

## TAXONOMIC AND MOLECULAR STUDY OF THE WIDOW SPIDER GENUS *LATRODECTUS* WALCKENAER, 1805 (ARANEAE: THERIDIIDAE) IN IRAQ

Hayder Badry Ali<sup>1</sup>, Hula Younis Fadhil<sup>2</sup>, Ishraq Mohammed Baker<sup>1</sup>

<sup>1</sup>Department of Biology, College of Science, University of Baghdad, Iraq

<sup>2</sup>Tropical-Biological Research Unit College of Science, University of Baghdad, Iraq

### ARTICLE INFORMATION

Received: December 17, 2017

Received in revised form: March 04, 2018

Accepted: June 06, 2018

### \*Corresponding Author:

Hayder Badry Ali

E-mail: [hayder.ali1130@yahoo.com](mailto:hayder.ali1130@yahoo.com)

### ABSTRACT

The widow spider, *Latrodectus*, (Araneae: Theridiidae) comprise about 31 currently recognized species, some species are very hard to distinct; in this study morphological and molecular method has been used to confirm the diagnosis of the spiders. Phylogenetic tree was constructed using the neighbor-joining of some other *Latrodectus* sp. sampled, including taxa occurring in the Middle East, Australia, New Zealand, North and South America and Europe, and with the two genera *Steatoda* Sundevall, 1833 and *Asagena* Sundevall, 1833 outgroup. The results of the analysis show the highest identity (90%) for *Latrodectus thoracicus* Nicolet 1849 represented by three specimens and for *L. pallidus* O.P.-Cambridge, 1872 represented by one specimen in addition to (85%) for the false widow *Asagena phalerata* (Panzer, 1801) represented by one specimen, these three species is recorded in Iraq for the first time.

**Keywords:** *Latrodectus*, Taxonomy, Iraq Spider Fauna, Widow Spiders

### INTRODUCTION

The genus *Latrodectus* Walckenaer, 1805 (Araneae: Theridiidae) is a worldwide distribute genus (Graudins *et al.*, 2001), it includes a group of species commonly referred to as widow spiders. It's considered a taxonomically complex genus as the status of several forms had not been properly evaluated and specific boundaries are not well defined or understood (Levi, 1959; 1967; Garb *et al.*, 2001), therefore, in multiple cases, populations has been uncritically referred to as different taxa. Discriminating between *Latrodectus* species using morphology has always been problematic (Levi, 1983), it is difficult taxonomically and readily separated from members of other Theridiid genera (Mirshamsi, 2005). The Genus *Asagena* Sundevall, 1833 was revalidated by Wunderlich (2008), this genus was earlier considered as a junior synonym of *Steatoda* Sundevall, 1833.

The information and knowledge about the widow spiders in Iraq are very limited; in general spider in Iraq was neglected and rarely studied until the last few years, recently the interest in studying of this group was increased because of the emergence of several cases of bites, which was supposed to be caused by black widow spiders, researchers have revealed the existence of three *Latrodectus* species in Iraq, *L. scelio*

(Abdul-Rassoul *et al.*, 2012), *L. hasselti* (Al-Hadlag and Najim, 2015), while Zamani and El-Hennawy (2016) rejected these two species from the list of Iraqi spiders fauna and attributed them to *L. renivulvatus*. The difficulties of diagnosis underlines the importance of molecular characters in creating a valid phylogeny for this genus, this study characterizes the first phylogenetic relationship to the *Latrodectus* genus in Iraq and is made by sequence of the partial mt DNA-COI gene. The aim of the present study is to provide a taxonomic status of the widow spider species based on molecular analysis for the first time in Iraq.

### MATERIALS AND METHODS

#### Spiders collection

Widow spiders were collected using forceps, preserved in 70-80 % ethanol, ten individual spider specimens obtained from different locations in Baghdad, Najaf and Babylon provinces. All the examined specimens were deposited in the Iraq Natural History Research Center and Museum, University of Baghdad. Identification was carried out by using morphological characteristics according to several diagnostic keys (Mirshamsi, 2005; Levi and Randolph, 1975; Sutton, *et al.*, 2006).

### DNA extraction

DNA was extracted from (1-2) legs removed with clean forceps from each specimen with the Geneaid DNA Mini extraction kit for (Tissue), according to the standard protocol recommended by the manufacturer, with some modification. Extracted DNA was being stored at -20°C until use.

