-proximate localities, suggesting reproductive isolation. The *palmatus* clade included two distinct subclades of specimens from localities adjacent to the Dead Sea. Three other clades, comprising specimens from the most northeastern localities, were tentatively assigned to subspecies previously recorded in neighboring Jordan and Syria. The morphological, behavioral and genetic evidence supports previous suggestions that *Scorpio maurus* is a species complex and justifies the following taxonomic emendations: *Scorpio fuscus* (Ehrenberg, 1829), stat. nov.; *Scorpio kruglovi* Birula, 1910, stat. nov.; *Scorpio palmatus* (Ehrenberg, 1828), stat. nov.; *Scorpio propinquus* (Simon, 1872), stat. nov.

1. Introduction

Scorpio Linnaeus, 1758 is one of four genera in the Old World scorpion family Scorpionidae Latreille, 1802 (Prendini et al., 2003; Prendini and Wheeler, 2005). The genus originally

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accommodated a number of species (e.g., Simon, 1872; Pocock, 1900), some of which were subsequently transferred to other genera (e.g., *Heterometrus* Ehrenberg, 1828 and *Pandinus* Thorell, 1876). By 1910, however, only a single species, *Scorpio maurus* Linnaeus, 1758, containing 11 subspecies, was recognized (Birula, 1910). Additional subspecies were subsequently added (Pallary, 1928; Werner, 1929, 1932, 1936; Schenkel, 1949; Bouisset and Larrouy, 1962) but the genus remained monotypic for almost a century, by which time *S. maurus* comprised 19 recognized subspecies, distributed from West Africa, throughout the Maghreb and the Middle East, to Iran (Fet, 2000).

Levy and Amitai (1980) stated that there are no reliable characters to distinguish the subspecies of *S. maurus*, and agreed with Vachon (1950) that the absence of such characters precludes their recognition as species. Nevertheless, the mostly allo- or parapatric subspecies of *S. maurus* could be distinguished on the basis of somatic characters presented in several identification keys (Birula, 1910; Vachon, 1950, 1952; Levy and Amitai, 1980) leading Prendini et al. (2003) to suggest that they are in fact diagnosable (i.e., phylogenetic) species. The limited ecological data available also suggested that multiple ecological species, with different climatic requirements and ecological niches, might be involved across the broad distribution of *S. maurus*. For example, *Scorpio maurus palmatus* Ehrenberg, 1828 occurs near the Dead Sea, 400 m below sea level, whereas *Scorpio maurus fuliginosus* (Pallary, 1928) occurs at 3000 m in the Atlas Mountains, thousands of kilometers away (Levy and Amitai, 1980). Although all subspecies construct and live in burrows with characteristic oval or crescent-shaped entrances (Levy and Amitai, 1980; Prendini et al., 2003), burrows vary in length, depth and configuration (straight, or with twists and turns).

More recently, considerable divergence in DNA sequences from the mitochondrial Cytochrome c Oxidase Subunit I (COI) gene, used for DNA barcoding (Hebert et al., 2003), was reported among subspecies of *S. maurus* in Morocco (Froufe et al., 2008). The following year, seven Moroccan subspecies of *S. maurus* were elevated to the rank of species (Lourenço, 2009), while four new species of *Scorpio* were described from Cameroon, Chad, Niger and Sudan, extending the known distribution of the genus within sub-Saharan Africa (Lourenço, 2009; Lourenço and Cloudsley-Thompson, 2009, 2012; Lourenço et al., 2012).

