



Molecular study of *Hemiscorpius* Peters (Scorpiones: Hemiscorpiidae) in Hormozgan province, South of Iran

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How to cite this article: Shahi M, Barahoei H. Molecular study of *Hemiscorpius* Peters (Scorpiones: Hemiscorpiidae) in Hormozgan province, South of Iran. *Archives of Razi Institute*. 2024;79(1):211-217. DOI: 10.32592/ARI.2024.79.1.211



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ABSTRACT

Three species of *Hemiscorpius* were identified in Hormozgan province, for which the available antivenoms lack the efficacy required for treating patients. Consequently, an exact identification of the existing species was deemed necessary as the first step in managing treatment procedures. Considering the morphological similarities among the species, the aim of this research was the molecular study of the samples to accurately identify the species. *Hemiscorpius* specimens were collected from various locations in Hormozgan province between 2021 to 2023. The Cytochrome c oxidase subunit I gene was amplified and sequenced. Four sequences were obtained from *Hemiscorpius* specimens collected from Hormozgan province, and three sequences were sourced from the NCBI for analysis. Bayesian inference and Maximum likelihood phylogenetic trees showed similar results, positioning the base of *Hemiscorpius enischnochela* tree as an older species and *Hemiscorpius lepturus* adjacent to *Hemiscorpius acanthocercus*, identified as the newest species at the tree's tip. The results confirmed the validity of three species, namely *H. acanthocercus*, *H. enischnochela*, and *H. lepturus*. *Hemiscorpius acanthocercus* and *H. lepturus* are known for having dangerous venom for humans with reported deaths due to their stings. Considering the importance of the members of this genus from the medical point of view, a comprehensive examination of all species is imperative.

Keywords: Bayesian inference, Cytochrome c oxidase subunit I, Genetic distance, *Hemiscorpius*, Hormozgan

Article Info:

Received: 17 July 2023

Accepted: 28 October 2023

Published: 29 February 2024

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1. Introduction

The family Hemiscorpidae Pocock, 1893 (Arachnida: Scorpiones) consists of a single genus, *Hemiscorpis*, encompassing 17 globally described species (1), with seven of them distributed in Iran (2). All species reported in Iran have been characterized based on morphological studies. A morphological investigation of the Hemiscorpidae family in Hormozgan province revealed the existence of three *Hemiscorpis* species including *H. acanthocercus* Monod & Lourenço, 2005, *H. enischnochela* Monod & Lourenço, 2005 and *H. shahii* Kovařík et al., 2017 (3). Limited research with molecular basis has been conducted on the Iranian scorpions, primarily focusing on Buthidae species. Mirshamsi et al. (2013) and Azghadi et al. (2014) revised the genus *Odontobuthus* Vachon, 1950 and described a new species (4, 5). Jolodar (2019) sequenced the Cytochrome c oxidase subunit I (*COI*) gene of *H. lepturus*, collected from Khuzestan province (6). A simultaneous morphological and molecular study of *Androctonus crassicauda* (Olivier, 1807) was also conducted in Khuzestan (7). Jafari et al. (2020b) explored the molecular analysis of various Orthochirus Karsch, 1891 species (2020b) (8). Additionally, Soltan-Alinejad et al. (2021) investigated six scorpion species molecularly (9). A study by Barahoei et al. (2022) involving morphological, morphometric, and molecular analysis of *Odontobuthus* resulted in the description of three new species (10). Scorpionism poses a health concern in specific countries, particularly Iran. Predominantly, scorpion stings in southern Iran are attributed to members of *Hemiscorpis*. *Hemiscorpis lepturus* Peters, 1861, is responsible for more than one-third of the annual stings in Ahvaz (11, 12). The first death report by *H. acanthocercus* Monod & Lourenço, 2005, was documented in Hormozgan province (13). Different populations in regions with distinct weather conditions have different venom compositions (14). Consequently, available antivenoms lack proper efficacy for treating patients, underscoring the need to identify different species in each region to manage the treatment of patients. Given the morphological similarities among certain important species, there is an urgent demand for molecular investigation of these species. Several studies have used the *COI* gene sequences from different genera for the precise scorpion species determination (10, 15-17). Furthermore, the *COI* gene has been useful for genealogical studies at the species level. Molecular studies not only affirm the validity of described species but also offer the potential to introduce new species and identify synonyms.

