Acta Scientific Biotechnology

Volume 4 Issue 4 August 2023

Research Article

Molecular Phylogeny of Scorpions in UAE

Raghad Motaz Faisal*, Hadil Aziz Saeed, Salama Khalid Ibrahim, Fatima Abdalla Ahmed, Muram Ahmed Saeed, Ali Aref Ahmed, Mustafa Anwar Sami and Abdulrahman Jamal Saeed

Department of Applied Biology Research Project Course, University of Sharjah College of Science, UAE

***Corresponding Author:** Raghad Motaz Faisal, Department of Applied Biology Research Project Course, University of Sharjah College of Science, UAE.

Received: July 19, 2023

Published: November 09, 2023

Abstract

In phylogenetic investigations, mitochondrial DNA (mtDNA) has been frequently used. Scorpion samples were obtained from several locations across the UAE for this investigation. The 16S mitochondrial gene, a component of mitochondrial DNA, was used to identify the scorpions genetically. *Androctonus australis* (Linnaeus, 1758), *A. amoreuxi* (Audouin, 1826), *A. crassicauda* (Olivier, 1807), and *A. bicolor* (Ehrenberg, 1828) are the four primary medically noteworthy scorpion varieties. Phylogenetic analysis of the obtained sequences confirmed the morphological characterisation. For the first time, the 12S rDNA sequences of *Hottentotta saulcyi, Mesobuthus caucasicus, Androctonus crassicauda,* and *Mesobuthus eupeus,* as well as the 16S rDNA sequences of *Hottentotta saulcyi*, are published. We came to the conclusion that mitochondrial identification markers are useful for identifying these medically important scorpion species. The small genetic variation between the groups was validated, and the taxonomic validity of scorpions was confirmed using molecular data, according to the findings of this study. According to the 16S rRNA sequencing data, the genus appears to be represented in the UAE by a single scorpion species. The goal of this research was to identify the nucleotide sequence of the 16S rRNA mitochondrial gene in several scorpion species found in the United Arab Emirates, as well as to see if there was any genetic correlation with the colour differences observed among various species of these regions. The current study's nucleotide variations are the first descriptive report for scorpions in UAE.

Keywords: *Mesobuthus eupeus; Androctonus australis*; UAE

Introduction

Scorpion

Scorpion, any of roughly 1,500 extended spider species distinguished by a segmentation curving tail topped with a deadly stinger at the base of the spine and a set of gripping pincers at the front. Scorpions, mainly nocturnal, are frequently depicted as evildoers in tales and folklore. The Greeks revered scorpions; thus, the constellation Scorpius, a zodiac sign, was named after them [1]. Though scorpions are the most widespread and diversified in deserts, they may also be found in various other environments. Scorpions have been amended to subtropical, temperate, andtropical ecosystems such as savannas, grasslands, and forests, in additional to desert environments [1].

Scorpion morphology has altered slightly since Silurian Period. As a result, their body layout is quite rudimentary. During evolution, segments and accompanying features were lost or merged from primitive arthropods and arachnids to more highly developed successors. Scorpions get more sections than every arachnid and have a highly segmented heart and nerve system. The use of book lungs instead of tracheae for breathing is likewise primitive. The prosoma, mesosoma, and metasoma are the three primary sections

that make up the body, from front to back. The abdomen,or opisthosoma, is formed by mesosoma and metasoma. The prosoma is divided into six segments,each with two appendages. The pincer-like three-segmented chelicerae emerge from masticating food's first section [1]. They are some of the dominant terrestrial arthropods, including average size of 6 cm. An African variety native to Guinea, with a length of around 18 cm and a weight of 60 grams.

Scorpions are indiscriminate predators that ingest any little animal they can catch. Spiders, Insects,and other arachnids, comprising other scorpions, are common prey. Snails, pillbugs, and small vertebrates such as snakes, lizards, and rodents are less frequent but regular prey. The only notorious specialized scorpion is the Australian spiral burrow scorpion which hunts only on burrowing spiders. Many scorpions are apex predators who wait patiently for a good target to moveinto an assault region. Scorpions lack typical jaws and have peculiar eating patterns. The second set of pincer-like appendages are toothed, and the scorpion grinds the prey with all these claws andthe sharp corners of adjoining jaw-like appendages (maxillae) coxae) as gastric juices released from the midgut flow over it [1].

Medical uses

According to the research, scorpion venom is a rich source of bioactive chemicals, and soits toxins are of significance to the biotech and pharmaceutical sectors [2]. Despite the reality thatsignificant research is being conducted and the possibilities for scorpion-derived medicinalpeptides are highly intriguing, chlorotoxin seems to be the only neurotoxin from scorpion venomtested in clinical trials [3]. In 1991, research stated that the packing structure of charybdotoxin, a KTx discovered from *L. quinquestriatus* hebraeus venom, was very comparable to the insect antibacterial component defending [4]. Stigmurin, which was discovered and synthesized after a transcriptome study of the *T. stigmurus* venom gland, has antibacterial and antifungal action. It isefficient towards Gram-positive species of bacteria, include methicillin-resistant strains of *S. aureus*. Stigmurin has been shown to be effective against the fungus *Candida albicans*, *Candida krusei*, and *Candida glabrata*, with negligible toxicity to healthy human erythrocytes [5]. Mucroporin-M1, a variant of mucroporin from Lychas mucronatus venom, hos antiviral activity towards three RNA viruses; SARS-CoV, measles (MeV), and influenza H5N1. Chlorotoxin (CTx) is a chemical found in the venom of *L. quinquestriatus* that reacts with chloride channels. CTx wasfirst scorpion-derived drug to be shown to suppress glioma cell invasion and migration. It also shown the ability to intercept deep into tumour tissue [6].

Phylogeny

Scorpions are a varied and prolific group of Arachnids, with over 2581 species now identified [7]. In addition, there are over 120 identified fossilized scorpion varieties [8]. They dateback to the Silurian period, about 400 million years ago, rendering them the oldest unequivocallyknown arachnids [9]. In their "Catalog of Scorpions of the World," Fet and Lowe (2000)recognized 73 genera and 529 species, although it is likely that several species occur in nature [10]. This group also contains one of the most important evolutionary lineages of extant scorpions, known as orthobothriotaxic Type A [11]. Androctonus bicolor Ehrenberg, 1828 is among the mostperplexing species because its specimens have never been re-examined, and the taxonomicclassification was never changed as per contemporary standards. The first issue is that the initial explanation is quite inadequate and includes unclear facts that most subsequent authors have misconstrued or disregarded [12].

Historically, much of our understanding of Saudi Arabia's scorpion fauna relied on specimens gathered randomly around the kingdom. Numerous writers have accumulated and consolidated significant knowledge on Saudi Arabia's scorpion fauna and biology throughout the last decades [13-15]. Hendrixson studied the phylogeny of Saudi Arabia's buthid scorpion genera. His findings confirm the existence of 17 nominal buthid species among 10 genera, including the description offive species (Figure 6). Although its relevance, this work represents the aspects of Saudi Arabia's geographical areas. Several articles in the decades after that have made substantial contributions to our developing understanding of the ecology, composition and bio-geography of scorpions in Saudi Arabia [16,17].

The four main medically significant scorpion varieties are *Androctonus australis* (Linnaeus, 1758), *A. amoreuxi* (Audouin, 1826), *A. crassicauda* (Olivier, 1807) and *A. bicolor* (Ehrenberg, 1828) [18]. The morphological characterization was confirmed by phylogenetic analysis of the acquired sequences. The 12S rDNA sequences of *Hottentotta saulcyi, Mesobuthus caucasicus, Androctonus crassicauda* and *Mesobuthus eupeus,* as well as the 16S rDNA sequences of *Hottentotta saulcyi*, are presented for the first time. We concluded that mitochondrial identification markers are beneficial

for the species identification in these medically relevant scorpion species [19].

