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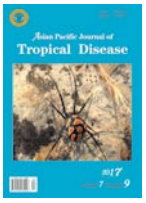

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Molecular investigation on Iranian widow spider *Latrodectus tredecimguttatus* based on DNA barcode analysis

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ABSTRACT

Objective: To identify the caught samples of *Latrodectus tredecimguttatus* (*L. tredecimguttatus*) to the species level and to compare the obtained sequences with those of them that have been submitted in GenBank in Bojnurd district, located in north-east part of Iran.
Methods: Samples were collected from different places of Bojnurd district using direct search method and were transferred to insectary. After then, by using valid morphological keys, samples were identified to the species level. Moreover, the DNA related to some samples was extracted by the use of different methods such as Collins, phenol-chloroform, salting-out and G-Spin kit. Finally, COI gene was studied by PCR amplification.
Results: Totally, two egg sacs were collected as well as two mature female spiders. According to the lab results, by the use of molecular methods, 50 spiderlings belonging to an egg sac was evaluated. The results of PCR assay revealed that the best way for DNA extraction was salting-out method. Finally, the sequence of the partial mtDNA-COI of *Latrodectus tredecimguttatus* (*L. tredecimguttatus*) sample was submitted to GenBank. The results showed an identity of 99% of the studied samples with those of GenBank.
Conclusions: Widow spiders are widely spread in different parts of the world and their bites cause death. It is important to study these samples by the use of molecular methods and then to produce them in a mass volume in order to fight and extract their venom for provision of anti-serum. To the best of our knowledge, this is the first molecular survey on spiders in Iran. Finally, the assessment of the human protection against the specific Iranian *L. tredecimguttatus* anti-venom is suggested.

1. Introduction

Spiders of *Latrodectus* Walckenaer genus (family: Theridiidae) are prevalent in most countries including Iran[1]. To date, around 31 species of this genus have been identified[2]. As black widows (*Latrodectus* genus) are able to cause injuries to humans due to defensive bites resulting in systemic effects like severe muscle pain and tachycardia, they have a very essentially medical importance[3]. Spider bite caused by widow *Latrodectus tredecimguttatus* (*L. tredecimguttatus*) is one of the main spider-related injuries[4]. In humans, *Latrodectus* bites may result in severe cramps, muscle pain and nausea, but it is important to mention that they are only occasionally fatal[5].

Investigators have studied the taxonomic aspects of this species as well as their toxins and venums due to their importance[6-8]. However, the taxonomy of *Latrodectus* genus has experienced a great difficulty associated with recognizing discrete morphological boundaries between members of the genus[9]. Slight variation in characters like somatic coloration, presence and shape of abdominal patterns, and setae length and abundance, were used in diagnosing species in early studies[10,11].

To date, an increasing number of systematic studies of spiders are using molecular methods and submitted sequence data in GenBank to investigate phylogenetic relationships among lineages within this tremendously diverse order[9].

In this investigation, a molecular assay has been carried out based on the mitochondrial (mt) gene cytochrome c oxidase

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subunit I (COI) to identify the caught samples to the species level and to compare the obtained sequences with those of them that have been submitted in GenBank[9] in Bojnurd district, located in north-east part of Iran.

2. Materials and methods

2.1. Study area

Bojnurd is the capital city of North Khorasan Province, Iran. The city is noted for its multicultural background. North Khorasan Province is a province located in Northeastern Iran. This province is bordered with Turkmenistan (Figure 1).

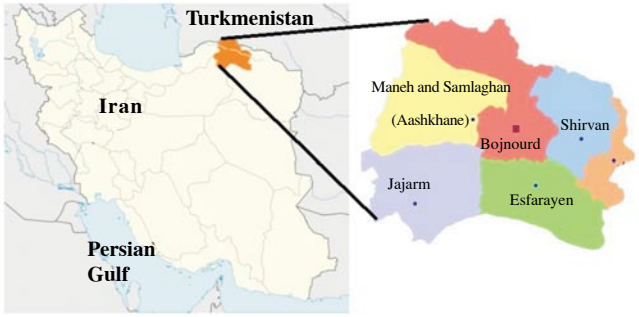

Figure 1. North Khorasan Province is located in north-east of Iran.

