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Genetic diversity within *Scorpio maurus* (Scorpiones: Scorpionidae) from Turkey

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Abstract. In this study, DNA sequence diversity of the mitochondrial (mt) cytochrome oxidase 1 (CO1) gene was investigated in *Scorpio maurus* specimens from across southeastern Turkey. Nucleotide sequences included 508 conserved sites and 126 variable sites, and the mean nucleotide variation within species was 7.9%. Intraspecific pairwise divergences ranged from 0.5% to 10.7%. Phylogenetic analysis indicated high divergence among specimens. This study is the first mtDNA sequence analysis for Turkish scorpions.

Key words: *Scorpio maurus*, intraspecific variation, mitochondrial DNA, CO1, Turkey.

Introduction

Scorpio maurus L. is one of the first species described by Linnaeus (1758). It has long been known as a widespread, polymorphic species (Fet 2000). Birula (1910) first reviewed all *S. maurus* populations in northern Africa and the Middle East. His research indicated that all forms of *S. maurus* are subspecies, but all forms were classified into two groups as "sectio maurus" [(*S. m. maurus* L. (= *S. maurus* L.), *S. m. tunetanus* Birula, 1910 (= *S. punicus* Fet, 2000), *S. m. palmatus* (Ehrenberg, 1828), *S. m. fuscus* (Ehrenberg, 1829), *S. m. subtypicus* Birula, 1910 (= *S. birulai* Fet, 1997), *S. m. magadorensis* Birula, 1910 (= *S. magadorensis* Birula, 1910), *S. m. hesperus* Birula, 1910 (= *S. hesperus* Birula, 1910)] and "sectio propinquus" [*S. m. arabicus* (Pocock, 1900), *S. m. propinquus* (Simon, 1872), *S. m. kruglovi* Birula, 1910, *S. m. towsendi* (Pocock, 1900)]. According to Birula (1910), the members of "sectio maurus" are distributed in Africa, and the members of "sectio propinquus" are distributed in Asia, with the exceptions that *S. m. palmatus* (found in the south of Israel and Sinai) and *S. m. fuscus* (found in the middle and north of Israel) (Levy & Amitai 1980) were classified within the "*maurus*" group. Subsequently, Vachon (1952) reviewed northern African populations, and Levy & Amitai (1980) reviewed populations in Israel and Sinai, and they all accepted Birula's (1910) classification. Fet (2000) reviewed the entire family Scorpionidae and listed nineteen subspecies belonging to *S. maurus*. According to Fet (2000), twelve of the subspecies occur in Africa. Prendini et al. (2003) conducted a molecular analysis of all scorpionid genera based on 12S, 16S, CO1 and 28S rDNA sequence data that included *S. m. fuscus* (Ehrenberg, 1829) from Israel and *S. m. palmatus* (Ehrenberg, 1828) from Egypt. More recently, Froufe et al. (2008) examined CO1 diversity within *S. maurus* from Morocco. Their study included *S. maurus birulai* Fet, 1997, *S. maurus fuliginosus* (Pallary, 1928) and another unidentified subspecies and detected 8.0% sequence divergence within *S. maurus fuliginosus*. Lourenço (2009) reviewed Vachon's (1952) taxa and elevated all eight subspecies that were studied in detail by the latter author and that occur in North Africa to species level but refrained from making taxonomical changes to the remaining 11 subspecies. However, despite these previous studies, the position of Asian populations remains unclear. Additionally, there are no molecular studies of these populations except for those in Israel (Prendini et al. 2003), and Desouky & Alshammari (2011) analyzed *S. m. kruglovi* Birula, 1910 from Saudi Arabia using 16S. Recently, Talal et al. (2015) reviewed *Scorpio* populations in Israel. They confirmed the presence of *Scorpio maurus fuscus* (Ehrenberg, 1829) and *Scorpio maurus palmatus* (Ehrenberg, 1828) in Israel and added *Scorpio maurus propinquus* (Simon, 1872) and *Scorpio maurus kruglovi* Birula, 1910 to the Israeli fauna. Additionally, Talal et al. (2015) elevated these taxa to species level.