Molecular phylogenetic study of COI with 1000 bootstrap repetitions using the MaximumLikelihood technique. The tree having greatest log probability (4375.20) is displayed. The final dataset had total of 698 locations. The percentage of trees with the related taxa group is presentednear the branches. The tree is presented to scale, and branch lengths are measured relative to the number of mutations per site. Asterisks denote sequences retrieved as part of this effort (Figure 1)[19].

Molecular markers

Although, many molecular markers have been used for the phylogenetic studies of the species in the last few decades. Some of which include Nuclear ribosomal genes (5S rRNA, 16S rRNA, 28S rRNA), Mitochondrial genes (mtDNA) (Mitochondrial 12S, Cytochrome oxidase I/II(COI/II), Cytochrome-b), and Chloroplast genes (matK, rbcL, rpl16, ndhF). These genes are conserved in sequences and show less variation than the genera. So it is easy to use these markersto identify the species and analyze the phylogenetic relationship of species. After obtaining the sequences of the respective genetic markers, phylogenetic trees were constructed using different analysis methods. Some methods are character-based methods (Maximum Likelihood and Maximum Parsimony) and some distance-based characters (Neighbor-Joining, Generalized NJ, UPGMA, Minimum evolution). Even though many phylogenetic markers are accessible, scientistsshould not be constrained to these genes alone. In reality, it needs the development of new phylogenetic markers.

Mitochondrial DNA (mt-DNA)

Mitochondrial DNA data has the potential to be extremely helpful in solving species-level phylogenies. The sequence of genomes in the mitochondrion varies, and hugenoncoding DNA sections isolate them. The mitochondrial DNA often reorganizes itself, allowing many reshuffled forms to coexist in the same cell body. The practice of mtDNA has developed progressively popular in the population genetic and phylogenetic studies because of i) the use of restriction enzymes to identify nucleotide differences, ii) improvements in methodology for mtDNA isolation, iii) applicability of the universal primers for amplification of mt-DNA, and iv) the advances of the PCR methodologies in molecular biology [20].

The enzyme cytochrome C oxidase is a very crucial electron transport chain proteinpresent in both mitochondria and bacteria. The COI and COII genes encode two of the cytochrome c oxidase complex's seven polypeptide subunits. The COI gene is approximately 894 bps long. Compared to other protein-coding mitochondrial genes, the COI gene evolves gradually and is commonly utilized to estimate molecular phylogenies [21]. It is an excellent performer for recovering an anticipated tree [22]. The examination of mitochondrial 12S rRNA gene sequences is widely employed in molecular taxonomy and phylogeny. Previously, the mitochondrial 12S rRNA gene sequence was utilized to determine species in wildlife forensic biology [23]. The cytochrome-b gene (1,143 bp) has been considered the most helpful marker in reconstructing phylogenetic connectionsamong closely related taxa; however, it can lose clarity at deeper nodes [23].

Methodology

DNA extraction

Genomic DNA was extracted from each specimen's legtissue using G- spin DNA extraction kit (CAT NO: 17045). The leg tissue of 25 mg was measured and transferred to 1.5 ml tube using spatula. Buffer CL of 200ul, proteinase K of 5ul and RNase A of 5 ul provided by the kit is added to the tube containing scorpion tissue. The mixture was then subjected to heat at 56˚ C for 10 – 30 minutes. When lysis is completed, 200ul of Buffer BL is added and the mixture was incubated at 70˚ C for 5 minutes. The sample was centrifuged at 13,000 rpm for 5 minutes to remove the unlysed tissue particles. Then Carefully, 350 – 400ul of the supernatent was added to a new 1.5 ml tube. Absolute ethanol of 200ul is added to lysate and it is mixed well by vortexing. The solution was then transferred to column tube. Then the washing step takes place. After Washing, The DNA binded to column is eluted by elution buffer.

PCR – polymerase chain reaction

Master mix Firepol 5X 12.5mM Mgcl2 (CAT: 04-11-00125) was used and the PCR conditions for amplification of all fragments were as follows: initial denaturation at 95 °C for 5 min; 35 cycles of 95 °C for 40 s, 48 °C for 40 s, 72 °C for 1 min 30 s and a final extension at 72 °C for 10 minutes.

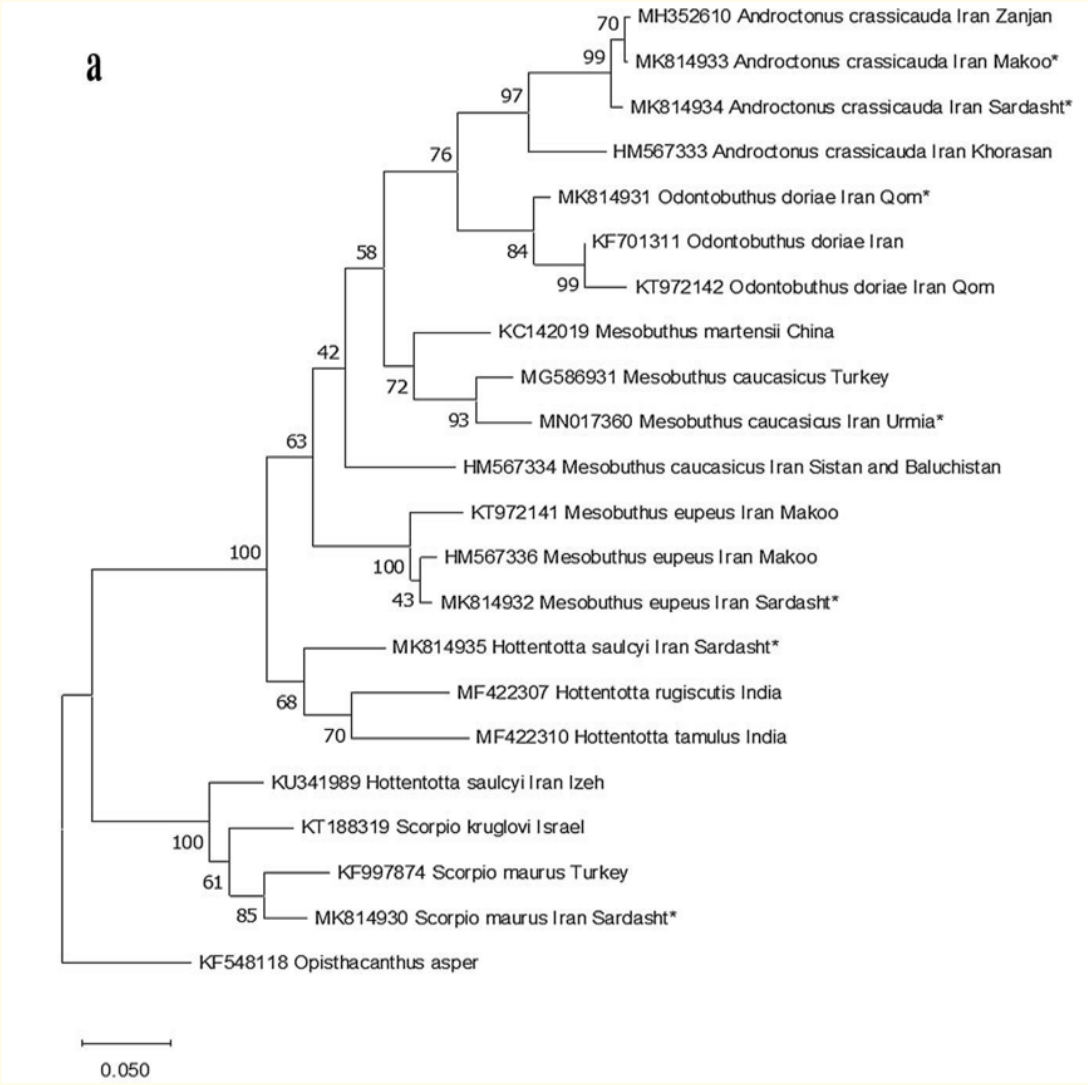


Figure 1: Molecular phylogenetic study of COI with 1000 bootstrap repetitions using the Maximum Likelihood technique.