2.2. Molecular analysis

After samples determination by the use of valid keys, DNA was extracted by the use of various methods for optimization. In the present investigation, Collins, phenol-chloroform, salting-out and G-Spin kit methods were used to find the best optimized DNA extraction method. After DNA extraction, the samples were electrophoresed in 1% gel agarose.

PCR reactions was performed on extracted DNA on barcode mtDNA-COI. For PCR, Premix kits (Intron, South Korea) including 2 μL gel loading buffer, *Taq* DNA polymerase, dNTPs, $MgCl_2$ and reaction buffer (10×) were used. Total volume of each reaction was 20 μL (forward primer: 2 μL; reverse primer: 2 μL; ddH_2O: 11.5 μL; DNA: 2 μL; Premix: 2 μL; $MgCl_2$: 0.5 μL).

In this investigation, designed primers by Folmer *et al.* was used[12]. These primers altogether were used for amplification. The following primer pair consistently amplified a 710-bp fragment of COI which were efficient across the broadest array of invertebrates[12]: FCOI: 5'-TTAAACTTCAGGGTGACCAAAAAATCA-3' and RCOI: 5'-GGTCAACAAATCATAAAGATATTGG-3'. The thermal cycle program was as follow: initial denaturation: 94 ˚C for 2 min, denaturation: 94 ˚C for 40 s, annealing: 45 ˚C for 40 s, extension: 72 ˚C for 1