Very little is known about Turkish *Scorpio maurus* populations. Birula (1898) first reported *Scorpio maurus* from Turkey (from Gülek Pass, Mersin and Bolkar Mountain) and identified it as *S. m. fuscus*. Later, Birula (1910), Vachon (1947a, 1947b, 1951), Tolunay (1959), Kinzelbach (1984)

and Crucitti & Vignoli (2002) followed and accepted Birula's (1898) identification. More recently, Talal et al. (2015) reported some specimens tentatively as *Scorpio kruglovi* (Ehrenberg, 1829) from Turkey, but they did not provide locality or diagnostic information about the Turkish specimens. However, the known species seems is *Scorpio fuscus* in Turkey at the moment, and it is unclear whether they reported additional records for Turkey or were referring to the population as known *Scorpio fuscus* in Turkey. Additionally, both species are not yellow and do not comprise yellow populations from Turkey. Therefore, we use the name of *Scorpio maurus* for all Turkish populations examined herein.

The mitochondrial (mt) cytochrome oxidase subunit I (CO1) gene is characterized by its relatively large size and the inclusion of both highly conserved and variable regions with different ranges of mutational rates. Because of these properties, it has become a marker of choice to clarify the taxonomic status of species and for molecular identification (Lunt et al. 1996). The CO1 gene has also been extensively used to investigate genetic diversity within species because of its rapid evolutionary rate (Li et al. 2009; Froufe et al. 2008; Xiao et al. 2008). In *S. maurus*, this gene has thus far only been used to assess the diversity within the Moroccan subspecies *S. m. birulai* and *S. m. fuliginosus* (Froufe et al. 2008).

The objective of this study was to determine the COI diversity of *Scorpio maurus* species from southeastern Turkey. Nucleotide variation found in this study was compared to other mitochondrial surveys of scorpions.

Materials and methods

Sampling and DNA isolation

Eleven specimens of *Scorpio maurus* were collected from eleven different geographic locations (Fig. 1). Detailed locality data and GenBank accession numbers are provided in Table 1. The scorpions were preserved in 96% ethanol. Total DNA was extracted from preserved muscle tissue (a leg) using a standard high-salt protocol (Sambrook et al. 1989).

Amplification and sequencing of mitochondrial DNA fragments

A fragment of the mitochondrial cytochrome *c* oxidase subunit I gene was amplified by polymerase chain reaction (PCR) using the primers LCO1490 ((5'GGGTCAACAAAATCATAAAGATATTGG3') and HCO2198 (5'TAAACTTCAGGGTGACCAAAAAATCA3') (Folmer et al. 1994). DNA was amplified at a 50 µl final volume containing 0.9X PCR buffer, 2.5 mM MgCl2, 10 mM of each dNTP, 0.1 µM forward and 0.1 µM reverse primer, 50 ng template DNA and 1 µL Taq polymerase. Amplifications were performed with an initial denaturation at 94ºC for 1 min, 5 cycles of 94ºC for 1 min 30 sec, 53ºC for 30 sec, 72ºC for 1 min, followed by 35 cycles of 94ºC for 30 sec, 53ºC for 1 min, 72ºC for 1 min and completed after a final elongation at 72 ºC for 5 min After purification of the PCR product, sequences were obtained on an ABI 310 automated sequencer.

Sequence analyses

All new DNA sequences were submitted to GenBank [http://www.ncbi.nlm.nih.gov] (Table 1). Thirteen CO1 mtDNA sequences published previously by Froufe et al. (2008) and Prendini et al. (2003) (AY156585. AY156584. FJ198057, FJ198058. FJ198059, FJ198064. FJ198060, FJ198065, FJ198066, FJ198061, FJ198062, FJ198063, AY156575) were extracted from GenBank and used in subsequent analyses. As an outgroup we selected *Heterometrus swammerdami* Simon, 1872, classified in the genus of the family Scorpionidae, which is distributed widely across tropical and subtropical southeastern Asia (Prendini et al. 2003; Kovařík 2004).