2. Materials and Methods

Hemiscorpis Peters (Scorpiones: Hemiscorpidae) specimens were collected from Hormozgan province

between 2021 to 2023. The samples were preserved in 96% ethanol and species were identified using the identification key (2) based on morphological traits. The second and third legs on the left side of the scorpion were separated and placed in tubes containing 99.8% ethanol. DNA extraction from the fixed tissue was carried out using the FavorPrep™ Tissue Genomic DNA Extraction Mini Kit (Pingtung, Taiwan). A fragment of the *COI* was amplified by polymerase chain reaction (PCR) using two primers

including LCO1490(GGTCAACAAATCATAAAGATATTGG) and HCO2198(TAAACTTCAGGGTGACCAAAAAATCA)

(18). The thermal cycler profiles used for amplification were as follows: step1: 94°C for 2 minutes and 30 seconds; step 2 (35 cycles): 94°C for 45 seconds, 52°C for 60 seconds and 72°C for 60 seconds; step 3: 72°C for 5 minutes. Purification and sequencing of amplified genes were done by Niagene Noor Company (Tehran). The quantity and quality of the sequences were assessed by BioEdit 7.1.9 (19) and then prepared for further analysis. The nine newly generated *COI* sequences have been deposited in GenBank [<http://www.ncbi.nlm.nih.gov>] with the accession numbers OR803737 to OR803739, OR828045 to OR828049 and OR800364 (Table 1). Genetic distances within and between species were investigated with MEGA 7.0 (20). Bayesian inference (50,000,000 generations) implemented using the CIPRES website (<http://www.phylo.org>) and Maximum likelihood tree (10,000 iterations) on the website <http://www.atgc-montpellier.fr/phyml> (Guindon et al., 2010) were conducted. All voucher specimens are stored at the Research Institute of Zabol (RIOZ).

3. Results

The analysis involved nine sequences obtained from *Hemiscorpis* specimens collected in Hormozgan province and three sequences from the National Center for Biotechnology Information (NCBI) *Odontobuthus tavighiae* used as outgroup (Table 1). The aligned *COI* sequences are 728 bp in length, with (79,1) 44 positions conserved (70,9) 194, variable, and 107 (17,03) parsimony-informative. Both Bayesian inference (BL) and Maximum likelihood (ML) phylogenetic trees showed similar results. *Hemiscorpis enischnochela* was placed at the base of the tree (Figure 1), as an older species of the genus and a sister group of two other species (*H. acanthocercus* and *H. lepturus*). *Hemiscorpis lepturus* placed the sister group of *H. acanthocercus* as the newest species at the tip of the tree. These two species are morphologically similar to each other (Figure 1).

Table 1. Data of scorpion specimens were used for sequencing of *COI* gene of *Hemiscorpius* and *Odontobuthus tavighiae* (as outgroup).

Species	Collection	Locality	Genbank
<i>Hemiscorpius acanthocercus</i>	RIZ-Hem-098A	IRAN, Hormozgan province, Bandar Abbas-Rudan Road	OR803737
<i>Hemiscorpius acanthocercus</i>	RIZ-Hem-098B	IRAN, Hormozgan province, Bandar Abbas-Rudan Road	OR803738
<i>Hemiscorpius acanthocercus</i>	RIZ-Hem-151	IRAN, Hormozgan province, Bandar Abbas	OR803739
<i>Hemiscorpius enischnochela</i>	RIZ-Hem-098A	IRAN, Hormozgan province, Parsian	OR828045
<i>Hemiscorpius enischnochela</i>	RIZ-Hem-098B	IRAN, Hormozgan province, Parsian	OR828046
<i>Hemiscorpius enischnochela</i>	RIZ-Hem-148	IRAN, Hormozgan, Bandar Abbas, Genow village	OR828047
<i>Hemiscorpius enischnochela</i>	RIZ-Hem-098	IRAN, Hormozgan province, Bandar Abbas-Rudan Road	OR828048
<i>Hemiscorpius enischnochela</i>	RIZ-Hem-087	IRAN, Hormozgan province, Khamir, Ruydar	OR828049
<i>Hemiscorpius lepturus</i>	RIZ-Hem-162	IRAN, Khuzestan, Dezful	OR800364
<i>Hemiscorpius lepturus</i>	-	IRAN, Khuzestan	KU341987
<i>Hemiscorpius lepturus</i>	-	IRAQ	MT230874
<i>Odontobuthus tavighiae</i>	-	IRAN, Bushehr province, Daylam	MW655764