### Amplification for COI Gene

The Cytochrome (C) Oxidase I gene was amplified with LepF (5'ATTCAACCAATCATAAAGATATTGG-3') (forward primer) and LepR (5'-TAAACTTCTGGATGTCCAAAAATCA-5') (reverse primer) (Brandon-Mong, et al., 2015). The DNA fragments to be analyzed were amplified using AccuPower<sub>2</sub> PCR PreMix (BIONEER, Corp., Daejeon, Korea) in 50µl reaction mixtures containing 0.4 µM each primer and 100-200 ng of genomic DNA template. The PCR amplification protocol was performed according to the following procedure: initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 60 s; annealing temperature 46.7°C for 30 s; and extension at 72°C for 60 s and final extension at 72°C for 5 min.

### RESULTS AND DISCUSSION

The results of PCR tests on 10 spider's specimens showed that LepF and LepR primers amplify one part of the mtDNA-COI gene with the length about 710 bp. only, 5 PCR products of studying spiders were sequenced. The alignment of acquired sequences with submitted sequences in Gene Bank showed that these sequenced samples belong to *Latrodectus thoracicus* for (BAB-1, KUF-3 and BAG-5) specimens and *L. pallidus* for KUF-2 specimen with Identities (90%), and belongs to *Asagena phalerata* for BAB-4 specimen with Identities (85%).

The nucleotide sequences of (BAB-1) submitted to Gene Bank under accession number MG645011 for *L. thoracicus*, nucleotide sequences of KUF-2 submitted under accession number MG645012 for *L. pallidus*, and nucleotide sequences of BAB-4 submitted under accession number MG645013 for *A. phalerata*. Sequencing ID in Gene Bank, score, expect and compatibility of Cytochrome Oxidase Subunit 1 (COI) gene, partial cds; mitochondrial sequences of the isolates spiders were summarized as in Table 1.

**Table 1**

Sequencing ID in GenBank, score, expect and compatibility of Cytochrome Oxidase Subunit 1 (COI) gene, partial cds; mitochondrial sequences.

*isolates	Organisms	Sequence ID	Score	Expect	Identities	Position in Gene Bank
BAB-1, KUF-3, BAG-5	<i>Latrodectus thoracicus</i>	GU112105.1	826	0.0	90%	1 to 639
KUF-2	<i>Latrodectus pallidus</i>	KY007713.1	821	0.0	90%	1 to 624
BAB-4	<i>Asagena phalerata</i>	KY269726.1	593	2e-165	85%	71 to 658

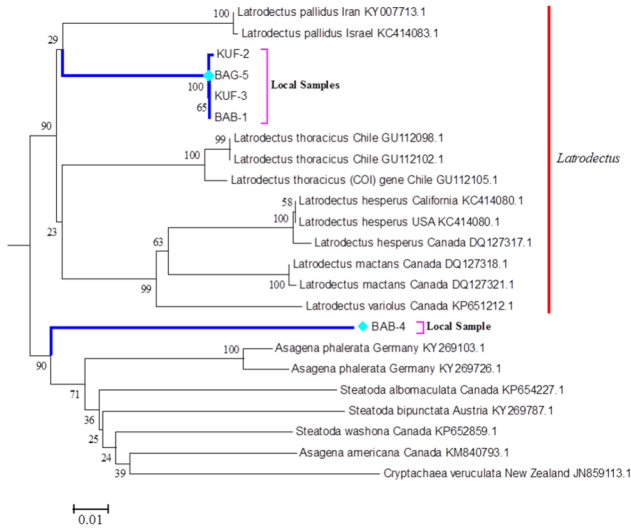
**Table 2**

Sequence Identity Matrix of the partial mtDNA-COI gene.

Seq<-	BAB-1	BAB-4	BAG-5	KUF-2	KUF-3
BAB-1	ID				
BAB-4	0.996	ID			
BAG-5	1.000	0.996	ID		
KUF-2	0.810	0.994	0.994	ID	
KUF-3	1.000	0.996	1.000	0.994	ID

### Phylogenetic Analysis

Phylogenetic relationship of these widow spiders was analyzed (Fig.1). The presented phylogenetic analysis distinct two clades ("*Latrodectus*" clade and "*Steatoda*" clade) all *Latrodectus* species in this phylogeny due to the "*mactans*" clade as resolved by the phylogenetic tree of Garb et al. (2004). From the Sequence Identity Matrix Table 2 results support the convergence between *L. thoracicus* and *L. pallidus*, similarly, that appears as sister group, the phylogenetic tree which presented by Mollaiizadeh et al. (2017) distinct this convergence, although they didn't present as sisters, Garb et al. (2004) interpreted the relationships and why *L. pallidus* is distinct from many other *Latrodectus* spp. in the "*mactans*" clade.