All sequences were imported into MEGA 6 (Tamura et al. 2013) and aligned using ClustalW (Thompson et al. 1994) to determine genetic distance and conduct Maximum Parsimony (MP) and Maximum Likelihood (ML) analyses. Node support was determined with 1000 bootstrap pseudoreplicates for both the MP and the ML analyses. For the ML analysis, the Tamura-Nei + G model was identified as the best substitution model using MODELTEST version 3.7 (Posada & Crandall 1998). This model of evolution was also used to construct the NJ tree (Saitou & Nei 1987). Tamura and Nei genetic distances (Tamura & Nei 1993) were used to estimate sequence divergence values.

Results

A total of 634 base pairs from 11 new specimens from different locations in southeastern Turkey were sequenced and aligned with the outgroup and 12 previously sequenced specimens. The aligned sequences, including specimens from southeastern Turkey, comprised 508 constant sites and 126 variable sites, 75 of which were parsimony informative.

The CO1 sequences were AT rich, with an average incidence of A=20.2%, C=13.9%, G=22.8% and T=43%. These nucleotide frequencies are consistent with those from the 12 *Scorpio maurus* sequences from GenBank (A=20.2%, C=13.7%, G=22.9%, and T= 43.1%). The average nucleotide diversity (P*i*) for the 11 sequences was 0.079. The

Table 1. Localities and GenBank accession numbers for *Scorpio maurus* and the outgroup *Heterometrus swammerdami*.

Population	Code	Taxon	Sex	Country	Province	Location	GenBank	Habitat	Color
1	SeqAT301	*Scorpio maurus*	♀	Turkey	Hatay	36°57'03"N, 36°14'56"E, 297m, Erzin, İçmeler Area	KF997866	Habitat comprising pine forest and *Quercus coccifera* bushes	Red
2	SeqAT302	*Scorpio maurus*	♂	Turkey	Adıyaman	37°47'39"N, 37°36'55"E, 1000m, Gölbaşı, Akçalar Village	KF997867	Steppe habitat includes *Quercus* spp. bushes	Red
3	SeqAT303	*Scorpio maurus*	Juv.	Turkey	Malatya	37°58'30"N, 38°08'10"E, 1457m, Doğanşehir, Kurucuova Village	KF997868	An open steppe area	Red
4	SeqAT304	*Scorpio maurus*	?	Turkey	Gaziantep	36°54'10.7"N, 38°00'3.9"E, 347m, Karkamış, Gürçay Village	KF997869	An open steppe area	Yellow
5	SeqAT305	*Scorpio maurus*	?	Turkey	Şanlıurfa	36°54'01"N, 38°03'16"E, 391m, Birecik, Çiçekalan Village	KF997870	An open steppe area	Yellow
6	SeqAT306	*Scorpio maurus*	♀	Turkey	Şanlıurfa	36°49'53"N, 39°22'10" E, 426m, Harran, Tektek Mountains	KF997871	Steppe habitat that includes rocky areas	Yellow
7	SeqAT307	*Scorpio maurus*	Juv.	Turkey	Şanlıurfa	37°32'26"N, 39°49'26" E, 1303m, Siverek, Karacadağ Mountain	KF997872	Steppe habitat that includes rocky areas	Yellow
8	SeqAT308	*Scorpio maurus*	?	Turkey	Diyarbakır	38°10'39"N, 40°05'20"E, 832m, Eğil, Kalkan Village	KF997873	Steppe habitat that includes *Quercus* spp. bushes	Yellow
9	SeqAT309	*Scorpio maurus*	♂	Turkey	Diyarbakır	38°26'59"N, 40°26'36"E, 1200m, Hani, Kalaba Village	KF953944	Steppe habitat that includes *Quercus* spp. bushes	Yellow
10	SeqAT310	*Scorpio maurus*	♀	Turkey	Mardin	37°28'33"N, 41°22'19"E, 694m, Midyat, Barıştepe Village	KF997874	An open steppe area	Yellow
11	SeqAT311	*Scorpio maurus*	Juv.	Turkey	Kilis	36°52'47"N, 36°47'42"E. 755m, Musabeyli, Hasancalı Village	KF997875	Steppe habitat that includes *Quercus* spp. bushes	Red
12		*Heterometrus swammerdami*					AY156575		

