Molecular analysis of the mitochondrial markers COI, 12S rDNA and 16S rDNA for six species of Iranian scorpions

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Abstract

Objectives: Annually, 1.2 million humans are stung by scorpions and severely affected by their venom. Some of the scorpion species of medical importance have a similar morphology to species with low toxicity. To establish diagnostic tools for surveying scorpions, the current study was conducted to generate three mitochondrial markers, Cytochrome Oxidase I (COI gene), 12S rDNA and 16S rDNA for six species of medically important Iranian scorpions: Androctonus crassicauda, Hottentotta saulcyi, Mesobuthus caucasicus, M. eupeus, Odontobuthus doriae, and Scorpio mauros.

Results: Phylogenetic analyses of the obtained sequences corroborated the morphological identification. For the first time, 12S rDNA sequences are reported from Androctonus crassicauda, Hottentotta saulcyi, Mesobuthus caucasicus and M. eupeus and also the 16S rDNA sequence from Hottentotta saulcyi. We conclude that the mitochondrial markers are useful for species determination among these medically important species of scorpions.

Keywords: mtDNA, Scorpionidae, Buthidae, Iran

Introduction

Scorpions comprise more than 1500 species of which fifty species are considered of medical importance [1]. Annually, 1.2 million people are stung by scorpions [2], and severely affected by their venom [3]. The geographical and climatic diversity in Iran provides suitable conditions for a substantial number of scorpion species; as many as 52 species have been reported from Iran, although this number is not definitive [4]. Of these, at least seven species are dangerous to humans: Androctonus crassicauda Olivier, 1807; Apistobuthus pterygocercus Finnegan, 1932; Hottentotta saulcyi Simon, 1880; Hottentotta schach Birula, 1905; Hemiscorpius lepturus Peters, 1861; Mesobuthus eupeus Koch, 1839 and Odontobuthus doriae Thorell, 1876 [4]. The difficulties in identifying species based on morphological techniques [5], as well as the low number of studies [4], are two of the reasons for the uncertainty regarding the number of scorpion species existing in Iran. Using molecular markers in addition to morphological methods could be useful for a more accurate assessment of species diversity in Iran. One example where potential misidentification of scorpion species could be of importance, regards H. saulcyi and Scorpio mauros. The two species are similar in appearance, but while the venom for H. saulcyi has an LD50 value of 0.73 mg/kg and is considered a risk for humans, S. maurus has a venom with an LD50 value of 9.37 mg/kg that is considered harmless [6].

Among available molecular markers, the mitochondrial markers have shown to be useful for both species identification and phylogenetic evaluation of scorpion species.
For example, analysis of intraspecific divergence between Androctonus scorpions using three mitochondrial markers (the COI gene, 12S rDNA and 16S rDNA), showed that these markers could explain the deep divergence sub-clades between Androctonus amoreuxi, A. australis and A. mauretanicus that also reflect differences in their venom production [7]. Also, COI and 16S rDNA markers have been used for investigating the evolution of Mesobuthus gibbosus in the Northeastern Mediterranean [8].

The current study was carried out to determine the COI, 12S rDNA and 16S rDNA sequences of six medically important Iranian scorpions and to evaluate the utility of these markers for identification in comparison to morphology.

Main text
Material and methods
Taxon sampling
Six species of medical importance were collected: Androctonus crassicauda Olivier, 1807; Hottentotta saulcyi Simon, 1880; Mesobuthus caucasicus Nordmann, 1840; Mesobuthus eupeus Koch, 1839; Odontobuthus doriae Thorell, 1876 and Scorpio maurus Ehrenberg, 1828. The samples were collected from the West Azerbaijan Province (Northwestern Iran) and the Qom Province (Central Iran; Additional files 1, 2: Tables S1, S2). The scorpions were captured at night using ultraviolet light (wavelength 366.3 nm), which causes maximum fluorescence of their epicuticles. All collected scorpions were stored in 96% ethanol. Specimens were identified using the keys of Farzanpay [9] and Dehghani and Valaie [10]. From the identified samples, one specimen from each species at each site was selected for molecular investigations (Additional files 1, 2: Table S1, S2) and one leg was then removed from each specimen for DNA extraction.

DNA extraction. COI, 12S rDNA and 16S rDNA amplification
Genomic DNA was extracted from each specimen’s leg tissue using AccuPrep® Genomic DNA Extraction kit (Bioneer, South Korea). The primers LCO1490 5’-GGT CAAACCAATCATATAAGATATTG-3’ and HCO2198 5’-TAAACTTCAGGGTGACCAAAAAATCTCA-3’ were used for amplification of the COI gene [11]. The primers Sc-12F 5’-AGAGTGACGGGCAATATGTG-3’ and Sc-12R 5’-CAGCCGCTCGGTTATAC-3’ were used for amplification of 12S rDNA [12]. The primers Sc-16F 5’-CGATTGGACTCAGATCA-3’ and Sc-16R 5’-GTGAAGGTTGACTAAT-3’ were used for amplification of 16S rDNA [13]. PCR conditions for amplification of all fragments were as follows: initial denaturation at 94 °C for 5 min; 30 cycles of [94 °C for 30 s, 48 °C for 30 s, 72 °C for 30 s] and a final extension at 72 °C for 7 min.