The primers used,	
a) 28Sa	GACCCGTCTTGAAGCACG
28Sbout	CCCACAGCGCCAGTTCTGCTTACC
b) 12S rDNA	
12Sai	AAACTAGGATTAGATACCCTATTAT
12Sbi	AAGAGCGACGGGCGATGTGT
c) 16S rDNA	
16Sbr	CTCCGGTTTGAACTCAGATCA
16Sar	CGCCTGTTTATCAAAAACAT
d) Cytochrome Oxidase I	
HCO	TAAACTTCAGGGTGACCAAAAAATCA
LCO	GGTCAACAAATCATAAAGATATTGG
e) HCOEXTa	GAAGTTTATATTTTAATTTTACCTGG
HCOEXTb	CCTATTGAARAACATARTGAAAATG

Table a

Polymerization of 22 sequences was performed with above primers (Table 1).

Sequence ID	Sequence
>SC-1 28S F	AAAAAGRCWSTGCSAGTMKTCCWYCTTTGGATACGCCTGACGGCGCTRTG CTTCTGGTCTTGAAMTCRWTGWCACTCGGGAK-AGGCTTACCGCCCCGTCC TGCCTGGTTGCCATCRCTTTCTCTCCCAGWCTAACATTCTATCGGTTTCC ATAATTAACTGACAS-GTTGGGACCAAATGGATGGTGTGGGATGCCTGGC AGGACGAGGCCAGAGGAAACTCTGGTGGAGGTCCGCAGCGATTCTGAC GT GCAAATCGATCGTCAGACCTGGGTATAGGGGCGAAAGACTAATCGAACA TCTAGTAGCTGGTTCCCTCCGAAGTTTCCCT-CAGGATAGCTGGCGCTGA AGGGAGTCAGTCTCATCCGGTAAAGCGAATGATTAGAGGCCTTGGGGCCG AAACGACCT-CAACCTATTCTCAAACTTTCAATGGGTGAGAAGTCCGGTTT ACATGACTGAAGCCCGGACAATTCTCAGGATGTGAGT-GCCCAGTGGGCCA CTTTTGGTAAGCAGAACTGGCGCTGTGGGA
>SC-1 28S R	AGGYCTARGGGGGAAACSATCCWGGKATTGTCCGGGCTTCAGTAATGCWA ACYGGACTTCTCRACCTTTGAAAGTTTGARA-AAAGGTGGAGGTCGTTTCG ACCCCAGGCCTCTAATCWTTCGYTTTACCGGAGGARACTGACTCCCTTCK AGCGCCAAMTATCCT-GASGGAAGCTTCRGAAGGAACCAGCTACTAGATCG TTCGATTAGTCTTTCGCCCCTATACCCAGGTCTGACGATCGATTTGCACG TCARAATCGCTGCGGACCTCCACCARAGTTTCCTCTGGCCTCGTCCTGCC CAGGCATAGTTCACCATCTTTCGGGTCCCAACGT-GTATGCTCTCGCTCG TCCCACTGACGTTGCARACGGGACGGGCCGTTGGTGCGCCCCAGCCACTA GGGACCGGGGATCCCAAC-GCARACRACAACGAGGTCSCCCTTCACTTTCA TTGCGCCTGCAGGCCTGTTCAAGACCCGACGACTCGCACACATGTTARAC TCCTTGGTCCGTGCTTCAARACGGGTMAAAA
>SC-1 ExA F	CWTTTCTCAMATTATTAGTCKCATKCTGGAAGAGGGAACCTTTTGGRG CT TTGGGAATGGTTTATGSRATGGTT-GCKATTGGATTTTTAKGTTTTGTT GT TTGAGCTCATCWTATGTTTACTGWTGGAATARATGTTGATACTCKWGC TT ATTTTACTGCTGCTACWATGGTTATTGCTGTTCCTACTGGAATTAAAA TT TTTAGATGAWTRGCTACTTTATTTG-GAKCTTATTTTGAGTTTACTCCT CC TTTATTATGAGCWTTAKGATTTGTWTTTTTGTTTACTGTAKGAGGATT AA CCG-GARTAATTTTATCTAATTCTTCTTTASATRTTGTTCTTCATGATA CT GATSTTGTTCATTTTCATTTTSTTTAARKSWMARG-GAATAGG

>SC-1 ExA R	TKGAARAATTCTMTATTATTASTCGTCATACAGGGAAGAGGGAGCCTT TT GGGGCATTAGGAATAGTGTATGCTATGGTTGC-TATTGGAGGGTTGGGT TT TATTGTTTGGGCTCATCATATGTTTACTGTTGGGATAGATGTTGATAC TC GAGCTTATTTTACTG-CAGCTACTATAATTATTGCTGTTCCTACAGGAA TT AARATTTTTAGTTGGTTASCTACTTTGCATGGCTCATATATTCCGTTT AG TTCGGCGGTGTTGTGGTCTTTGGGATTTGTATTTTTATTTACAGTAGG AG GGTTGACTGGGGTTATTTTASCTAATTCTTCTT-TAGATATTGTTCTTC AT GATACTTATTATGTTGWGGCTCATTTTCACTATGWTYTWTCAATAGGA ATAGGA
>SC-4 12S R	GTKWWWMCCCMTCAGCACTGCTCATTTTCCTGACTACTTCCAAATCCACC TTMAATTCTTCTTTCAAAAAAT-TATTCWTATTTCTTAAAGACAAKGTAAC TCACGTTTCCCTTAAATATAACTGAACCTTGATCTGTAATTTTTTATTTA ATAATTTACAATATACACTTTAAAGTTTTATTTTTACRACRATATACAAA CAAAATTTAAGTAARATTAAACGGGTA-ATTTCGAGTTATAACGCAAGTTC CTCTGGTAARATTAAAACACCGCCAAATTCTTTAAGTTTCAAGACTTCTA CWACTACCTA-ATTCCTTTACTCTCAAWAATAATAGGGTATCTAATCCTAG TTTAATAA
>SC-4 16S F	ASAWRAYASGRKAGTMTTAGATTCTATATTGATGATGATATCACTGTAGG CTAAAAACATTTTTAAAAGTTAAATAAATTTTT-GTATTGAGAGCTGGTAT TTGTTATATTTTCATAATAATTATTTAAAGTTAGACTAAGGTGTCACCAT TTGTTATTTAGTAACT-GAATTTTAATTTTTGACTAATTTTCCGAAATAAA AATTTGCCTTGATCTGAGTTCAAACCGGAG
>SC-4 16S R	GCCGGACTTGCGATTAGTCAAAATTAAAATTCAGTTACTAAATAACAAAT GGTGACACCTTAGTCTAACTTTAAATAATTATTAT-GAAAATATAACAAAT ACCAGCTCTCAATACAAAAATTTATTTAACTTTTAAAAATGTTTTTAGCC TACAGTGATATCATCAT-CAATATAGAATCTTAAGACTATTCACAGTTCTT TTGTAGAATGTTTTTGATAAACAGGCGA
>SC-4 28S R	ARGAAWAAAGGGGMCTCACATCCTGGAGAATCGTCCGGGCTTCAGTTATG
	TAAACCGGACTTCTCACCCATTGAAAGTTTGAGAATAGGTTGAGGTCGTT TCGGCCCCAAGGCCTCTAATCATTCGCTTTAC-CGGATGAGACTGATTCCA CTCGAGCACCAGCTATCCTGAGGGAAACTTCGGAGGGAACCAGCTACTAG ACGGTTCGATTAGTC-TATCGCCCCTATACCAAGGTCTGACGATCGATTTG CACGTCARAATCGCTGCGGACCTCCACCAGAGTTTCCTCTGGCCTCGTCC TGCCCAGGCATAGTTCACCATCTTTCGGGTCCCAACGTGTACGCTCTCGC TCCGTCCCGCTGACGTGGCAGGTGGGACGGGC-CGTTGGTGCGCCCGGAAC CGAGGTCCCGGGATCCCAACGCARATGCCGGCACGGGCATCCTTCACTTT CATTGCGCCTCT-GAGCCTGTTCAAGACTCACTGACTCGCGCACATGTTAR ACTCCTTGGTCCGTGCTTCAGACGGGTMAA
>SC-4 ExA F	TGGGGGAGRTTCTCTATTATTASTCGTCATACAGGGAAGAGGGAGCCTTT TGGSGCATTARGAATASTGTATGCTATGGTTGC-TATTGGASGGTTGGGTT TTATTGTTTGGGCTCATCATATGTTTACTGTTGGGATAGATGTTGATACT CKAGCTTATTTTACTGC-MGCTACTATAATTATTGCTGTTCCTACRGGAAT TAARATTTTTAGTTGGTTASCTACTTTGCATGGCTCAWATATTCCGTTTA RTTCGGCGGTGTTGTGGTCTTTGGGATTTGTATTTTTATTTACAGTASGA GGGTTGACTGGGGTTATTTTASCTWATTCTTCTT-TAGATATTGTTCTTCA TGATACTTATTATGTTGAGGCTCAWTTTCACTATGATCTWTCAATAGGAA CGG
>SC-4 ExA R	CWAWWAAAGGTAWTMATGAAGACAATATCTAAAGAAGAATTAGCTAAAAT AACCCCAGTCAACCCTCCTACTG-TAAATAAAAATACAAATCCCAAAGACC ACAACACCGCCGAACTAAACGGAATATATGAGCCATGCAAAGTAGCTAAC CAACTAAAAATCTTAATTCCTGTAGGAACAGCAATAATTATAGTAGCTGC AGTAAAATAAGCTCGAGTATCAACATC-TATCCCAACAGTAAACATATGAT GAGCCCAAACAATAAAACCCAACCCTCCAATAGCAACCATAGCATACACT ATTCCTAAT-GCCCCAAAAGGCTCCCTCTTCCCTGTATGACGACTAATAAT ATGAGAAATCATCCCAAACCCAGGTAAAATTAAAAATAAAC-WMRYWATWT CAATAGGA
>SC-4 HCO R	AYTKMSGGGGAWKAYCAAWATCTYYAWCCCCTTCSTTKCCCCTCGAWRAA RKTATTAAAATCCCAATCACTTAACAACATAA-TAWTAGCCCCCAGTCAAT ACTGGTAAAGATAACAATAACAAAACTGTCACAAAAAGAGACCAAACAAA TAACAGAACCTTATC-CAAAGTCATCCCAATACTCTGCATATTAATAATAG TCATAATAAAATTGATAGCTCTCAAAATTGAAAAAATTCCCACAAAATGG AAAGAAAAAATAGTCAAATTAACCAACTTCCCTATATATGCCAAAGACAA WGGAAGATAAACTGTCCAGCCTGTCCCTACTC-CACTTTCCAACAAAGCAG AAGACAACAACAAAAAAAAGCAGGTGGCAACAATCARAAACTCATATTAT TAATCTA-AGGAAAAGTTATATCAGGTGTCCCACCATCAAACACACCAATC AATTTCCAAATCCTCAATCATGATAGGTATAACCATA-AAAAAATCATAAC AAAAGCATAAGCTGTCACTACCACATTATAAACCTGATCATCCCCACCAA
>SC-7 12S R	AWWAAWCCAGCCTAGGGTGCTCGCTTTTCCTGACATACTTCCAAATCC GC CTTCAAATTCTTCTTTCAAAAAAT-TATTCATATTTCTTAAAGACAAKG TA ACTCACGTTTCCCTTAAATATAACTGAACCTTGATCTGTAATTTTTTA TT TAATAATTTACAATATACACTTTAAAGTTTTATTTTTACRACGATATA CA AACAAAATTTAAGTAARATTAAACGGGTA-ATTTCGAGTTATAACGCAA GT TCCTCTGGTAARATTAAAACACCGCCAAATTCTTTAAGTTTCAAGACT TC TACWACTACCTA-ATTCCTTTACTCTCAAWAAWAAKAGGGTATCTAATC CT AKTTTWAWAA