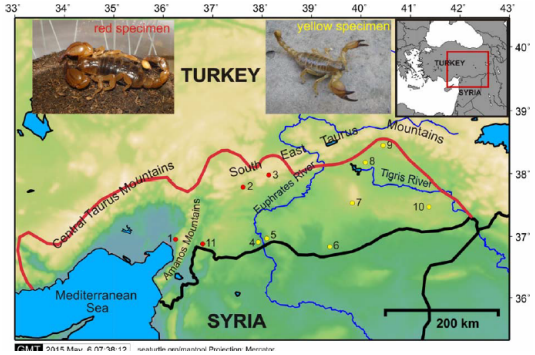

Figure 1. Map showing the collecting localities of the *Scorpio maurus* specimens from southeastern Turkey. The numbers correspond to the localities in Table 1. The red line indicates the northern limit of *Scorpio maurus* in Turkey. Red and yellow dots indicate red and yellow specimens of *Scorpio maurus*.

Table 2. Genetic distances between *Scorpio maurus* specimens from Turkey.

	SeqAT 301	SeqAT 302	SeqAT 303	SeqAT 304	SeqAT 305	SeqAT 306	SeqAT 307	SeqAT 308	SeqAT 309	SeqAT 310
SeqAT301										
SeqAT302	0.040									
SeqAT303	0.042	0.057								
SeqAT304	0.067	0.094	0.094							
SeqAT305	0.087	0.096	0.100	0.055						
SeqAT306	0.076	0.097	0.106	0.087	0.097					
SeqAT307	0.081	0.107	0.109	0.088	0.101	0.068				
SeqAT308	0.078	0.098	0.107	0.089	0.103	0.005	0.066			
SeqAT309	0.081	0.098	0.106	0.092	0.104	0.055	0.084	0.061		
SeqAT310	0.055	0.079	0.081	0.068	0.093	0.053	0.070	0.054	0.058	
SeqAT311	0.064	0.086	0.092	0.093	0.107	0.059	0.077	0.065	0.063	0.058

calculated pairwise genetic distances between the 11 new sequences are presented in Table 2. The maximum pairwise divergence is 10.9%.

The MP and the ML were nearly identical. The MP and ML trees did not differ regarding well-supported clades (Fig. 2a-b). The trees indicate two major clades, one comprising specimens from Turkey, Egypt and Israel (clade 1), and the other comprising specimens from Morocco (clade 2). Clade 1 has 80% bootstrap support in the MP analysis and 91% bootstrap support in the ML analysis. Specimens from Turkey were divided into three subgroups, but these are not well-supported. A minimum genetic distance of 8.9% occurs between subgroup 1 (localities 6–11 in Fig. 1) and subgroup 2 (localities 1–3 in Fig.1), and a maximum genetic distance of 9.4% occurs between subgroup 1 and subgroup 3 (localities 4 and 5 in Fig. 1). Clade 2 comprises specimens from Morocco, but this clade is poorly supported in both analyses (41% and 44% for MP and ML analyses, respectively).