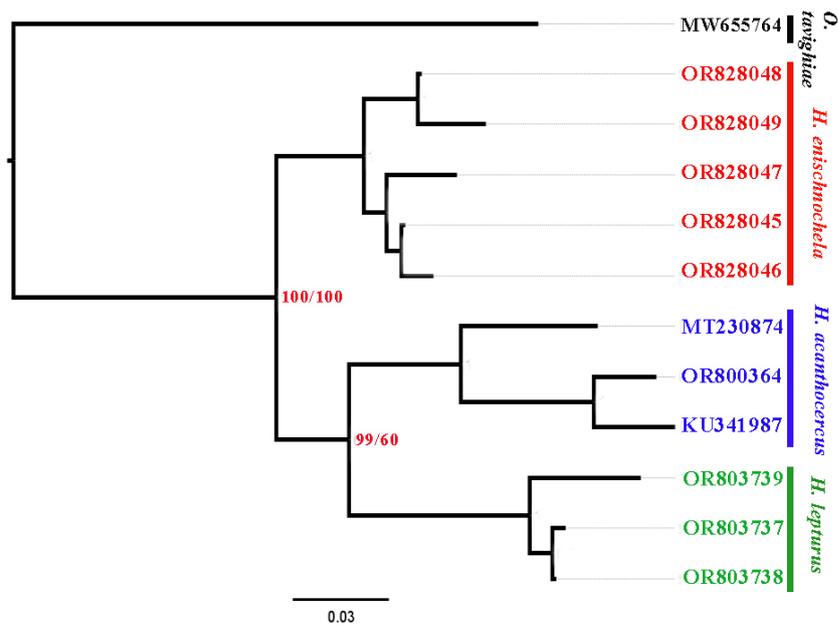


Figure 1. Bayesian inference (BI) was obtained by analysis of *COI* gene of *Hemiscorpius* spp. *Odontobuthus tavighiae* is used as outgroup. Posterior probability values from BI and bootstrap values from maximum likelihood (ML) analyses were added for each node, respectively.

The smallest genetic distance was observed between *H. acanthocercus* and *H. enischnochela* (0.011). Meanwhile, The highest genetic distance was between *H. acanthocercus* and Iraqi *H. lepturus* (0.112) (Table 2).

The genetic distances confirmed the validity of three species, namely *H. acanthocercus*, *H. enischnochela*, and *H. lepturus* (Table 2).

Table 2. Average Kimura 2-parameter (K2P) genetic distances among and within (bold) species of *Hemiscorpius* and two outgroups sequencing of *COI* gene. Standard error estimates are shown above the bold diagonal.

	<i>Hemiscorpius acanthocercus</i>	<i>Hemiscorpius enischnochela</i>	<i>Hemiscorpius lepturus</i> (Iraq)	<i>Hemiscorpius lepturus</i> (Iran)	<i>Odontobuthus tavighiae</i>
<i>Hemiscorpius acanthocercus</i>	0.026	0.110	0.125	0.123	0.247
<i>Hemiscorpius enischnochela</i>	0.081	0.034	0.123	0.123	0.210
<i>Hemiscorpius lepturus</i> (Iraq)	0.112	0.106	N/A	0.114	0.233
<i>Hemiscorpius lepturus</i> (Iran)	0.088	0.083	0.092	0.045	0.251
<i>Odontobuthus tavighiae</i>	0.235	0.193	0.233	0.229	N/A

4. Discussion

Twenty-five samples of *H. acanthocercus* (9 samples), *H. enischnochela* (10 samples), *H. lepturus* (2 samples) and *H. Shahii* (4 samples) were utilized for *COI* gene sequencing. However, only nine sequences were obtained from these samples, including three sequences from *H. acanthocercus*, five sequences from *H. enischnochela* and one sequence from *H. lepturus* (Table 1, Figure 1). Samples collected over a year ago, such as *H. Shahii* in this study, did not yield successful sequencing. The storage conditions of samples also play an important role in sequencing. Successful sequencing may be unattainable in instances where the sample is desiccated, the ethanol amount is diminished, the ethanol quality is subpar, or the ethanol is excessively diluted. Optimal results are achieved by using live specimens or freshly fixed samples in 96 or 99% ethanol for less than 6 months. The findings of the study confirmed the validity of three species including *H. acanthocercus*, *H. enischnochela*, and *H. lepturus* (Figure 1; Table 2). Notably, *Hemiscorpius lepturus* was positioned as a sister group of *H. acanthocercus*, this two species are morphologically similar (Figure 2), having three trichobothria on the ventral surface of their pedipalp patella. In contrast, *Hemiscorpius enischnochela* (Figure 3) specimens were characterized by their lighter pigmentation and had 10-12 trichobothria on the ventral surface of their pedipalp patella. *Hemiscorpius acanthocercus* and *H. lepturus* are distributed in the east and west of the Zagros Mountains, respectively (3). In addition, both species have dangerous venom for humans resulting from their stings, with documented cases of fatalities (13, 22). Given the variation in venom composition among different species, both the identification of each species within this genus and the range of distribution of each species are necessary for scorpion sting prevention and patient treatment, involving the development of specific antivenoms tailored