>SC-7 28S F	GGGGGGSARRCTGGSGSGAGTCAGTGAGTCTTGRACAGGCTCAGAGGC GC AATGAAAGTGAAGGATGCCCGTGCCGGCATCT-GCGTTGGGATCCCGGG AC CTCGGTTCCGGGCGCACCAACGGCCCGTCCCACCTGCCACGTCAGCGG GA CGGAGCGAGAGCGTA-CACGTTGGGACCCGAAAGATGGTGAACTATGCC TG GGCAGGACGAGGCCAGAGGAAACTCTGGTGGAGGTCCGCAGCGATTCT GA CGTGCAAATCGATCGTCAGACCTTGGTATAGGGGCGATAGACTAATCG AA CCGTCTAGTAGCTGGTTCCCTCC-GAAGTTTCCCTCAGGATAGCTGGTG CT CGAGTGGAATCAGTCTCATCCGGTAAAGCGAATGATTAGAGGCCTTGG GG CC-GAAACGACCTCAACCTATTCTCAAACTTTCAATGGGTGAGAAGTCC GG TTTACATAACTGAAGCCCGGACGATTCTCCAGGAT-GTGAGTGCSCAGT GG GCCAATTTTGGTAAGCAGAACTGGCGCTGTGGGA
>SC-7 ExA F	GGAKATCTCATATTATTAGTCGTCATACAGGGAAGAGGGAGCCTTTTG GG GCATTAGGAATAGTGTATGCTATGGTTGCTATTG-GAGGGTTGGGTTTT AT TGTTTGGGCTCATCATATGTTTACTGTTGGGATAGATGTTGATACTCG GG CTTATTTTACTGCARC-TACTATAAWTTWTTKGCTGKTTCCYWCMRGGA AW TWAAGAWTTTTWRKTTGGKTAGCTACTTTTACATGGKTCATATATTCC SK TTARTTCSGCGGKGTTKKGGKCTTTGGGATTTGTATTTTTATTTACAG TG GGGAGGGKTGASYGGGKTWWTTTASYAATYC-TYCTTWRAWTTGTTCTT CA TGATACTATTATGTTGTGGCTCATTTCACTATGTTCTWYCATAGGRCGGG
>SC-7 ExA R	CWAATWAAGGTATCMATGAAGACAATATCTAAAGAAGAATTAGCTAAA AT AACCCCAGTCAACCCTCCCACTGTAAATA-AAAATACAAATCCCAAAGA CC ACAACACCGCCGAACTAAACGGAATATATGAACCATGTAAAGTAGCTA AC CAACTAAAAATCT-TAATTCCTGTAGGAACAGCAATAATTATAGTAGCT GC AGTAAAATAAGCCCGAGTATCAACATCTATCCCAACAGTAAACATATG AT GAGCCCAAACAATAAAACCCAACCCTCCAATAGCAACCATAGCATACA CT ATTCCTAATGCCCCAAAAGGCTCCCTCTTCCCT-GTATGACGACTAATA AT
	ATGAGAAATCATCCCAAACCCAGGTAAAATTAAAAAAATAAACTMAYM AT ATCAATAGGA
>SC-10 12S F	TGTCCGCCCCGCATGTACGGSGTAGTAGAGTCTTGAAACTTAAAGAATTT GGSGGKGTTTTAATCTTACCAGAGGAACTTGSGT-TATAACTCGAAATTAC CCGTTTAATCTTACTTAAATTTTGTTTGTATATCGTCGTAAAAATAAAAC TTTAAAGTGTATATTGTA-AATTATTAAATAAAAAATTACAGATCAAGGTT CAGTTATATTTAAGGGAAACGTGAGTTACATTGTCTTTAARAAATATGAA TAATTTTTTGAAARAARAATTTGAAGGTGGATTTGGAAGTATGTCAGGAA AAGTTAGTTTGGTTGAATGAGCAATAARACATG-CACACATCGCCCGTCGC TCTATWWAA
>SC-10 12S R	GTGTATCCTACGACTCTCTGTCCCTTTTCCTGACATACTTCCAAATCCAC CTTCAAATTCTTCTTTCAAAAAAT-TATTCATATTTCTTAAARACAATGTA ACTCACGTTTCCCTTAAATATAACTGAACCTTGATCTGTAATTTTTTATT TAATAATTTACAATATACACTTTAAAGTTTTATTTTTACRACGATATACA AACAAAATTTAAGTAARATTAAACGGG-TAATTTCGAGTTATAACGCAAGT TCCTCTGGTAARATTAAAACACCGCCAAATTCTTTAAGTTTCAAGACTTC TACTACTACCTA-ATTCCTTTACTCTCAATAATAATAGGGTATCTAATCCT AGTTTAACAA
>SC-10 16S F	CCKKSMSKSSATAGRTCTTAGATTCTATATTGATGATGATATCACTGTAG GCTAAAAACATTTTTAAAAGTTAAATAAATTTTT-GTATTGAGAGCTGGTA TTTGTTATATTTTCATAATAATTATTTAAAGTTAGACTAAGGTGTCACCA TTTGTTATTTAGTAACT-GAATTTTAATTTTTGACTAATTTTCCGAAATAA AAATTTGCCTTGATCTGAGTTCAAACCGGAGA
>SC-10 16S R	>SC-10 16S R GSTTTGAACRKSSGCGAATTAGTCAAAATTAAAATTCAGTTACTAAATAA CAAATGGTGACACCTTAGTCTA-ACTTTAAATAATTATTATGAAAATATAA CAAATACCAGCTCTCAATACAAAAATTTATTTAACTTTTAAAAATGTTTT TAGCCTA-CAGTGATATCATCATCAATATAGAATCTTAAGACTATTCACAG TTCTTTTGTAGAATGTTTTTGATAAACAGGCGA
>SC-10 28S F	GGGCAWCTCTGCGCGAGTCAGTGAGTCTTGRACAGGCTCARAGGCGCAMT GARRGTGAAGGATGCCCGTGCCGGCATCTGC-GTTGGGATCCCGGGACCTC GGTTCCGGGCGCACCAACGGCCCGTCCCACCTGCCACGTCAGCGGGACGG AGCGAGAGCGTACAC-GTTGGGACCCGAAAGATGGTGAACTATGCCTGGGC AGGACGAGGCCAGAGGAAACTCTGGTGGAGGTCCGCAGCGATTCTGACGT GCAAATCGATCGTCAGACCTTGGTATAGGGGCGATAGACTAATCGAACCG TCTAGTAGCTGGTTCCCTCCGAAGTTTCCCT-CAGGATAGCTGGTGCTCGA GTGGAATCAGTCTCATCCGGTAAAGCGAATGATTAGAGGCCTTGGGGCCG AAACGACCT-CAACCTATTCTCAAACTTTCAATGGGTGAGAAGTCCGGTTT ACATAACTGAAGCCCGGACGATTCTCCAGGATGTGAGT-GCCCAGTGGGCC AATTTTGGTAAGCAGAACGSSGSSSYKKKGGGRA
>SC-10 28S R	AKCATGGGCCTCACATCCTGGAGAATCGTCCGGGCTTCAGTWATGTAMAC CGGACTTCTCACCCATTGAAAGTTT-GAGAATAGGTTGAGGTCGTTTCGGC CCCAAGGCCTCTAATCATTCGCTTTACCGGATGAGACTGATTCCACTCGA GCACCAGCTATCCTGAGGGAAACTTCGGAGGGAACCAGCTACTAGACGGT TCGATTAGTCTATCGCCCCTATAC-CAAGGTCTGACGATCGATTTGCACGT CARAATCGCTGCGGACCTCCACCAGAGTTTCCTCTGGCCTCGTCCTGCCC AGGCATAGTTCACCATCTTTCGGGTCCCAACGTGTACGCTCTCGCTCCGT CCCGCTGACGTGGCAGGTGGGACGGGC-CGTTGGTGCGCCCGGAACCGAGG TCCCGGGATCCCAACGCARATGCCGGCACGGGCATCCTTCACTTTCATTG CGCCTCT-GAGCCTGTTCAAGACTCACTGACTCGCGCACATGTTAGACTCC TTGGTCCGTGCTTAGAAAAGGGGTCAAA