Two subspecies of *S. maurus*, distinguished by the coloration of the cuticle and subtle differences in the shape and carination of the pedipalp chelae, have long been recognized from the eastern Mediterranean region, including Israel, Sinai, the Golan Heights and the Palestinian territories (Levy and Amitai, 1980). *Scorpio maurus fuscus* (Ehrenberg, 1829) is dark brown to greenish black in color (Fig. 1A) whereas *S. m. palmatus* is light olive-brown to yellow (Fig. 1B). The two subspecies are parapatric, occurring in different habitats, and appear to have distinct ecological requirements (Fig. 2A). *Scorpio m. fuscus* is restricted to areas with a Mediterranean climate, where the average rainfall may exceed 800 mm, in the northern coastal plain, northern Israel and Lebanon, and occurs mostly on terra rossa, basalt, rendzina and brown-red sandy soils. *Scorpio m. palmatus* is restricted to arid and semi-arid habitats of the southern coastal plain, Negev and Judaean deserts, extending through Sinai to Egypt, where the annual rainfall may average less than 50 mm, and occurs on brown-red sandy soils, loess, alluvial soils and in stony desert. Burrow architecture and depth also appear to differ between the two subspecies (Levy and Amitai, 1980). *Scorpio m. fuscus* usually constructs a simple, straight tunnel to a depth of about 40 cm and the burrow entrance is usually situated at the base of a stone, whereas *S. m. palmatus* usually constructs a spiral burrow up to 70 cm deep, with the opening always situated in open ground. Physiological studies revealed that *S. m. fuscus* and *S. m. palmatus* differ in several traits concerning water relations, conferring better desiccation resistance to *S. m. palmatus*, which occurs in more arid areas (Gefen and Ar, 2004; Gefen, 2011; Kalra and Gefen, 2012).

Levy and Amitai (1980) reported that both subspecies co-exist in the northern Negev and Judaean foothills (Fig. 2A), where intermediate color morphs are found, and suggested this was evidence for hybridization (and, hence, a single biological species). Morphological and ecological differences between the two subspecies, mixed color populations (Fig. 1C and D), and suggestions that *S. maurus* is a species complex (Prendini et al., 2003; Froufe et al., 2008) motivated us to examine morphological variation, burrow architecture, and genetic divergence among populations of *S. m. fuscus* and *S. m. palmatus* in the eastern Mediterranean region to assess whether they are conspecific and, if not, how many species might be involved. We analyzed DNA sequences from four gene loci, both mitochondrial and nuclear, separately and in combination.

2. Material and methods

2.1. Fieldwork and material

Scorpions were collected from 39 sites in Israel, the Golan Heights and the Palestinian territories, hereafter referred to as the 'study area' (Fig. 2B; Supplementary material S1), by turning stones and excavating burrows during the day, and by detection with ultraviolet flashlights on warm, moonless nights. After collection, scorpions were preserved individually in 50 ml vials of 99% ethanol and frozen at −20 °C until DNA extraction.

Tissue samples were deposited in the Ambrose Monell Collection for Molecular and Microbial Research at the American Museum of Natural History (AMNH), New York (Supplementary material S2). Adult voucher specimens, collected from the same populations, were deposited in the AMNH Arachnida and Myriapoda Collections.

2.2. Morphological characters

Cuticle base coloration and condition of the digital carina of the pedipalp chela (partially costate vs. entirely granular) were noted for individuals collected (Supplementary material S1, S2). Coloration was assessed by eye in freshly-collected specimens. Pectinal tooth counts and seven measurements, i.e., carapace length and six measurements capturing dimensions of the pedipalp chela (Supplementary material S3), were recorded for 12 male and 35 female specimens from 14 sampling sites. The six pedipalp chela measurements of female specimens were normalized against the first measurement, carapace length, a standard proxy for total body size, and plotted to assess their utility as diagnostic characters.

2.3. Burrow architecture and depth

Sixty burrows of *S. m. fuscus* and *S. m. palmatus* (thirty per subspecies at two sites each) were excavated and examined for burrow structure and depth at four locations across a 33 km north–south transect in southwestern Israel: *S. m. fuscus* at Gevim (site 18) and Nahal Bohu (site 20), and *S. m. palmatus* at Sharsheret (site 25) and Tze'elim (site 29) (Supplementary material S1, Fig. 2B). Each burrow was carefully excavated to expose the burrow structure, from the entrance to the basal chamber. Burrow depth was measured along a perpendicular line, from the surface of the entrance to the surface of the terminal chamber, using a spirit level and a ruler. The configuration of each burrow was captured by a count of the number of curves with angles greater than 30°.