to each species. In a study by Jolodar (2019), the *COI* sequence of *H. lepturus* was compared with another sequence from the *H. lepturus* collected from Izeh, Khuzestan province (accession number: KU341987.1) revealing a genetic distance of 0.06 between them (6). However, due to the shortness of the sequence, it was excluded from the present research. Similarly, the sequence of *H. persicus* Birula, 1903 (accession number: KU341988.1) was not utilized in this study as it displayed complete similarity to the sequence belonging to *H. lepturus* (accession number: KU341987.1). The genetic distance observed within and between species of the Hemiscorpiidae family surpasses that of the Buthidae family (Table 2). The genetic distance between Iranian and Iraqi samples of *H. lepturus* (0.092) is too large (Table 2), potentially contributing to the limited number of sequences available for these species of each population and the absence of sequences for all species within this genus. Therefore, sequencing other species within this genus is necessary. Once the sequences of all species have been examined, a comprehensive discussion of this matter can be conducted. Additionally, it raises the possibility to propose the scenario regarding the origin of the *Hemiscorpius* genus and the distribution map of its species in the plateau of Iran.

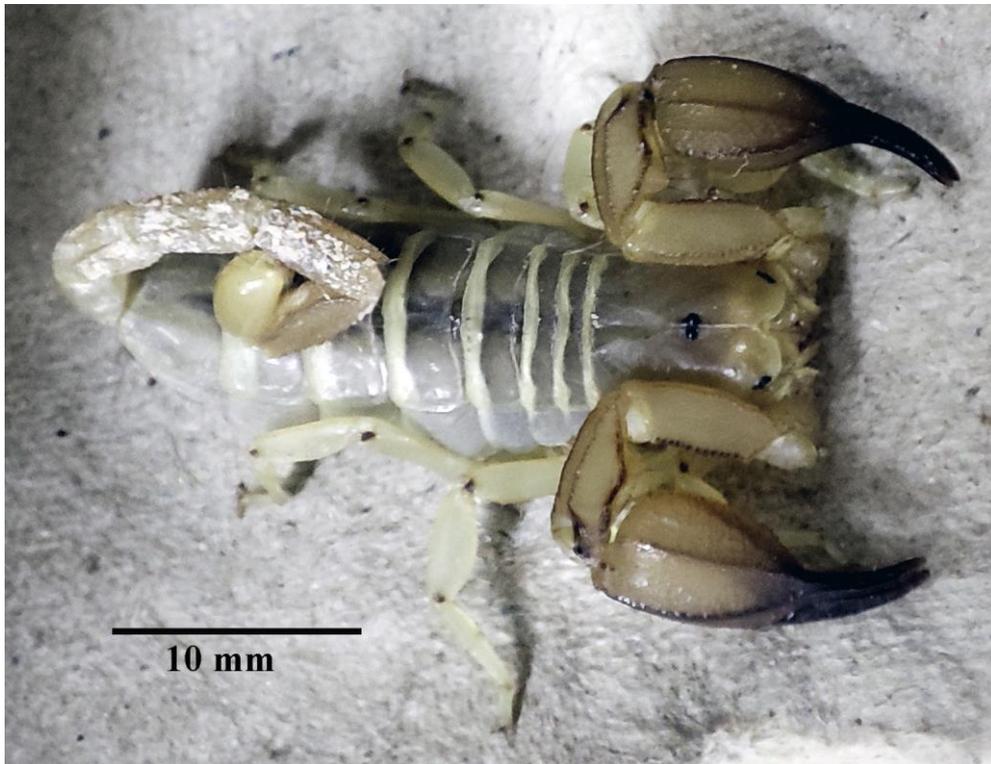


Figure 2. Habitus of a female alive specimen of *Hemiscorpius acanthocercus* collected from Hormozgan province, Bandar Abbas County, Geno village.



Figure 3. Habitus of a female alive specimen of *Hemiscorpius enischnochela* collected from Hormozgan province, Bandar Abbas County, Geno village.

Acknowledgment

This study was funded by the Vice-Chancellor for Research and Technology at Hormozgan University of Medical Sciences, Iran. The authors would like to express their sincere gratitude for the invaluable support provided by this deputy, which was instrumental in the successful completion of our research.

Authors' Contribution

Collecting samples and revising the manuscript: Mehran Shahi. Laboratory operations, drafting the manuscript, and correspondence: Hossein Barahoei. All authors read and approved the final version of the manuscript.

Ethics

The protocol was accepted by the Research Institute of Zabol, Zabol, Iran.

Conflict of Interest

The authors affirm that there is no conflict of interest concerning the publication of this paper.

Grant Support

This research was granted by the Hormozgan University of Medical Sciences, Project No. ۹۹.۰۰.۲ (IR.HUMS.REC.1400.099).

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