Phylogenetic analysis
All amplicons of the three fragments were sequenced and acquired consensus sequences were analyzed using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) in order to find and include similar sequences in the phylogenetic analysis. The phylogenetic relationship of the scorpions in the current study was inferred by using the Maximum Likelihood tree building algorithm and the Tamura-Nei model [14]. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a
matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. Evolutionary analyses were conducted in MEGA X [15].

Results and discussion
In the current study, six species of Buthidae and Scorpionidae scorpions from northwestern and central Iran were identified based on morphological characteristics (Fig. 1). We amplified the COI gene, 12S rDNA and 16S rDNA and a representative subset was deposited in GenBank; accession numbers are shown in Additional files 1, 2: Table S1, S2 and in Fig. 2. The acquired sequences of 12S rDNA fragments in the species Androctonus crassicauda, Hottentotta saulcyi, Mesobuthus caucasicus, M. eupeus as well as 16S rDNA in Hottentotta saulcyi are novel. We also consider our COI sequence of H. saulcyi to be the first for this species, since the existing COI sequence in GenBank (KU341989) seems to be from a misidentified specimen (Fig. 2a). Additionally, the sequences of 16S rDNA of A. crassicauda, M. caucasicus, and M. eupeus, the 12S rDNA sequence of Odontobuthus doriae, and all three mitochondrial gene sequences of Scorpio maurus are the first reported from Iran.

In this study, we successfully used 16S rDNA to differentiate between genera, and sequences clustered well on the species level (Fig. 2b). Likewise, Cytochrome Oxidase I (COI) and 16S rDNA fragments had the appropriate capability for the differentiation of four Eurasian scorpion species (Mesobuthus caucasicus, M. cyripius, M. eupeus, and M. gibbosus; [16], which is in accordance with our results showing effective differentiation of Mesobuthus at the species level using all three mitochondrial markers (Fig. 2).

There has been taxonomic controversy over the placement of Mesobuthus caucasicus, which has been proposed to belong to the genus Olivierius [4, 9, 17–19] while other researchers prefer to maintain the original scientific name Mesobuthus caucasicus [16, 20–22]. Our phylogenies of all three mitochondrial markers strongly suggest that Mesobuthus caucasicus should indeed be included in the genus Mesobuthus (Fig. 2) and confirmed by placing Opisthacanthus asper as a rooted outgroup (Fig. 2).

In all, we have in this paper shown that COI, 12S rDNA and 16S rDNA are efficient markers for phylogenetic discrimination of these Iranian scorpions and set the foundations for the correct identification of these medically important species.

Limitations
Due to the fact that the samples analyzed in this analysis were collected from limited areas, the collection of samples and species from wider areas could better indicate potential differences in morphological characteristics and genetic markers. Also, the selection of one sample of any scorpion species in each location for molecular investigation, as a limiting factor in the present study, should be considered.

(See figure on next page.)

**Fig. 2** Molecular phylogenetic analysis of a COI, using the Maximum Likelihood method with 1000 bootstrap replications. The tree with the highest log likelihood (−4375.20) is shown. There were a total of 698 positions in the final dataset. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Asterisks indicate sequences obtained in this project. b 16S rDNA, using the Maximum Likelihood method with 1000 bootstrap replications. The tree with the highest log likelihood (−3635.89) is shown. There were a total of 489 positions in the final dataset. Asterisks indicate sequences obtained in this project. c 12S rDNA, using the Maximum Likelihood method with 1000 bootstrap replications. The tree with the highest log likelihood (−4080.84) is shown. There were a total of 524 positions in the final dataset. Asterisks indicate sequences obtained in this project.
Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13104-021-05449-3.

Additional file 1: Table S1. Summary of scorpion taxa collected, sampling localities and accession numbers of acquired 125 and 16S sequences.

Additional file 2: Table S2. Summary of scorpion taxa collected, sampling localities and accession numbers of acquired COI sequences.

Abbreviations

COI: Cytochrome Oxidase 1; 125 rDNA: 12S ribosomal DNA; 16S rDNA: 16S ribosomal DNA.

Acknowledgments

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Authors’ contributions

ARC designed and supervised the study. PSA, JR, FD and PO did the field and laboratory activities. ARC and OT analyzed the Data. Also, ARC and OT wrote the draft and finalized the Draft. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This project was approved by the Ethics Committee of Urmia University of Medical Sciences.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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