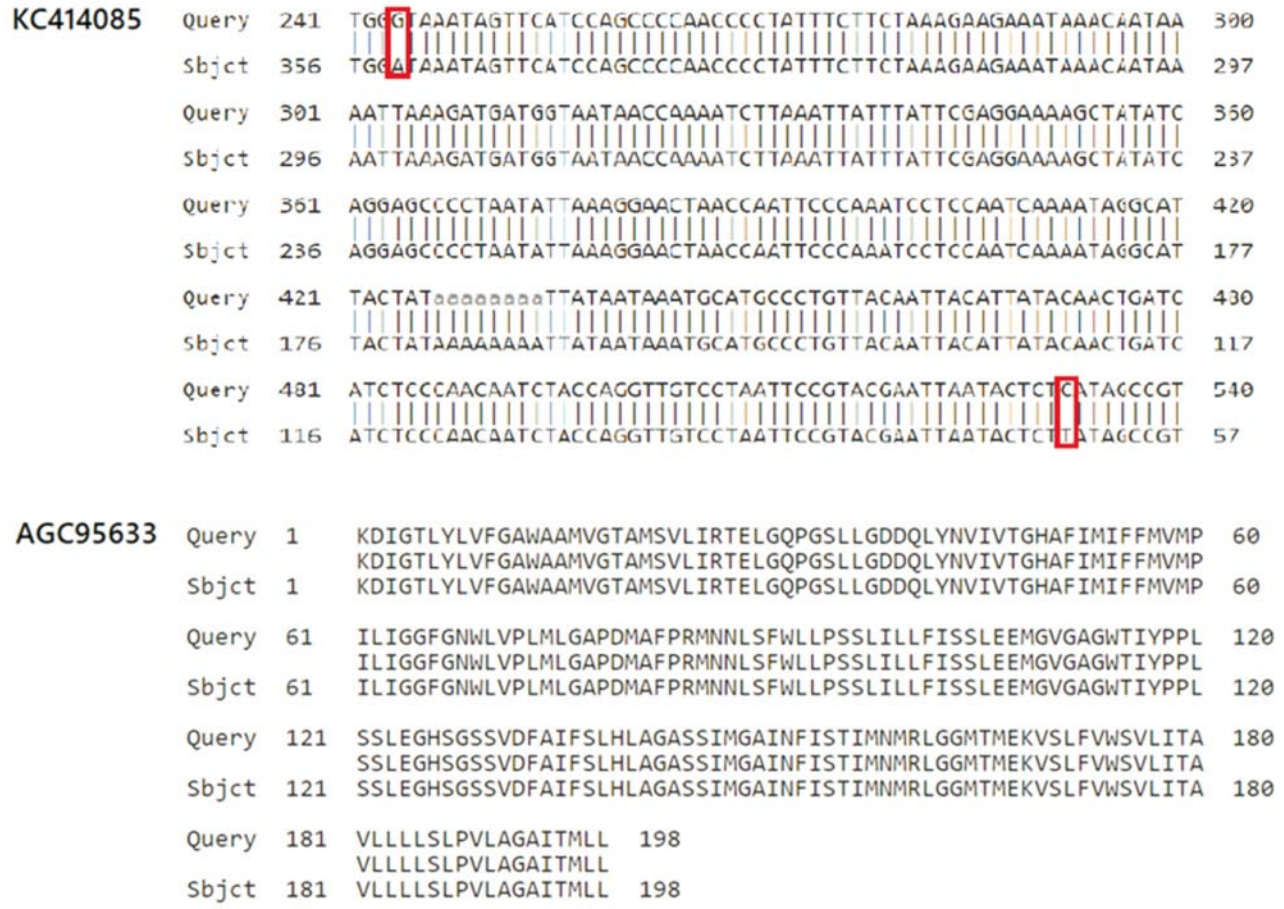

Figure 2. As it is obvious, the sequence analysis of the Iranian black widow (AN: KC414085) has an identity of 594/596 nucleotides (99%) with the submitted sequences in GenBank. These two nucleotides do not affect the protein structure.

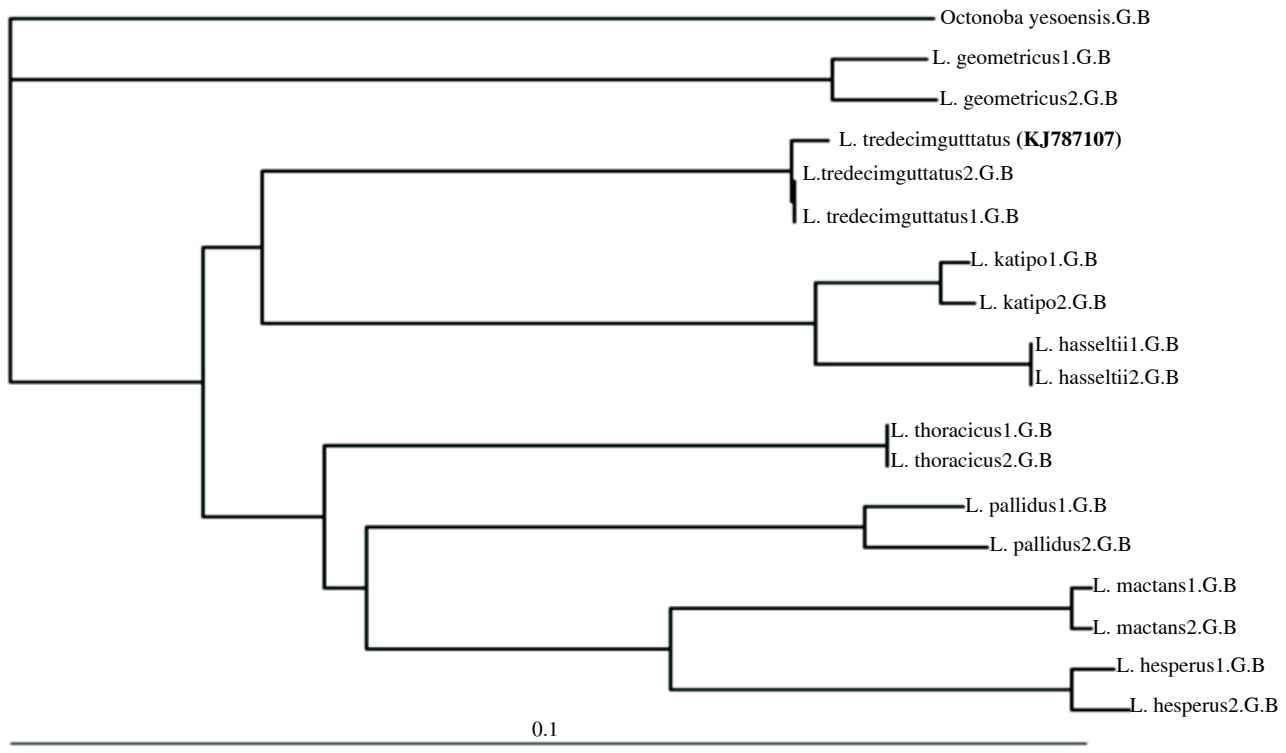

Figure 3. Phylogenic analysis of mtDNA-COI gene in black widow.

min (5 cycles), and denaturation: 94 ℃ for 40 s, annealing: 51 ℃ for 40 s, extension: 72 ℃ for 1 min (35 cycles) and finally, a final extension of 72 ℃ for 5 min.