Discussion

Sequence analysis of a partial mitochondrial CO1 gene segment has been used to detect the genetic diversity of *Scorpio maurus* from Turkey. The CO1 sequences revealed a high degree of intraspecific variability with no shared haplotypes between localities. The maximum uncorrected pairwise divergences ranged from 0.5% (SeqAT306–SeqAT308) to 10.9% (SeqAT305–SeqAT311 and SeqAT307–SeqAT302) (Table 2). This result is consistent with the results of Froufe et al. (2008) who found highly divergent mtDNA lineages in Moroccan samples, especially in *S. maurus fuliginosus* (8.0%), and detected approximately 10% maximum uncorrected pairwise divergence. As Froufe et al. (2008) commented, these results support that *S. maurus* may be a species complex. More recently, considerable genetic divergence was reported in *S. maurus* from 39 sites in Israel, Gallon Heights and Palestinian territories. Subspecies of *Scorpio maurus* has been recognized as a species under the phylogenetic, ecological and biological species concepts by evaluating diagnostic differences in morphology and burrowing behavior, and considering cuticle color, pedipalp chela digital carina and burrow architecture (Talal et al. 2015). Oppositly Levy and Amitai (1980), they didn't detected any hybrid populations and they found all subspecies allopatric or parapatric. Therefore Talal et al. (2015) desided that subspecies of *Scorpio maurus* in Israel are one each biological species. Subsequently, this species complex was resolved by Lourenço (2009) for North African specimens using hemispermatophore and genital operculum morphology.

According to Birula's (1910) classification, the "maurus" group includes North African populations and also includes the Middle Eastern subspecies *S. m. palmatus* and *S. m. fuscus*. In our study, these subspecies are grouped with the subspecies that are included in the "propinquus" group (*S. m. arabicus*, *S. m. propinquus*, *S. m. kruglovi*, *S. m. towsendi*). These results indicate that all Asian populations most likely can be grouped in the "propinquus" group, which is a more parsimonious hypothesis than each group comprising specimens from both Africa and Asia. Additional morphological and molecular studies that include a broader sampling of the eastern diversity of the genus may justify this hypothesis in the future.

Froufe et al. (2008), using Bayesian phylogenetic methods, found that the largest divergence occurred between Asian and African populations.