>SC-10 ExA F	TGTKAATTTCTMTATTATTAGTCGTCATACAGGGAAGAGGGAGCCTTTTG GGGCATTAGGAATAGTGTATGCYATGGTTGC-TATTGGAGGGTTGGGTTTT
	ATTGTTTGGGCTCATCATATGTTTACTGTTGGGATAGATGTTGATACTCG AGCTTATTTTACTGCAGCTACTATAATTATT-GCTGTTCCTACAGGAATTA AGATTTTTAGTTGGTTAGCTACTTTACATGGCTCATATATTCCGTTTAGT TCGGCGGTGTTGTG-GTCTTTGGGATTTGTATTTTTATTTACAGTGGGAGG

Table 1: Sequence of mtDNA of samples.

PCR and sequencing are used to determine the nucleotide content of the 12S data sequences (Table 2).

Domain: Data																				
	T (U)	C	A	G	Total	T-1	C-1	A-1	G-1	Pos#1	T-2	C-2	A-2	G-2	Pos#2	T-3	C-3	A-3	G-3	Pos #3
SC 4 1	37.	16.	37.	8.	245.	33	22.	33.	10.	84.0	46	15.	32.	6.3	79.0	34	12.	46.	7.3	82.0
	6	7	6	2	0		6	3	7			2	9				2	3		
SC 7 1	37.	16.	37.	8.	247.	33	22.	33.	10.	84.0	45	15.	32.	7.5	80.0	34	12.	47.	7.2	83.0
	2	6	7	5	0		6	3	7			0	5				0	0		
SC 4 2	37.	16.	37.	8.	245.	33	22.	33.	10.	84.0	46	15.	32.	6.3	79.0	34	12.	46.	7.3	82.0
	6	7	6	2	0		6	3	7			2	9				2	3		
SC 7 2	37.	16.	37.	8.	247.	33	22.	33.	10.	84.0	45	15.	32.	7.5	80.0	34	12.	47.	7.2	83.0
	2	6	7	5	0		6	3	7			0	5				0	0		
SC 10 1	37.	16.	36.	9.	247.	33	22.	33.	10.	84.0	45	14.	29.	11.	82.0	33	12.	48.	6.2	81.0
	2	6	8	3	0		6	3	7			6	3	0			3	1		
SC 10 2	37.	16.	37.	8.	247.	33	22.	33.	10.	84.0	46	14.	32.	7.4	81.0	34	12.	47.	6.1	82.0
	7	6	7	1	0		6	3	7			8	1				2	6		
SC 10 3	37.	16.	36.	9.	247.	33	22.	33.	10.	84.0	45	14.	29.	11.	82.0	33	12.	48.	6.2	81.0
	2	6	8	3	0		6	3	7			6	3	0			3	1		
SC 10 4	37.	16.	37.	8.	247.	33	22.	33.	10.	84.0	46	14.	32.	7.4	81.0	34	12.	47.	6.1	82.0
	7	6	7	1	0		6	3	7			8	1				2	6		
MK170424.1	36.	16.	37.	9.	251.	33	22.	32.	11.	84.0	43	14.	32.	10.	84.0	34	12.	48.	6.0	83.0
Androctonus crassicauda	7	3	5	6	0		6	1	9			3	1	7			0	2		
KT972135.1	39.	20.	32.	8.	253.	38	30.	22.	9.4	85.0	43	16.	29.	10.	84.0	37	13.	44.	6.0	84.0
Odontobuthus	1	2	0	7	0		6	4				7	8	7			1	0		
doriae																				
Avg.	37.	17.	36.	8.	247.	34	23.	32.	10.	84.1	45	15.	31.	8.6	81.2	34	12.	47.	6.6	82.3
	5	0	9	6	6		4	1	7			0	5				3	0		

Table 2: Nucleotide composition of the 12S data sequences.

GEL electrophoresis

Finally, amplification products were electrophoretically separated in a 1 percent agarose electrophoretic gel, and PCR samples were purified from the agarose gel. The purpose of the agarose gel in this situation is to concentrate a sample so that all of the reaction product can be placed into a single well of the gel for subsequent assays (Figure 4).