After PCR assay, 30 μL of each selected sample was prepared and finally sequenced (Bioneer, South Korea). Sequencing was performed using an ABI 3730 sequencer machine (Bioneer, South Korea). The ambiguous sequences were corrected with Chromas software and the consensus sequences obtained using DNASTAR Lasergene (SEQMAN and EDITSEQ). The sequences were aligned using ClustalW (http://www.ebi.ac.uk/clustalw) and BLAST software tool (http://www.ncbi.nih.gov.org/blast) to explore possible identity percent and polymorphisms and finally were submitted to GenBank.

3. Results

In order to conduct molecular studies and using PCR technique, it was needed to extract DNA from samples. In this study, various methods of DNA extraction were tested. Comparison of DNA quality according to PCR showed that salting out is better than other methods.

To conduct PCR tests, FCOI and RCOI primers were used and products were electrophoresed on 1% agarose gel. Results showed that these primers amplify one part of the mtDNA-COI gene with the length of 711 bp.

Totally, 25 PCR products of studied spiders were sequenced (Bioneer, South Korea). The alignment of acquired sequences with submitted sequences in GenBank showed that these sequenced samples belong to *L. tredecimguttatus* with an identity of 99% with submitted sequences in GenBank (Figure 2). This sequence submitted to GenBank under accession number KJ787107. Finally, the phylogenetic relationship of black widow was analyzed (Figure 3).

4. Discussion

L. tredecimguttatus is highly venomous black widow spider with toxicity coming from venomous glands as well as other parts of its body and also newborn spiderlings and eggs[13]. Due to the medical importance of *L. tredecimguttatus*, transcriptome analysis to understand the toxicity of this species'eggs was carried out in 2016[13]. Widow spiders are difficult taxonomically species and their bites are relatively common in northeastern part of Iran, causing morbidity and rarely mortality[14]. In Iran, *L. tredecimguttatus* spiders mostly appear in northeast part of the country[15]. In 2006, Sahra introduced a renew checklist of Iranian spiders[16]. To date, at least 244 species of spiders belonging to 33 families have been recorded from Iran. Out of these 244 recorded species, at least 62 of them are reported for the first time in recent years[16]. Morphological studies on the Iranian spiders have been carried out in Iran[15-18]. In an investigation in 2007, Rafijenad *et al.* studied on systematics, bio-ecology, and medical importance of widow spiders in northeastern part of Iran[17]. The results of that investigation revealed that the most prevalence bited cases were observed in mid-age (20–55 years old) and particularly among farmers (36.4%)[17]. Rafijenad *et al.* collected *L. tredecimguttatus*,

Latrodectus dahli, *Latrodectus geometricus* and *Latrodectus pallidus* with prevalence of 62%, 32%, 5% and 1% respectively from 15 counties of northeast of Iran[17]. They also revealed that 65% of spiders were collected in summer[17]. In an investigation carried out by Valikhanfard-Zanjani *et al*. in 2016, the effects of *Latrodectus dahli* venom on various serum biochemical parameters were determined which revealed a significant rise in liver and kidney function tests[5]. Spiders are unlikely vectors of bacterial transmission to humans when they bit; however, physicians should not casually associate bacterial infection with spider bites[19]. Morphological identification of spiders is a controversial way for investigators. Investigators suggest that morphological identification of spiders could be confirmed by molecular methods[20,21]. To the best of our knowledge, this is the first molecular study on spiders in Iran and this would be a base for future alternative studies like assessment of the human protection against the specific Iranian *L. tredecimguttatus* anti-venom.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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