

1-1-2020

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Recommended Citation

JAFARI, HEDIEH; SALABI, FATEMEH; and FOROUZAN, ALIREZA (2020) "A study of genetic diversity among different population of Orthochirus sp. based on cytochrome C oxidase subunit I and 16srRNA sequencing," *Turkish Journal of Zoology*: Vol. 44: No. 1, Article 8. <https://doi.org/10.3906/zoo-1904-9>
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A study of genetic diversity among different population of *Orthochirus* sp. based on cytochrome C oxidase subunit I and 16srRNA sequencing

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Received: 08.04.2019 • Accepted/Published Online: 12.09.2019 • Final Version: 03.01.2020

Abstract: *Orthochirus* sp. (Family: Buthidae) is widespread throughout the world. There are 17 species, 7 of which have been reported in Khuzestan Province of Iran, including the most diverse species related to the scorpion. In the present study, specimens of *O. iranus*, *O. farzanpay*, *O. zagrosensis*, and *O. stockwelli* were collected from different areas in this province. Phylogenetic analysis was conducted through duplicating and sequencing the cytochrome c oxidase subunit I (COXI) and 16SrRNA genes and comparing their genetic diversity. *Orthochirus iranus* had the highest genetic distance from *O. stockwelli*, while the lowest genetic distance was observed between *O. farzanpay* and *O. stockwelli*. In addition, the highest interspecific divergence was reported between the species of *O. iranus* and *O. zagrosensis*, while the lowest interspecific divergence was found between the species of *O. farzanpay* and *O. stockwelli*. The results indicated that cytochrome c oxidase (COXI) and 16SrRNA genes have species identification value.

Key words: Scorpion, *Orthochirus* sp., genetic diversity, cytochrome c oxidase, 16SrRNA

Iranian scorpion fauna is among the most diverse faunas in the western Asian region, which is considered one of the richest sources for the study of this arthropod because of the high level of species and ecosystem diversity in this region (Navidpour, 2015). The *Orthochirus* sp. is a scorpion which can be found in Iran, Turkmenistan, India, Pakistan, Iraq, Oman, Turkey, Uzbekistan, and North Africa (Fet and Lowe, 2000). At least 6 species of this scorpion have been identified in Iran (Kovarik, 2004) through recognized similarities in the members of this genus. The mitochondrial 16S rRNA gene was first used in phylogenetic analysis of the genus *Euscorpis* (Gantenbein et al., 1998). The first molecular phylogenetic analysis on the family Buthidae was carried out by Fet et al. using the mitochondrial marker (16SrRNA), which showed 3 groups of scorpions of Buthidae: (a) *Compsobuthus*, *Mesobuthus*, *Liobuthus*, *Kraepelinia*; (b) *Hottentotta*, *Buthacus*; (c) *Orthochirus*, *Anomalobuthus* (Fet et al., 2003). Molecular phylogenetic analysis based on the mitochondrial marker demonstrated a close relation between the species of *O. iranus* (Iran) and *O. innesi* (Morocco) (Jafari et al., 2018). There are a limited number of studies on interspecies relations among the scorpions in Iran. Therefore, the specimens collected from Khuzestan Province were

identified through morphological and molecular analysis and evaluated based on cytochrome c oxidase subunit I and 16SrRNA genes. The results were applied in the biosystematics approaches of this genus in Khuzestan, and genetic divergence of the studied species was measured based on the sequence data of the mentioned genes.

Orthochirus sp. are small scorpions which live in sandy heights. The specimens of this genus were collected in the Khuzestan region based on dispersion and diversity in different regions in April–September 2016 and April–June 2017. The morphological analysis of the specimens was based on the terminology presented by Vashon (1974). Genomic DNA was extracted using an extraction kit (Sina Pure™, Tehran, Iran) according to the kit's instructions. Polymerase chain reaction was performed by duplicating 1 segment of about 700 nucleotides of cytochrome c oxidase subunit I (COXI) from the primers 5'GGTCAACAAATATATAATAGATAT and 5'CCGGTAAAAT'AAAATATAAA; amplification of this primer was carried out in 25- μ L reaction volumes containing 1–5 μ L of DNA template, 2.0 mM MgCl₂, 200 mM of each dNTP, 25 pmol of each forward and reverse primer, and 0.5 unit of Taq DNA polymerase. The cycling protocol included an initial hot start (94 °C for 3 min), 35 PCR cycles of 94 °C for 30 s, 58 °C for 30 s, 72 °C for 60 s, and