**Fig. 1**

Neighbor-joining phylogenetic analysis of mtDNA-COI gene in widow spiders, (BAB-1, KUF-3 and BAG-5) for *Latrodectus thoracicus* Iraq, (KUF-2) for *Latrodectus pallidus* Iraq, and (BAB-4) for *Asagena phalerata* Iraq

**Morphological Analysis**

**Key to widow spiders in this study:**

1a- lateral eyes contiguous or slightly separated; abdomen shinier and less globular; Leg short in comparison to body, tibia to metatarsus are tapered down .....*Asagena phalerata*

1b- lateral eyes separate by their diameter or more, Abdomen more globular, leg more longer, tibia very thick compared to very thin metatarsus .....(*Latrodectus*)... 2

2a - Abdomen white to creamy with two rows of dark spots; pale hourglass marking present at the venter of abdomen (female)..... *L. pallidus*

2b- Abdomen dull brown to black; Adult with large dorsal red marking dorsally; red hourglass marking ventrally (female)..... *L. thoracicus*

**Redescription of the recorded species**

*Latrodectus pallidus* O.P.-Cambridge, 1872 (white widow spider)

**Material examined:** 1♀ and 1 juvenile Babylon province, Al-Nael, June, 2016; 1♀ Al-Najaf province, Kufa, Aug, 2016; 1♀ Baghdad province, Abu Ghraib, Feb. 2017.

**Diagnosis:** Female: body length 10.0-12.5 mm, cephalothorax: carapace light brown to orange brown; legs very light brown with broad dark annular stripes; abdomen pale cream to white with several dark dots on dorsal side.

**Global distribution:** Cape Verde Islands, Libya to Central Asia (Platnick, 2014).

***Latrodectus thoracicus* Nicolet, 1849**

**Material examined:** 1♀ Babylon province, Al-Neel district, Jun. 2016; 1♂ Al-Najaf Province, Kufa, Aug. 2016; 1♀ and 1 juvenile Baghdad province, Abu Ghraib, Feb. 2017.

**Diagnosis:** Female: body length 10.2-11.4 mm cephalothorax: carapace and legs shiny black; abdomen black with large dorsal red marking from the middle of central line to the end of the abdomen; ventrally with red hourglass marking small in size.

Male: smaller than female, body length 2.8 mm; cephalothorax: carapace dull brown wider than longer; abdomen white with creamy parts, with several dark dorsal bands irregular in size and shape; legs longer than in female; color black to dark brown with broad white annular stripes.

**Global distribution:** Central and Southern parts of Chile (Aguilera *et al.*, 2009) and other region.

*Asagena phalerata* (Panzer, 1801)

It's a synonym to *Steatoda phalerata* (Panzer, 1801) (Le Peru, 2011).

**Material examined:** 1♀ and 1♂ and several specimens juveniles collected from Babylon province, Al-Neel district, Aug. 2016

**Diagnosis:** Female: body length 4.8 mm; cephalothorax: carapace shiny dark brown to black with numerous tiny tubercles; legs reddish brown with broad dark annular stripes. Palps reddish brown to brown; abdomen shiny dark brown with a pattern of yellow or white spots. Male: body length 4.2 mm. cephalothorax: carapace shiny dark reddish brown with many very fine tubercles on whole surface, length longer than wide; femora of legs I and II darker brown, than III and IV, all the other segments yellowish brown with broad dark annular stripes; abdomen uniformly black with pairs of definite yellowish white horizontal patterns, with small dots at the posterior part; ventral surface dark brown with no pattern.

**Habitat:** arid grasslands, agricultural areas, forests, and wetlands (Vona-Túri, *et al.*, 2016).

**Global distribution:** wide distribution from Europe and N Africa to Asia Minor, Karakorum and Trans-Baikal area (Namkung *et al.*, 1996).

**CONCLUSION**

This study characterizes the first phylogenetic relationship to the *Latrodectus* genus in Iraq and is made by the sequence of the partial mtDNA-COI gene; also phylogenetic tree was constructed. The results of the analysis show the highest identity for *L. thoracicus* and for *L. pallidus*, in addition to the false widow *A. phalerata* these three species are recorded in Iraq for the first time.

**ACKNOWLEDGEMENTS**

The authors would like to appreciate very much from the colleagues in the Iraq Natural History Research Center and Museum, University of Baghdad where the specimens were deposited. This research was carried out in the Tropical Biological Research Unit, College of Science, University of Baghdad.

**AUTHORS' CONTRIBUTION**

Hayder Badry Ali prepared the experimental design and wrote the manuscript. Hula Younis Fadhil Performed the DNA extraction, amplification for COI Gene, analyzed the bioinformatics data and approved the final manuscript; while Ishraq M. Baker collected the specimens.

