



New Record of Brown Widow Spider (*Latrodectus geometricus* Koch, 1841) in Ha'il Region, Saudi Arabia

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Abstract

Brown Widow Spider (*Latrodectus geometricus*) is recorded for the first time in Hail Region of Saudi Arabia. Three specimens of brown widow spider (*L. geometricus*) were found and collected on October, 2021 from inside a beehive in Al Hait city (Southern of Ha'il region of Saudi Arabia). The specimens were collected and submitted to Biology department of Hail University for the identification of morphological characters and DNA barcode sequence analysis of the specimen. Phylogenetic analysis was performed to compare the DNA sequence of the newly discovered female brown widow spider with the other species of *Latrodectus* genus.

Keywords: Brown Widow Spider; *Latrodectus geometricus*; Morphological DNA Barcoding; Saudi Arabia

Introduction

Brown widow Spider species belongs to genus *Latrodectus* Walckenaer, 1805 (Family: Theridiidae). The widow spiders consists of 30 currently recognized species of genus *Latrodectus* Walckenaer, 1805. *Latrodectus* is a genus of spiders that includes numerous species that are collectively known as true widows. The common term "widow spiders" comes from the predominance of sexual cannibalism. After mating, the female of brown widow spider eats the male) [1]. The *Latrodectus* genus consists of two phylogenetic groups, the *mactant* clade also known as black widow and the *geometricus* clade also known as brown widow [2]. The genus of *Latrodectus* has a global distribution, with species found on several continents and marine islands. Many species of *Latrodectus* have been discovered across the globe. Among these are *L. indistinctus* and brown widow *L. geometryus* in Africa; black widow (*L. mactans*) in North America; black wolf (*L. tredecimguttatus*) in Europe (Poland, Spain and Turkey); red widow (*L. bishopi*)

in the USA; northern widow (*L. variolus*) in the USA; and red-back spider (*L. hasselti*) in Australia, India, and Japan [3] argued that [2]. Brown widow spiders are members of the Theridiidae spider family [4]. This family is known as comb-foot spiders because of the broad curving bristles on the tarsal bones of their hind legs that seem like comb-like rows. Cobweb spiders are sometimes known as grey widow spiders because of the color of their webs [5].

However, it is widely accepted that the brown spider was initially discovered in South America. Its current pantropical range [6,7] presently covers many subtropical regions of the planet. They've established themselves across the United States as well as other countries like Japan and South Africa. Compared to female brown spiders, male brown spiders' bodies are much smaller [8,9]. According to Heeres, et al. [10], the patterns and hues of women vary widely; they might be practically black, white, grey or light brown. For whatever reason, moulting causes a change in colour, which may be due to the substrate colour changing [11].

mactans's venom may kill people, however the fatality rate of its venom is less than 1% [12]. A study published in 1992 by Clark, et al. [13] Humans have been killed by the brown widow's venom in Madagascar since 1991. The identification of the spider, the effects of delayed medical intervention, and the case's specifics remain a mystery to this day [14]. Fertilized females produced an average of twenty three egg sacs over their lifetimes. Brown widow egg sacs are distinguished from other *Latrodectus* species by white silk spikes on the outside [15]. The eggs of little spiders hatch around 14 to 21 days, although they remain in the egg sac during a period of time ranging from a few days to a month until they are ready to emerge. Small spiders that have just born are white and lack any patterning. They moult and develop patterns like as an hourglass, although the hourglass is white rather than orange as they emerge from their egg sac. In this study, we provide morphological features and DNA barcode analysis as methods for identifying the brown widow spider in Hail, Saudi Arabia.

Materials and Methods

- **Material Examined:** HUM1501, Al Hait city (Southern of Ha'il region of Saudi Arabia), 28.10.2021.
- **Remarks:** Three specimen of Brown Widow Spider were collected from inside an empty beehive can in open desert area in Al Hait city, 500 m from the highway road between Ha'il and Almadinah Almonorah City.

Demography of Location

The Hail principality is located in the Kingdom of Saudi Arabia's northern central region, between 25°17'N and 28°52'N and 39°18'E and 44°21'E. It covers an area of about 112,444 square kilometres. In the southern Hail area, the weather is often dry from February through December. With temperatures averaging over 38°C (100°F), August is the hottest month of the year in the area. With an average high temperature of 16°C (60°F), January is the coldest month of the year.

Collection of Specimen

The Specimens of *L. geometricus* were collected from small sweeping net from Saudi Arabia's Southern Hail region. The specimens of brown widow spider were found in a wooden box of Beehive. One of them along with eggs sac.

Preservation of Specimen

After collection of the specimens, they were transferred

to the research center at department of Biology of University of Hail, Saudi Arabia. The specimens were preserved in 70% isopropanol for further study.

Morphological Identification

We use morphological characteristics including body form, eye pattern, and epigyne structure to assess and identify spiders [16]. We confirmed the genus of our specimen by comparing the above morphological features according to morphological features reported by Vincent, et al. [16].

Molecular Identification

We used a sterilised sharp blade to cut the spider leg and remove the spider's tissue. Thermo Scientific GeneJet Genomic DNA Extraction & purification kit was used to extract and purify the DNA of spider following the manufacturer's instructions. PCR was used to amplify the COI mitochondrial gene's standard barcode region (681base pairs). Using gel electrophoresis, the PCR product was compared to a known-size ladder (Fermentas #1173) in order to verify the resulting bands. In order to see the bands under a UV illuminator, we added Ethidium bromide to our solution, visualized and sequenced according to Ashfaq, et al. [17].