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final extension at 72 °C for 7 min. Replication for 1 segment of about 400 nucleotides of mitochondrial gene of the major ribosomal RNA subunit (16SrRNA) was performed from the primers 5'CGATTAGACTCAGATCA and 5'GTGCAAAGGTAGCATAATCA; amplification of this primer was employed in 25- μ L reaction volumes containing 1–5 μ L of DNA template, 2.0 mM MgCl₂, 200 mM of each dNTP, 25 pmol of each forward and reverse primer, and 0.5 unit of Taq DNA polymerase. The cycling protocol included an initial hot start (94 °C for 3 min), 35 PCR cycles of 94 °C for 50 s, 45 °C for 50 s, 72 °C for 60 s, and final extension of 72 °C for 10 min.

The PCR product, along with the designed primers, was sent to Tekapozist Co. in Tehran for sequencing of the 16SrRNA and COXI genes and determining the sequences of the product using the primers. Phylogenetic analyses were performed on individual COX1 and 16srRNA datasets using the maximum likelihood (ML) method. The ML analysis was performed using MEGA 7.0; the nodes were tested for robustness with 10,000 bootstrap replicates. *Androctonus crassicauda* was used as the outgroup.

In total, 13 specimens including *O. iran* (n = 5), *O. zagrosensis* (n = 2), *O. farzanpay* (n = 3), *O. stockwelli* (n = 3), and a specimen of *Androctonus crassicauda* as an external group were sequenced.

The number of 517 conserved positions (77/39%), 151 variable positions (22.62%), and 95 positions (14.22%) had an information bar based on parsimony principle and 56 singleton positions among 668 nucleotides of the duplicated COXI gene.

The mean of the interspecies genetic distance (Kimura-2-parameter) indicated that the species of *O. farzanpay* and *O. stockwelli* had the lowest divergence (6.4%); in other words, they were genetically close. The highest genetic divergence (13.6%) was observed between the species of *O. iran* and *O. stockwelli*, which means that these 2 species had the highest divergence genetically. In addition, based on the mean of the intraspecies genetic distance, as shown in Table 1, the highest intraspecies divergence (1.2%) belongs to *O. zagrosensis*, and the lowest intraspecies genetic divergence (0) belongs to *O. stockwelli* and *O. farzanpay*.

Table 2 shows the number of intraspecies nucleotide sequences, nucleotides, haplotypes, nucleotide difference mean, nucleotide diversity, haplotype diversity, and separating positions (polymorphic) in the 5 species.

The number of 254 conserved positions (72.54%), 96 variable positions (27.42%), and 72 positions (20%) had an information bar based on the parsimony principle and 24 singleton positions among 350 nucleotide pairs of duplicated 16S gene for 14 specimens, using a specimen of *A. crassicauda* as the outgroup.

The mean of the interspecies genetic distance (Kimura-2-parameter) indicated that the species of *O. farzanpay* and *O. stockwelli* had the lowest divergence (4.4%); in other words, they were genetically close. The highest interspecies genetic divergence (13.5%) was observed between the species of *O. iran* and *O. farzanpay*, which means that these 2 species had the highest divergence genetically. In addition, based on the mean of the intraspecies genetic

Table 1. The mean of interspecies genetic distance in the COXI mitochondrial gene.

Species	<i>O. iran</i>	<i>O. zagrosensis</i>	<i>O. farzanpay</i>	<i>O. stockwelli</i>
<i>O. iran</i>	0.010			
<i>O. zagrosensis</i>	0.125	0.012		
<i>O. farzanpay</i>	0.127	0.103	0.000	
<i>O. stockwelli</i>	0.136	0.107	0.064	0.000
Outgroup	0.175	0.162	0.158	0.170

Table 2. The number of intraspecies nucleotide sequences, nucleotides, haplotypes, nucleotide difference mean, nucleotide diversity, haplotype diversity, and separating positions (polymorphic) in 4 studied specimens.

	<i>O. iran</i>	<i>O. zagrosensis</i>	<i>O. farzanpay</i>	<i>O. stockwelli</i>
The number of nucleotides sequenced	5	2	3	3
The mean of nucleotides differences	2.700	18.500	3.000	00.000
Nucleotides divergence	0.00897	0.04386	0.00953	0.0000
The number of separating sites	7	18	9	00

distance as shown in Table 3, the highest intraspecies divergence (0.5%) is that of *O. iranus* and the lowest intraspecies genetic divergence (0) belongs to *O. stockwelli* and *O. farzanpay*. The mean of the intraspecies distance of the *Orthochirus* genus was 0.004 ± 0.001 based on 16srRNA gene and the mean of the interspecies distance of this genus was 0.106 ± 0.003 .