## REFERENCES

- Abdul-Rassoul, M.S., B.H. Al-Jalely, K.T. Al-Nuaimi and L.K. Al-Ani, 2012. First record of red -back spider *Latrodectus scelio* Thorell, 1870 (Theridiidae: Araneae) in Baghdad, Bulletin of the Iraq Natural History Museum, 12: 1-5.
- Aguilera, M.A., M.E. Casanueva and G. D'elia, 2009. Revalidation of *Latrodectus Thoracicus* Nicolet, 1849 (Araneae: Theridiidae) Biol. Phyl. Ant. Gayana, 73:161-171.
- Al-Hadlag, K.S. and S.A. Najim, 2015. First record of red-back spider *Latrodectus hasselti* Thorell, 1870 (Araneae: Theridiidae) from Basrah, South of Iraq Journal of Purity, Utility Reaction and Environment, 4: 91-98.
- Brandon-Mong, G.J., H.M. Gan, K.W. Sing, P.S. Lee, P.E. Lim and J.J. Wilson, 2015. DNA metabarcoding of insects and allies: an evaluation of primers and pipelines. Bull. Entomol. Res., 105: 717-727.
- Garb, J.E., Gonzalez A. and R.G. Gillespie, 2004. The black widow spider genus *Latrodectus* (Araneae: Theridiidae): phylogeny, biogeography, and invasion history. Mol. Phyl. Evol., 31: 1127-1142.
- Graudins A, M., Padula, K. Broady and G.M. Nicholson, 2001. Red-back spider (*Latrodectus hasselti*) antivenom prevents the toxicity of widow spider venoms. Ann. Emerg. Med., 37: 154-160.
- Le Peru, B., 2011. The spiders of Europe, a synthesis of data: Atypidae to Theridiidae. Mémoires de la Société linnéenne de Lyon 2: 1-522.
- Levi, H.W. and D.E. Randolph, 1975. A key and checklist of American spiders of the family Theridiidae north of Mexico (Araneae). J. Arach., 3: 31-51.
- Levi, H.W., 1959. The spider genus *Latrodectus* (Araneae: Theridiidae). Trans. Amerc. Micr. Soc., 78: 7-43.
- Levi, H.W., 1967. Cosmopolitan and pantropical species of theridiid spiders (Araneae: Theridiidae). Pac. Insec., 9: 175-186.
- Levi, H.W., 1983. On the value of genitalic structures and coloration in separating species of widow spiders (*Latrodectus* sp.) (Arachnida: Araneae: Theridiidae). Veröffentlichungen fördert der Verein das Hamburgische, 26: 195-200.
- Mirshamsi, O., 2005. New records of three *Latrodectus* species found in Khorasan province (Araneae: Theridiidae). Ira. J. Anim. Biosyst., 1: 52-58.
- Mollaiizadeh, M., J. Rafinejad, M.A. Oshaghi, H. Bakhshi, S.S. Hashemi, S. Gholami and M.M. Sedaghat, 2017. Molecular investigation on Iranian widow spider *Latrodectus tredecimguttatus* based on DNA barcode analysis. Asian Pac. J. Trop. Dis., 7: 560-563.
- Namkung, J., M. Im and S. Kim, 1996. A Rare Species of the Spider, *Steatoda phalerata* (Panzer, 1801) from Korea (Araneae: Theridiidae). Act. Arachn., 45: 15-18.
- Platnick, N.I., 2014. The world spider cataloge, Version 14.5. American Museum of Natural History. <http://research.amnh.org/iz/spiders/catalog>.
- Sutton, M.E., B.R. Christensen and J.A. Hutcheson, 2006. Field identification of katipo. Science and Technical Publishing Wellington, New Zealand. pp.9
- Vona-Túri, D., T. Szmátóna-Túri, F. Kádár, B. Kiss, A. Weiperth and B. Gál, 2016. Ground-dwelling arthropod (Araneae: Coleoptera: Carabidae, Isopoda: Oniscidea) assemblages on Hungarian main road verges. Agric. Ecosyst. Environ., 8: 96-111.
- Wunderlich, J., 2008. On extant and fossil (Eocene) European comb-footed spiders (Araneae: Theridiidae), with notes on their subfamilies, and with descriptions of new taxa. Beiträge zur Araneologie, 5: 140-469.
- Zamani, A. and H.K. El-Hennawy, 2016. Preliminary list of the spiders of Iraq (Arachnida: Araneae). Arachnida, Rivista Aracnologica Italiana, 6: 12-20.