Sequencing

The Sequencing is done by three steps. 5ul of PCR product and 2ul of Exosap reagent are added separately for forward and reverse primer to a PCR tube and total 7ul is kept in PCR machine with PCR conditions as follows: 37˚ C for 15 minutes and 80˚ C for 15 minutes. After this, Cycle sequencing is done in which the primers are diluted to 1uM concentration and reaction volume is 20ul consists of Exosap Product 7ul, Primer 4 ul, Sequencing premix BIODYE Terminator 3.1 cycle sequencing RR-100 (LOT NO: 1712134)1ul, Sequencing buffer BIGDYE terminator (LOT NO: 1808296) 2 ul and make up to 20 ul with nuclease free water. PCR conditions are 96 C for 1 min, 25 cycles of 96 C for 10 s, 50 C for 5 s, 60 C for 4 mins. The last step is Ethanol/EDTA/sodium acetate precipitation, The PCR product from cycle sequencing is added to 96 well plate. 2 ul of cold 125mM EDTA and 2 ul of cold 3M sodium acetate is added to the wells. Then add 50ul of cold absolute ethanol. Seal the plate and invert the plate for 4 – 8 times. Keep the plate in room temperature for 15mins and centrifuge for 1650 g for 45mins at 4˚ C. Invert the plate and spin upto 185g and take it out. Add 70ul of 70% ethanol to each well. Centrifuge at 1650g for 15 minutes. Invert the plate and spin upto 185g for 1 minute. Resuspend the samples in Injection buffer, seal the plate and store it at 4˚ C. The plate is ready to go to sequencing machine for analysis. The results of DNA sequencing were examined via chromatograms (Table 3).

Blast

Basic Local Alignment Search Tool was used to edit and align the retrieved DNAsequences, and its software was used to align them (Figure 5).

Results

The scorpions gathered in the field were largely analysed under a stereo microscope for morphological investigations, and morphological study revealed that they all belonged to the UAE scorpion species. Four groups of samples were formed based on colour variation. For the species Leiurus abdullahbayrami, genetic distances were estimated 16rRNA. The sequence distance in the samples studied was calculated using available scorpions (Gen Bank Acc. AY226174, Fet et., *et al*. 2003). We discovered a sequence distance of 0.488 and 0,512 (percent). The interspecific distance between E1 and C4 was found to be the greatest. The distances between the UAE scorpion species and the outgroup species ranged from 48.8% to 51.2 percent. The highest distances between E1 and the outgroup were noted (Figure 2).

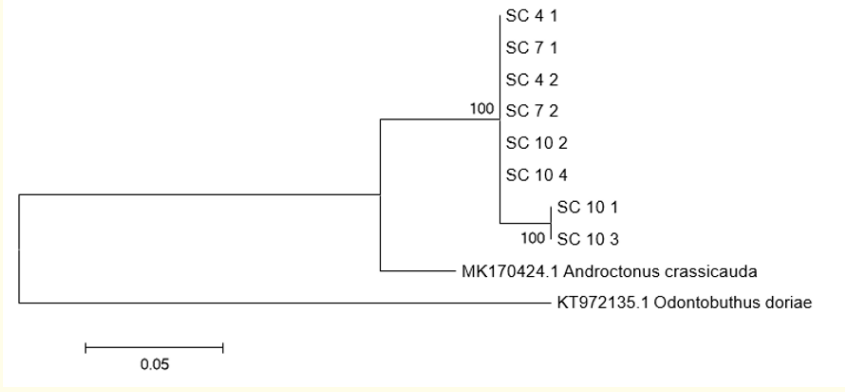


Figure 2: Molecular Phylogenetic analysis by Maximum Likelihood method of *Androctonus crassicauda* collected from UAE.

Evolutionary relationships of *Androctonus crassicauda* taxa collected in the United ArabEmirates was determined (Figure 3).

The electrophoretic results of PCR were determined through silica gel electrophoresis.

The received DNA sequences were edited and aligned using the Basic Local Alignment Search Tool's software, and the following findings were obtained. BLAST analysis was used to compare the acquired DNA sequences of the relevant gene region generated by PCR. When Group A and Group B were examined, DNA sequence analyses found 98 percent, 98 percent, 99 percent, 96 percent, and 98 percentsimilarity. A and C, A and D, A and E, A and H, A and I, A and J, A and K, A and G, and A and F were all in sync. The percentages of resemblance were 97 percent, 97 percent, 94 percent, and 96 percent, respectively. When Groups B and C, B and D, B and E, and B and F come together. The B and F groups werematched (Figure 5 - Table -3).

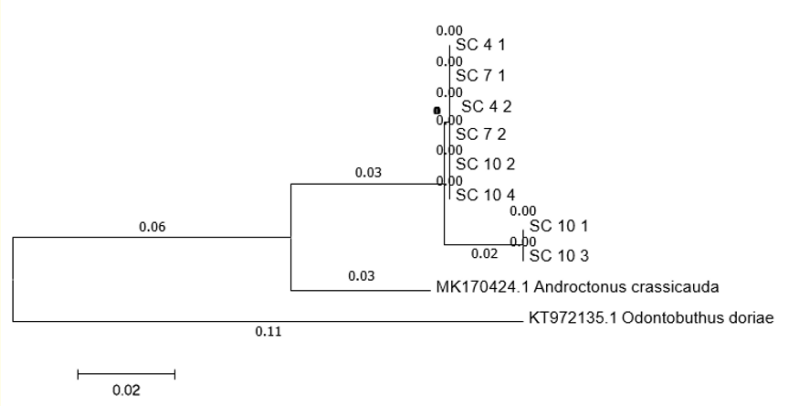


Figure 3: Evolutionary relationships of taxa of *Androctonus crassicauda* collected from UAE.

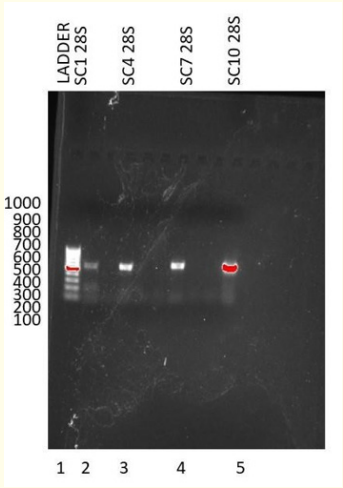


Figure 4a: Silica Gel Electrophoresis LADDER USED – 100bp Generuler, SC1 28S – SCORPION 1 28S PRIMER, SC4 28S – SCORPION 4 28S PRIMER, SC7 28S – SCORPION 7 28S PRIMER, SC10 28S – SCORPION 10 28S PRIMER.

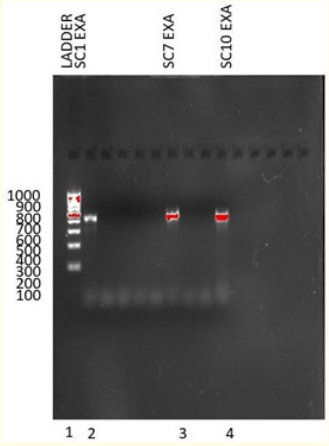


Figure 4b: Silica Gel Electrophoresis LADDER USED – 100 bp GENERULER, SC1 EXA – SCORPION 1 HCOEXT a PRIMER, SC4 EXA – SCORPION 4 HCOEXT a PRIMER, SC7 EXA – SCORPION 7 HCOEXT a PRIMER, SC10 EXA – SCORPION 10 HCOEXT a PRIMER.