Table 4 shows the number of nucleotide sequences, nucleotides, haplotypes, nucleotide difference mean, nucleotide diversity, haplotype diversity, and separating positions (polymorphic) in the 5 species.

The maximum likelihood was obtained using MEGA 7.0 software; 50% majority-rule consensus tree of ML with verification test of bootstrap was drawn with 2000 repetitions. The phylogenetic tree constructed based on maximum likelihood analysis of 16S rRNA and COXI gene revealed the grouping of *Orthochirus iranus*, *O. farzanpay*, *O. stockwelli*, *O. zagrosensis*, *O. innesi* (JQ514244.1 Morocco), and *O. bicolor* (KT716038.1 India), with the outgroup species *Androctonus crassicauda* (Figure). In the phylogenetic tree, there are 4 main subclades. Clade A comprises 3 subclades including *O. iranus*, *O. zagrosensis*, and *O. innesi*; clade B is subdivided into the 2 subclades of *O. farzanpay* and *O. stockwelli* species; *O. bicolor* is in subclade C in the tree.

The *Orthochirus* genus is one of the scorpions of the Buthidae family which can be found in sandy heights along water channels (Fet and Lowe, 2000). This small scorpion has a geographic distribution pattern in Iran, India, Afghanistan, Pakistan, Iraq, Oman, Turkey, Uzbekistan,

Turkmenistan, and North Africa (Fet, 1989). Seventeen species have been identified in these regions (Kovarik, 2004), 6 of which have been found in western and southwestern Iran (Navidpour, 2015). *Orthochirus farzanpay* is one of the species found in Iran in the provinces of Kerman, Khuzestan, and Sistan and Baluchestan (Navidpour et al., 2008; Navidpour et al., 2011). The earlier name of *Orthochirus farzanpay* was *Simonodeous farzanpay*, which was given in a review of the diagnostic features including the row of internal and external granules on the moving fingers of the pedipalp and the presence or absence of sensitive hair in the metasoma and telson area (Kovarik and Fet, 1989). Most isolates in the Khuzestan region include *Orthochirus iranus*, *O. farzanpay*, *O. stockwelli*, and *O. zagrosensis*. Analysis based on the morphological features of the species is regarded as a challenge due to the close morphological similarities. Thus, the molecular studies, along with the phylogenetic relations of species, can be considered as a solution for this challenge. In the present study, the sequences of the COXI gene with 668 nucleotide pairs and 16SrRNA gene with 350 nucleotide pairs were analyzed. There are 4 main clades in the phylogenetic tree. Clade A has 3 subclades including *O. iranus*, *O. zagrosensis*, and *O. innesi*; in clade B, there are 2 subclades, the *O. farzanpay* and *O. stockwelli* species; *O. bicolor* is in subclade C in the tree.

In general, as expected, all of the obtained isolates in the study confirmed the isolation of the 4 mentioned species. The results of the phylogenetic study demonstrated

Table 3. The mean of interspecies genetic distance in 16S gene.

Species	<i>O. iranus</i>	<i>O. zagrosensis</i>	<i>O. farzanpay</i>	<i>O. stockwelli</i>
<i>O. iranus</i>	0.005			
<i>O. zagrosensis</i>	0.065	0.0016		
<i>O. farzanpay</i>	0.135	0.118	0.000	
<i>O. stockwelli</i>	0.119	0.127	0.044	0.000
Outgroup	0.228	0.190	0.158	0.214

Table 4. The number of nucleotide sequences, nucleotides, haplotypes, nucleotide difference mean, nucleotide diversity, haplotype diversity, and separating sites (polymorphic) in 4 studied specimens.