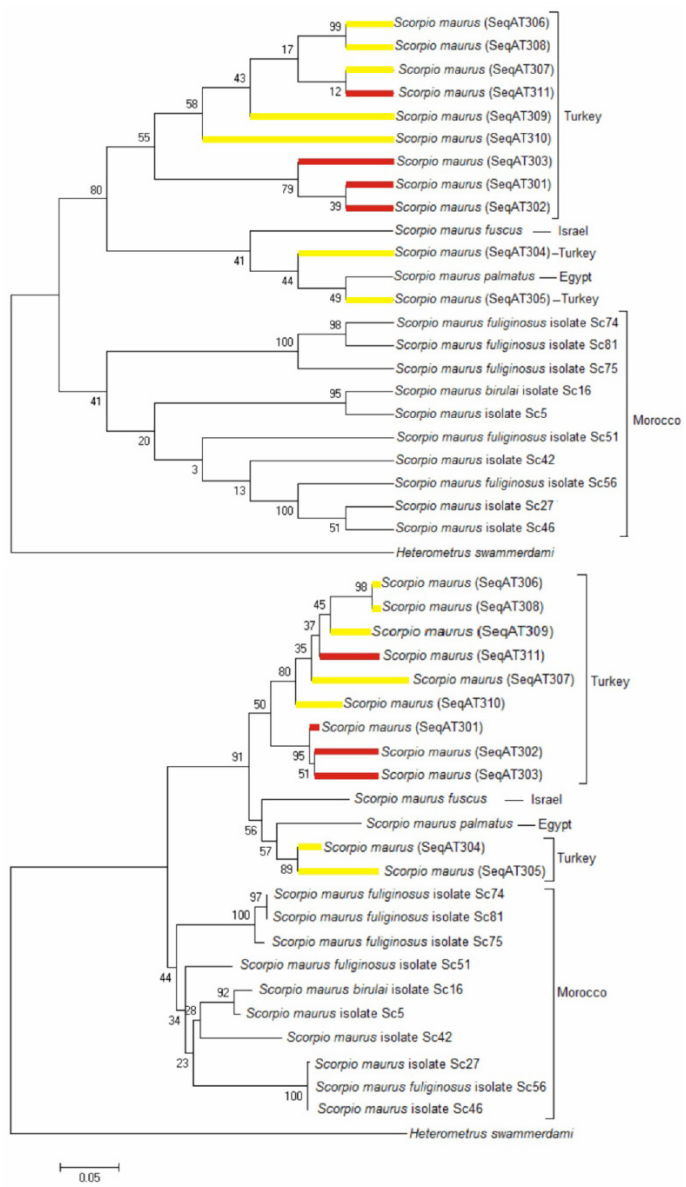

Figure 2. Phylograms showing the relationships estimated using Maximum Parsimony (A) and Maximum Likelihood (B). Bootstrap values (1000 pseudoreplicates) are indicated at the nodes. Red and yellow branches indicate red and yellow specimens of *Scorpio maurus*.

Our results are consistent with these results and indicate deep divergence between Asian and African populations. Phylogeographic analyses of COI and multilocus (28S rDNA, 12S rDNA, 16S rDNA, Cytochrome c Oxidase Subunit I) datasets recovered seven geographically-delimited clades of *Scorpio maurus*, corresponding to *S. m. fuscus, S. m. palmatus, S. m. kruglovi, S. m. propinquus* from Israel, Gallon Heights and Palestinian territories in the eastern Mediterranean region (Talal et al. 2015). Specimens from these clades were genetically distant from each other and had different burrow architecture and depth. Additionally, although they were in geographically proximate localities, they were reproductively isolated (Talal et al. 2015).

S. maurus populations are common in the eastern Mediterranean and southeastern Anatolian regions of Turkey. These populations comprise both red and yellow specimens. According to Levy & Amitai (1980) color variability is important for indicating subspecies of *S. maurus*. SeqAT301–03

and SeqAT311 are red specimens that were collected in redzina soil to the west of the Euphrates River. SeqAT305–10 are yellow specimens that were collected to the east of the Euphrates River in calcareous terra rosa soil. However, SeqAT304, a yellow specimen, was collected to the west of the Euphrates in calcareous terra rosa soil. But SeqAT307, a yellow specimen was collected from Karacadağ Mountain which is a volcanic area with dark soil. It appears that yellow and red specimens are typically found on substrates similar to their own habitus color, but this isn't always the rule. Our results indicate genetic divergence between yellow and red groups of specimens, and this color variability could represent species level differences but may also indicate localized natural selection. However, two yellow specimens, SeqAT304 (Gaziantep, Karkamış) and SeqAT305 (Şanlıurfa, Birecik), grouped most closely with *S. m. palmatus* (Egypt) (a yellow subspecies) and *S. m. fuscus* (Israel) (a dark red subspecies). These two specimens were collected at low elevations on opposite banks of the Euphrates River near Turkish-Syrian border. These specimens may be closely related, and their ancestral population may have dispersed along the semi-arid Syrian plain. All specimens from Turkey were more closely related to samples from Egypt and Israel than they were to specimens Morocco.

The specimens SeqAT302 and SeqAT303 from near Adıyaman and Malatya, respectively, are both red and are clustered in a subclade within the larger Turkish clade. Although these specimen found from northern and southern sides of Taurus Mountains.