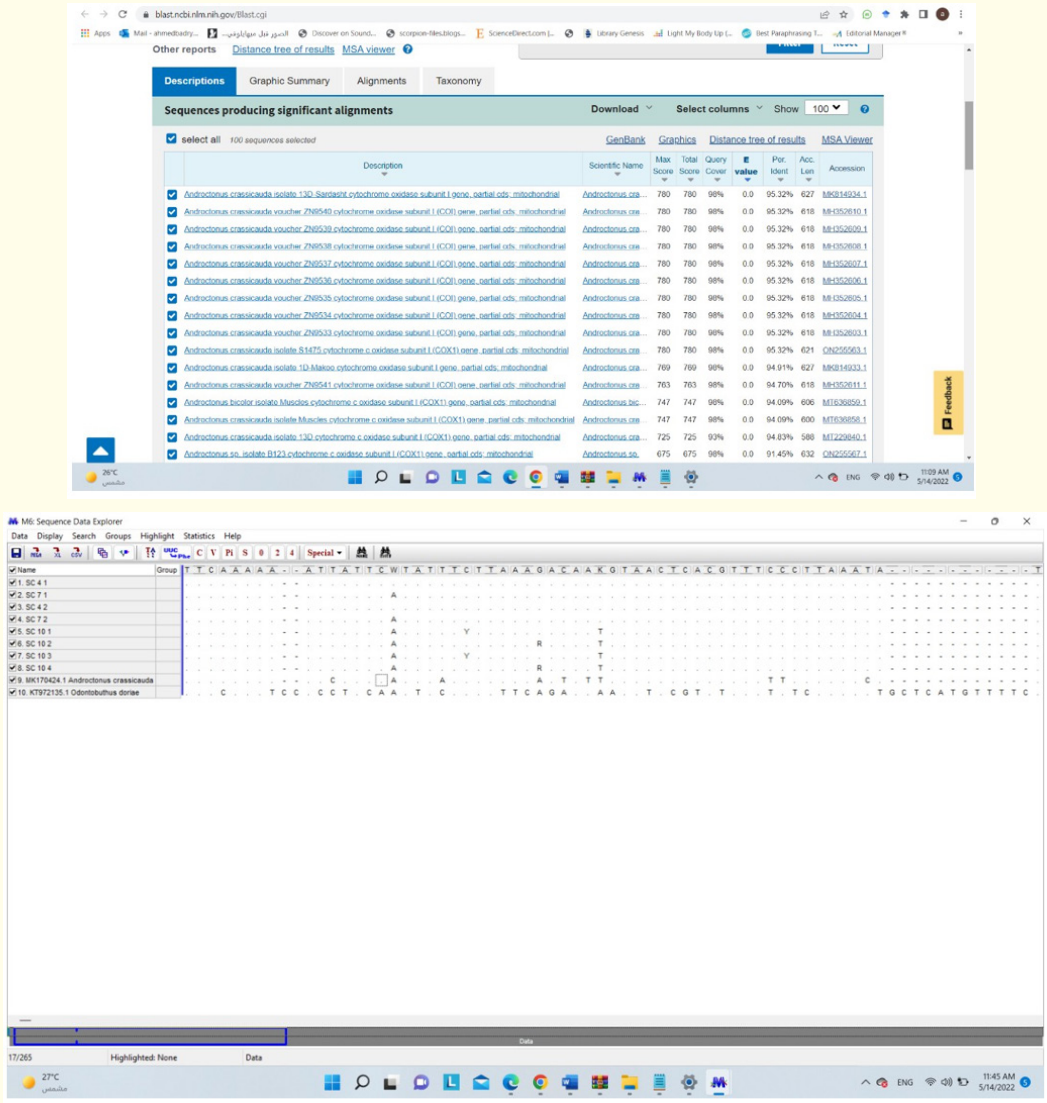


Figure 5: BLAST of sample sequences on the basis of similarity index and alignment using BLAST NCBI Tool.

	SC_4_1	SC_7_1	SC_	SC_	SC_	SC_	SC_	SC_	MK1	KT97
SC_4_1		0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.02
SC_7_1	0.00		0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.02
SC_4_2	0.00	0.00		0.00	0.01	0.00	0.01	0.00	0.01	0.02
SC_7_2	0.00	0.00	0.00		0.01	0.00	0.01	0.00	0.01	0.02
SC_10_1	0.02	0.02	0.02	0.02		0.01	0.00	0.01	0.02	0.03
SC_10_2	0.00	0.00	0.00	0.00	0.02		0.01	0.00	0.01	0.02
SC_10_3	0.02	0.02	0.02	0.02	0.00	0.02		0.01	0.02	0.03
SC_10_4	0.00	0.00	0.00	0.00	0.02	0.00	0.02		0.01	0.02
MK170424.1_Androctonus_crassicauda	0.06	0.06	0.06	0.06	0.08	0.06	0.08	0.06		0.03
KT972135.1_Odontobuthus_doriae	0.20	0.20	0.20	0.20	0.21	0.20	0.21	0.20	0.19	

Table 3: Pairwise genetic distance of 12s Data sequence of Androctonus crassicauda collectedfrom UAE.


SC-1


SC-2


SC-2


SC-2


Scorpion B


Scorpion C

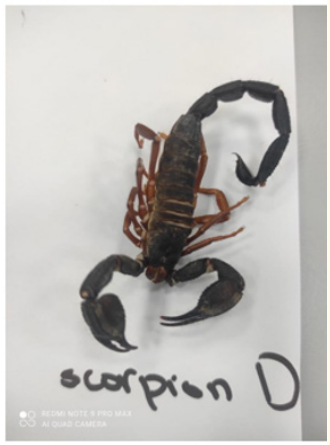

Scorpion C

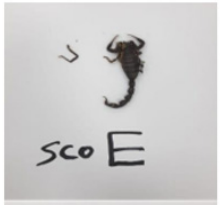

Scorpion A

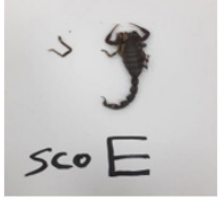

Scorpion A


Scorpion A

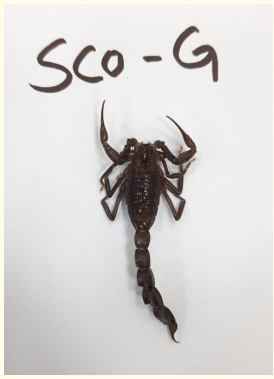

Scorpion B


scorpion D


sco E

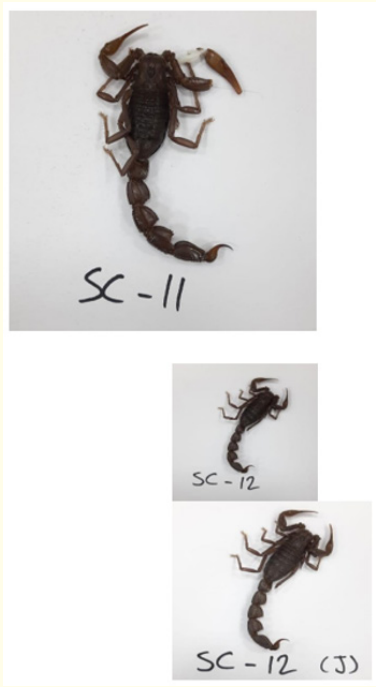

sco E

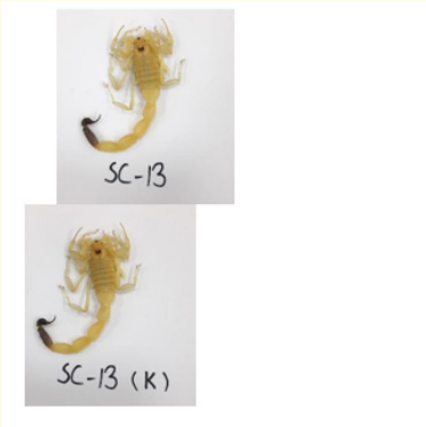

SCO F
SCO F
SCO F
SCO F


SCO-G


SC - H


SC - II (I)


SC - II
SC - 12
SC - 12 (J)


SC-13
SC-13 (K)