	<i>O. iranus</i>	<i>O. zagrosensis</i>	<i>O. farzanpay</i>	<i>O. stockwelli</i>
The number of nucleotides sequenced	5	2	3	3
The mean of nucleotides differences	5.000	1.630	0.000	0.000
Nucleotides divergence	0.0167	0.005	0.000	0.000
The number of separating sites	6	3	0	0

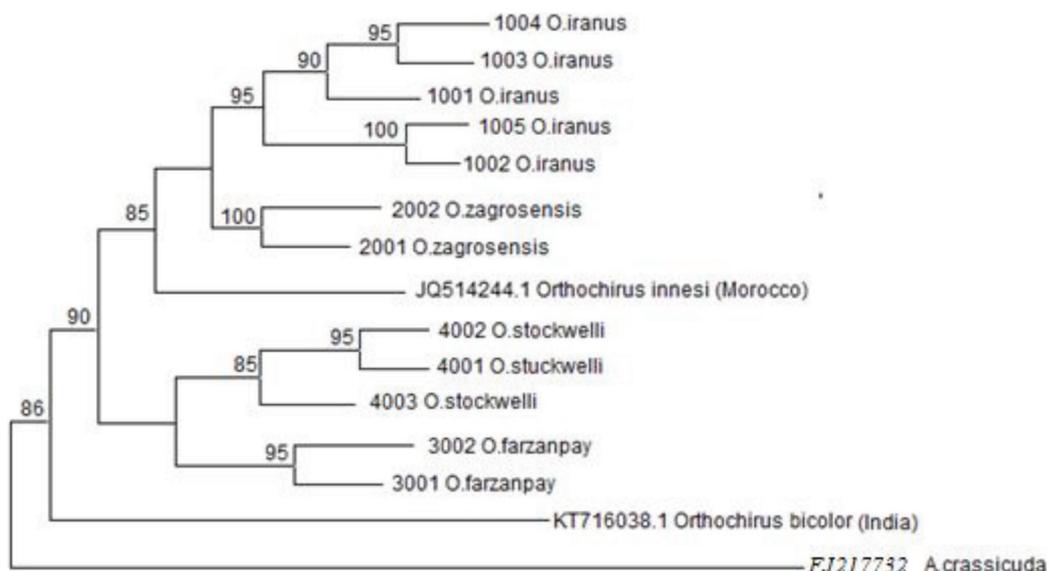


Figure. Phylogram showing phylogenetic relationships estimated using maximum likelihood analysis of 16S rRNA and COXI gene revealed the grouping of *Orthochirus iranus*, *O. farzanpay*, *O. stockwelli*, *O. zagrosensis*, *O. innesi* (JQ514244.1 Morocco), and *O. bicolor* (KT716038.1 India), with the outgroup species *Androctonus crassicauda* (FJ217732).

the mean of the genetic distance among these 4 species. *Orthochirus iranus* had the highest genetic distance from *O. stockwelli*, while the lowest genetic distance was observed between *O. farzanpay* and *O. stockwelli*. In addition, the highest intraspecies divergence was observed between the species of *O. iranus* and *O. zagrosensis*, while the lowest intraspecies divergence was found between the species of *O. farzanpay* and *O. stockwelli*. The molecular phylogenetic analysis based on mitochondrial marker indicated a close relation between *O. iranus* and *Orthochirus innesi* (Morocco) (Jafari et al., 2018).

In the first molecular phylogenetic analysis on the Buthidae family, which was conducted through the 16SrRNA marker, the findings of this study showed 3 groups including a) *Compsobuthus*, *Mesobuthus*, *Liobuthus*, and *Kraepelinia*; b) *Hottentotta* and *Buthacus*; and c) *Orthochirus* and *Anomalobuthus*. The phylogeny of this family indicates that *Orthochirus* sp. has a very different position which can be considered a subfamily of the Buthidae, and that there is a close relation between *Orthochirus* sp. and *Anomalobuthus* sp. (Fet et al., 2003).

The molecular phylogenetic analysis in Iran on *Mesobuthus eupeus* from the Buthidae family through the mitochondrial gene of cytochrome c oxidase subunit 1 revealed a considerable divergence among collected specimens from the northern and southern regions. To this end, geological studies of the Iranian plateau's history have been presented for the evolutionary and phylogenetic

structure of the *Mesobuthus* sp. scorpions. Two distinct clades of *Mesobuthus* sp., a collection of species with very similar morphological characteristics, were observed in the study (Mirshamsi et al., 2010). In the phylogenetic study of *Odonthobutus* sp. of Khorasan Province, at least 5 species have been reported in Iran, and morphological features, statistical morphometric analysis, and molecular evidence in this study confirmed the separation of these species (Mirshamsi et al., 2013). In another study, the intraspecies analysis of *Androctonus crassicauda* through cytochrome c oxidase indicated no intraspecies diversity in isolated specimens in Turkey (Ozkan et al., 2010).

In conclusion, the genetic structure of the concerned population should be identified and evaluated through molecular methods, and then a management plan should be developed and applied for conserving and restoring resources before implementing any management or production program. To this end, molecular and phylogenetic analysis should be applied to investigate the relative, demographic, and genetic structure of various species of scorpions in different regions of the country where there is a high diversity of scorpion species.