The fauna of southeastern Turkey is unique and geographically different from the rest of Turkey (Crucitti & Vignoli 2002; Yağmur et al. 2013). The distribution of *S. maurus* in this region is restricted by the South East Taurus Mountains in the north and the Amanos Mountains in the west. In southeastern Turkey, there are two important geographical barriers, the Euphrates River and the Amanos Mountains. However, these barriers do not appear to affect the distribution of *S. maurus*. Similarly, the Euphrates River is not a barrier to dispersal to the scorpion species *Androctonus crassicauda*, *Compsobuthus matthiesseni*, *Leiurus abdullahbayrami*, *Mesobuthus phillipsii* and *Calchas birulai*. By contrast, *Buthacus macrocentrus* is not found west of the Euphrates River, and *Mesobuthus nigrocinctus* is not found east of the Euphrates River. The Amanos Mountains also restrict scorpion species. For example, *Mesobuthus gibbosus* and *M. nigrocinctus* are distributed to the west and the east of Amanos Mountain, respectively. Only *Protoiurus asiaticus* is distributed along the foothills of the Taurus Mountains and may occupy a small area in southeastern Turkey (Kovařík et al. 2010). Additionally, two vertebrate species, the snakes *Rhynchocalamus melanocephalus saturnini* (Avcı et al. 2007) and *Eirenis lineomaculatus* (Göçmen et al. 2014) have similar distribution patterns to *S. maurus* in this region.

In conclusion, this study provides a first preliminary survey of the CO1 variability of *Scorpio maurus* from Turkey. This study indicates that Turkish *S. maurus* populations may be considered a species complex. Thus, additional detailed morphological data and genetic data, including more species and nuclear data, are required to resolve the species diversity of the *Scorpio* complex.

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References

Avcı, A., Ilgaz, Ç., Kumlutaş, Y., Olgun, K., Baran, İ. (2007): Morphology and distribution of *Rhynchocalamus melanocephalus satunini* (Nikolsky, 1899) in Turkey. Herpetozoa 20(1/2): 82-86.

Birula, A.A. (1898): Ein Beitrag zur Kenntnis der Skorpionenfauna Kleinasiens. Horae Societatis Entomologicae Rossicae 33: 132-140.

Birula, A.A. (1910): Über *Scorpio maurus* Linné und seine Unterarten. Horae Societatis Entomologicae Rossicae 35: 115-192.

Crucitti, P., Vignoli, V. (2002): Gli scorpioni (Scorpiones) dell'Anatolia sudorientale (Turchia). Bollettino del Museo Regionale di Scienze naturali 19(2): 433-480.

Göçmen, B., Geçit, M., Karış, M. (2014): New locality records of the Striped Dwarf Snake, *Eirenis lineomaculatus* Schmidt, 1939 (Squamata: Ophidia: Colubridae). Biherean Biologist 8(2): 126-128.

Desouky, M.M.A., Alshammari, A.M. (2011): Scorpions of the Ha'il Region, northern Saudi Arabia, and molecular phylogenetics of two common species, *Androctonus crassicauda* and *Scorpio maurus kruglovi*. Bulletin of the British Arachnological Society 15(6): 193-200.

Fet, V. (2000): Family Scorpionidae Latreille, 1802. pp. 427-486. In: Fet, V., Sissom, W.D., Lowe, G., Braunwalder, M.E. (eds.), Catalog of the Scorpions of the world (1758-1998). The New York Entomological Society.

Folmer, O., Black, M.B., Hoch, W., Lutz, R.A., Vrijehock, R.C. (1994): DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan

invertebrates. Molecular Marine Biology and Biotechnology 3(5): 294-299.

Froufe, E., Sousa, P., Alves, P.C., Harris, D.J. (2008): Genetic diversity within *Scorpio maurus* (Scorpiones: Scorpionidae) from morocco: Preliminary evidence based on CO1 mitochondrial DNA sequences. Biologia 63(6): 1157-1160.

Kinzelbach, R. (1984): Die Skorpionssammlung des Naturhistorischen Museums der Stadt Mainz, -Teil II: Vorderasien. Mainzer Naturwissenschaftliche Archiv 22: 97-106.

Kovařík, F. (2004): A Review of the Genus *Heterometrus* Ehrenberg, 1828, with Descriptions of Seven New Species (Scorpiones, Scorpionidae). Euscorpius 15: 1-60.

Kovařík, F., Fet, V., Soleglad, M.E., Yağmur, E.A. (2010): Etudes on iurids, III. Revision of the genus *Iurus* Thorell, 1876 (Scorpiones: Iuridae), with a description of two new species from Turkey. Euscorpius 95: 1-212.