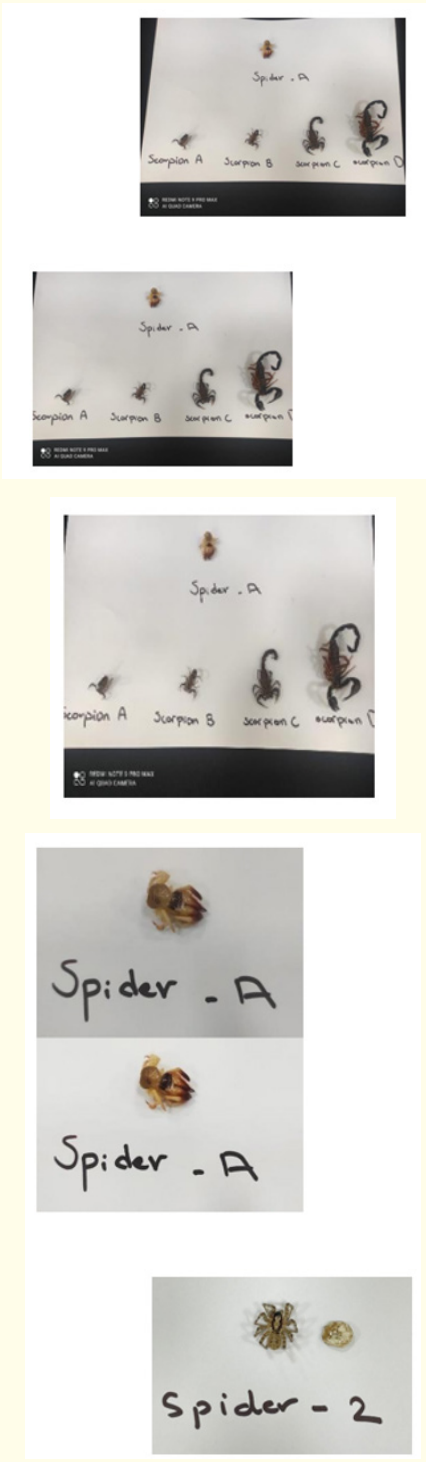


Figure 6: Scorpions samples in UAE used in this study.

Discussion and Conclusion

Molecular approaches have recently been utilised to identify new scorpion species or subspecies (mtDNA is often used in phylogenetic and phylogeographic studies). Studies on the taxonomic classification of scorpions and the relationship between phylogenetic trees are still ongoing. In addition to taxonomic studies of scorpions, scorpionism instances are an interesting research area. For the first time, the Molecular Phylogeny of Scorpions in the United Arab Emirates is presented in this study. The colouring is obviously varied. There is a significant deal of variation in hue, perhaps.as a result of local ecological and geographic adaptation. The acquired DNA sequences of the relevant gene area generated by PCR were compared using BLAST analysis. In this study,DNA sequence studies for revealed 98 percent, 98 percent, 99 percent, 96 percent, and 98percent similarity when Group A and Group B were compared. Groups A and C, A and D,A and E, A and H, A and I, A and J, A and K, A and G, and A and F were all aligned withone other. The results were 97 percent, 97 percent, 94 percent, and 96 percent similarity, respectively. When Group B and C, Group B and D, Group B and E, and Group B and F are combined. The groups B and F were matched. Another comparison revealed 100%, 96percent, and 99 percent similarity, respectively. The mtDNA 16S rRNA region has 96-100percent similarity among more than ten field samples, according to nucleotide comparisons.

The findings of this study contributed to the genetic knowledge available to evolutionary taxonomists working with scorpions. We want to incorporate a variety of genetic loci in our research. Phylogenetic investigations in the future to confirm the existing findings and to reveal the rationale of various morphologies. The genetic diversityand composition of the population will change in the future. The evolution of scorpion venom should be explored. In the development of more potent antivenom for scorpion envenomation The study of various populations could be beneficial. In the understanding of scorpion origins and current distribution the tentative tree that was shown. This study may provide insight into the relationships between different species in the UAE scorpion fauna.

Acknowledgment

This research was supported by the Research and Graduate Studies Institute, University of Sharjah. We would like to express our deep gratitude and appreciation to our research supervisor Dr Abdullah Fahad Al Mutery for his guidance, encouragement and

support of this research. We would also like to thank mero, our lab assistant, for her help and support throughout our research. Additionally, we would be remiss in not mentioning Mahmoud who helped us in collecting the scorpion samples.

Bibliography

1. Scorpion - Ecology and habitats | Britannica (2022).

2. Kerkis I., *et al*. "Toxin bioportides: exploring toxin biological activity and multifunctionality". *Cellular and Molecular Life Sciences* 74 (2017): 647-661.

3. 131-I-TM-601 Study in Adults With Recurrent High-Grade Glioma - Full Text View - ClinicalTrials.gov. (2022).

4. Bontems F., *et al*. "Refined structure of charybdotoxin: common motifs in scorpion toxins and insect defensins". *Science* 254 (1991): 1521-1523.

5. De Melo ET., *et al*. "Structural characterization of a novel peptide with antimicrobial activity from the venom gland of the scorpion Tityus stigmurus: Stigmurin". *Peptides* 68 (2015): 3-10.

6. Akef HM. "Anticancer and antimicrobial activities of scorpion venoms and their peptides". 38 (2017): 41-53.

7. The Scorpion files – Introduction (2022).

8. Dunlop J and Penney D. "Catalog DJ-T world spider, version undefined, 2012 undefined. A summary list of fossil spiders and their relatives". wsc.nmbe.ch (2022).

9. Anderson L. "Unmineralised arthropods" (2007).

10. Fet V., *et al*. "Catalog of the Scorpions of the World (1758-1998)". *Biological Sciences* (2000).

11. Vachon. Étude des caractères utilisés pour classer des familles et les genres de Scorpions (Arachnides). 1. La trichobothriotaxie en Arachnologie. Sigles trichobothriaux et types de trichobothriotaxie chez les Scorpions. Bull du Muséum Natl d'Histoire Nat Paris 3 (1974): 140.

12. Teruel R and Kovařík F. "Redescription of Androctonus bicolor Ehrenberg, 1828, and description of Androctonus turieli sp. n. from Tunisia (Scorpiones: Buthidae)". *Euscorpius* (2014): 1-15.

13. Badry A., *et al*. "On the scorpion fauna of Egypt, with an identification key (Arachnida: Scorpiones)". *Zoology in the Middle East* 64 (2018): 75-87.

14. Lourenço WR and Qi J xin. "Further considerations on the genus Butheolus Simon, 1882 and description of one new species from Saudi Arabia (Scorpiones: Buthidae)". 37 (2013): 91-97.

15. Kinzelbach R., *et al*. "Vorderer Orient, Skorpione (Arachnida: Scorpiones)= Middle East, scorpions (arachnida: scorpiones)". ixtheo.de (1985).

16. Kovařík F., *et al*. "Review of the genus Neobuthus Hirst, 1911 with description of a new species from Ethiopia (Scorpiones: Buthidae)". mds.marshall.edu (2012).

17. Alqahtani A., *et al*. "The scorpion fauna of the southwestern part of Saudi Arabia". journals.ekb.eg (2019).

18. Teruel R., *et al*. "A new species of Androctonus Ehrenberg, 1828 from northwestern Egypt (Scorpiones: Buthidae)". *Euscorpius* 2013 (2016): 1-12.

19. Soltan-Alinejad P., *et al*. "Molecular analysis of the mitochondrial markers COI, 12S rDNA and 16S rDNA for six species of Iranian scorpions". *BMC Research Notes* 14 (2021): 1-6.

20. Brown WM., *et al*. "Mitochondrial DNA sequences of primates: tempo and mode of evolution". *Journal of Molecular Evolution* 18 (1982): 225-239.

21. Russo CAM., *et al*. "Efficiencies of different genes and different tree- building methods in recovering a known vertebrate phylogeny". *Molecular Biology and Evolution* 13 (1996): 525-536.

22. Zardoya R and Meyer A. "Phylogenetic performance of mitochondrial protein-coding genes in resolving relationships among vertebrates". *Molecular Biology and Evolution* 13 (1996): 933-942.

23. Patwardhan A., *et al*. "Molecular Markers in Phylogenetic Studies - A review". *Journal of Phylogenetics and Evolutionary Biology* 2 (2014): 131.