Acknowledgments

This research was supported by the Department of Venomous Animals, Razi Vaccine and Serum Research Institute, Agricultural Research, Education, and Extension Organization (AREEO), Ahvaz, Iran.

References

- Fet V, Gantenbein B, Gromov AV, Lowe G, Lourenco WR (2003). The first molecular phylogeny of Buthidae (Scorpiones). *Euscorpius* 4: 1-10. DOI: 10.3109/24701394.2016.1149830
- Fet V (1989). A catalogue of scorpions (Chelicerata: Scorpiones) of the USSR. *Review Museum Society National Bergamo* 13: 73-171.
- Fet V, Lowe G (2000). Family Buthidae C.L. Koch, 1837. In: Fet V, Sissom WD, Lowe G, Braunwalder ME, editors. *Catalog of the Scorpions of the World (1758–1998)*. New York: New York Entomological Society, pp. 54-286.
- Gantenbein B, Fet V, Largiadèr CR, Scholl A (1998). First DNA phylogeny of the genus *Euscorpius* Thorell, 1876 (Scorpiones: Euscorpiidae) and its bearing on the taxonomy and biogeography of this genus. *Biogeographica* 75: 59-72. DOI: 10.3897/zookeys.219.3597
- Jafari H, Salabi F, Jelodar A, Navidpour SH, Jahanifard E et al. (2018). Phylogenetic study on *Orthochirus iranus* by using morphological and molecular methods (Scorpiones: Buthidae). *Journal of Entomology and Zoology Studies* 6(3): 304-309.
- Kovařík F (2004). Revision and taxonomic position of genera *Afghanorthochirus* Lourenço & Vachon, *Baloorthochirus* Kovařík, *Butheolus* Simon, *Nanobuthus* Pocock, *Orthochiroides* Kovařík, *Pakistanorthochirus* Lourenço, and Asian *Orthochirus* Karsch, with descriptions of twelve new species (Scorpiones, Buthidae). *Euscorpius* 16: 1-33.
- Kovařík F, Fet V (2006). Taxonomic position of the genus *Simonoides* Vachon et Farzanpay and description of a new species of *Orthochirus* Karsch from Iran (Scorpiones: Buthidae). *Euscorpius* 38: 1-10.
- Mirshamsi O, Sari A, Elahi E, Hosseinie S (2010). Phylogenetic relationships of *Mesobuthus eupeus* (C.L. Koch, 1839) inferred from COI sequences (Scorpiones: Buthidae). *Journal of Natural History* 44: 2851-2872. DOI: 10.1080/00222933.2010.512400
- Mirshamsi O, Sari A, Hosseinie S (2011). History and current checklist of Iran's scorpiofauna (Arachnida: Scorpiones). *Progress in Biological Sciences* 1: 16-28.
- Mirshamsi O, Azghadi S, Navidpour S, Aliabadian M, Kovařík F (2013). *Odontobuthus tirkari* sp. nov. (Scorpiones, Buthidae) from the eastern region of the Iranian Plateau. *Zootaxa* 3731: 153-170 DOI: 10.11646/zootaxa.3731.1.7
- Navidpour SH (2015). An annotated checklist of scorpions in south and southwestern parts of Iran. *International Journal of Fauna and Biological Studies* 2(3): 9-15.
- Navidpour SH, Ezatkah M, Kovařík F, Soleglad ME, Fet V (2011). Scorpions of Iran (Arachnida: Scorpiones). Part VII Kerman Province. *Euscorpius* 131: 1-32.
- Navidpour SH, Kovařík F, Soleglad ME, Fet V (2008). Scorpions of Iran (Arachnida, Scorpiones). Part I Khuzestan Province. *Euscorpius* 65: 1-41.
- Ozkan O, Ahmet C, Zafer K (2010). A study on the genetic diversity of *Androctonus crassicauda* (Olivier, 1807; Scorpiones Buthidae) from Turkey. *The Journal of Venomous Animals and Toxins including Tropical Diseases* 16(4): 599-606. DOI: 10.1590/S1678-91992010000400010
- Vachon M (1974). Étude des caractères utilisés pour classer les familles et les genres de Scorpions (Arachnides) La trichobothriotaxie en arachnologie. Sigles trichobothriax et types de trichobothriotaxie chez les scorpions. *Bulletin du Muséum National d'Histoire Naturelle* 3: 857-958.