Levy, G., Amitai, P. (1980): Fauna Palaestina, Arachnida I: Scorpiones. Israel Academy of Sciences and Humanities, Jerusalem.

Li, Y., Yang, B., Wang, H., Xia, R., Wang, L., Zhang, Z., Liu Y. (2009): Mitochondrial DNA analysis reveals a low nucleotide diversity of *Caligula japonica* in China. African Journal of Biotechnology 8(12): 2707-2712.

Linneaus, C. V. (1758): Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentis, synonymis, locis. 10th Edition. Laurentii Salvii, Holmiae.

Lourenço, W.R. (2009): Reanalysis of the genus *Scorpio* Linnaeus 1758 in sub-Saharan Africa and description of one new species from Cameroon (Scorpiones, Scorpionidae). Entomologische Mitteilungen Aus Dem Zoologischen Museum Hamburg 15(181): 99-113.

Lunt, D.H., Zhang, D.X., Szymura, J.M., Hewitt G.M. (1996): The insect cytochrome oxidase I gene: evolutionary patterns and conserved primers for phylogenetic studies. Insect Molecular Biology 5: 153–165.

Posada, A., Crandall, K.A. (1998): Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817-818.

Prendini, L., Crowe, T.M., Wheeler, W.C. (2003): Systematics and biogeography of the family Scorpionidae (Chelicerata: Scorpiones), with a discussion on phylogenetic methods. Invertebrate Systematics 17(2): 185-259.

Saitou, N., Nei, M. (1987): The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406-425.

Sambrook, J., Fritsch, E.F., Maniatis, T. (1989): Molecular cloning: A laboratory manual. Volume 2. Cold Spring Harbor Laboratory, New York.

Talal, S., Tesler, I., Sivan, J., Ben-Shlomo, R., Tahir, H.M., Prendini, L., Snir, S., Gefen, E. (2015): 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. Molecular Phylogenetics and Evolution 91: 226-237.

Tamura, K., Nei, M. (1993): Model selection in the estimation of the number of nucleotide substitutions. Molecular Biology and Evolution 10: 512-526.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. (2013): MEGA6: Molecular evolutionary genetics analysis Version 6.0.. Molecular Biology and Evolution 30: 2725–2729.

Thompson, J.D., Higgins, D.G., Gibson, T.J. (1994): CLUSTAL W: improving the sensitivity of progressive multiple alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22(22): 4673-4680.

Tolunay, M.A. (1959): Zur Verbreitung der Skorpione in der Türkei. Zeitschrift für Angewandte Entomologie 43 (4): 366-370.

Vachon, M. (1947a): Remarques préliminaires sur la faune des scorpions de Turquie. Bulletin du Museum National d'Histoire Naturelle 19(2): 161-164.

Vachon, M. (1947b): Repartition et origine des scorpions de Turquie. Comptes Rendus des Séances de Société de Biogéographie 24(206-208): 26-29.

Vachon, M. (1951): A propes de quelques Scorpions de Turquie collectés par M. le Professeur Dr. Curt Kosswig. İstanbul Üniversitesi Fen Fakültesi Mecmuası 16(4): 341-344.

Vachon, M. (1952): Études sur les scorpions. Publications de l'Institut Pasteur d'Algérie, Algeria.

Xiao, Y.S., Zhang, Y., Gao, T.X., Yabe, M., Sakurai, Y. (2008): Phylogenetic relationships of the lancelets of the genus *Branchiostoma* in China inferred from mitochondrial genome analysis. African Journal of Biotechnology 7(21): 3845-3852.

Yağmur, E.A., Soleglad, M.E., Fet, V., Kovařík, F. (2013): Etudes on iurids, VI. Further revision of *Calchas* Birula, 1899 (Scorpiones: Iuridae), with a description of a new genus and two new species. Euscorpius 159: 1-36.