Nucleotide-BLAST [18] was used to determine the most closely related sequences and species in our collection, as well. Using MEGA 11 [9] and 1000 bootstrap replications, we generated a neighbor-joining (NJ) tree (Figures 1 & 2).

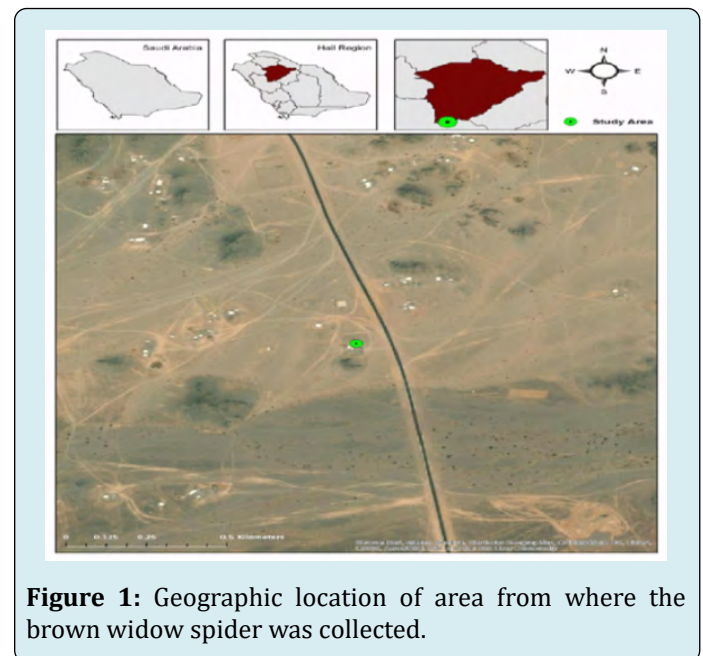


Figure 1: Geographic location of area from where the brown widow spider was collected.

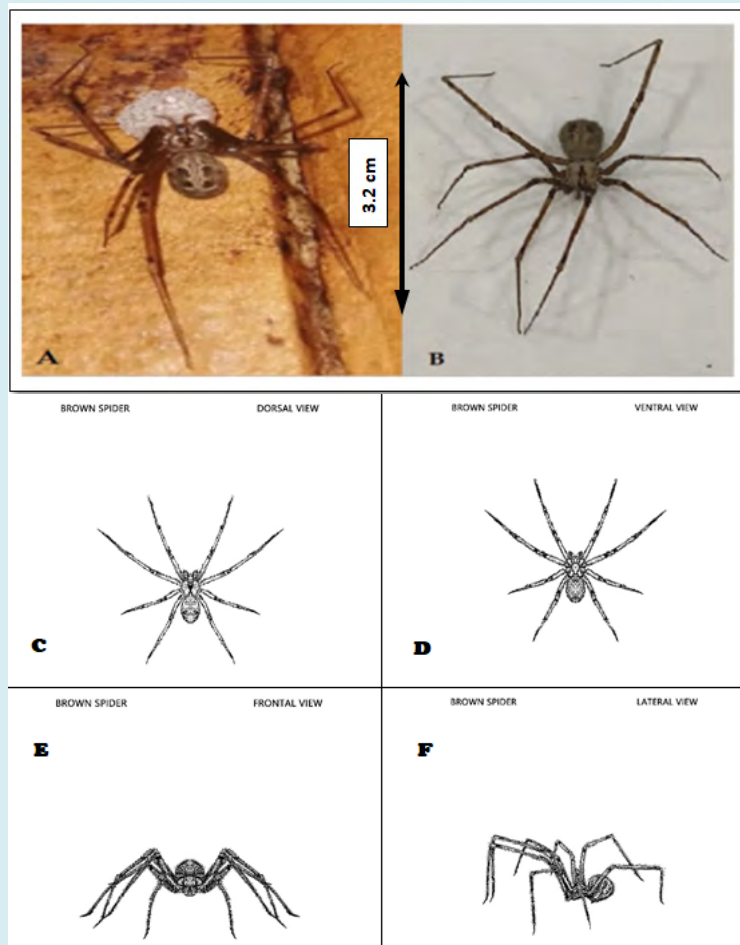


Figure 2: A&B: The Brown widow spider specimen (*Latrodectus geometricus*) with the egg sac. C,D,E&F Drawings of the dorsal, ventral, frontal and lateral views of the collected specimen.

Results & Discussion

Different morphological characters were proved that the collected species is the brown widow spider (*L. geometricus*). Morphological characteristics analysis determined that the collected species is *L. geometricus* such as egg sacs, length of legs, comb feet, eyes and geometrical marking underside the abdomen. *L. geometricus* has four pairs of eyes are arranged in two parallel rows, and the hind tarsal bristles form a comb-like pattern [16]. The bottom of the abdomen of the widow spider has an hourglass-shaped stripe that ranges from light yellow to dark yellow or orange in mature spiders of this species [20]. Figure 2 shows all the morphological characteristic observed of brown widow female spider.

Male brown spider differs in body size form the female

brown spider. The body length of the brown female spider was measured to be 12-16mm (excluding the legs) [16].

Molecular Identification

The mitochondrial cytochrome oxidase subunit I (COI) gene sequence was compared to NCBI GenBank records, and only the top four records are shown here in Table 1. An exact match has been found between the DNA sequence of a brown widow spider from Malaysia and a street in Akron. *L. geometricus*, a brown widow spider from California, shares 97 percent of its DNA sequence. In addition, a neighbor-joining (NJ) (Saitou and Nei, 1987) tree was created using MEGA 11 to investigate the evolutionary connection [21]. According to the results of a NJ tree, the sequences clustered together with those of the *L. geometricus* sequence, validating the species.

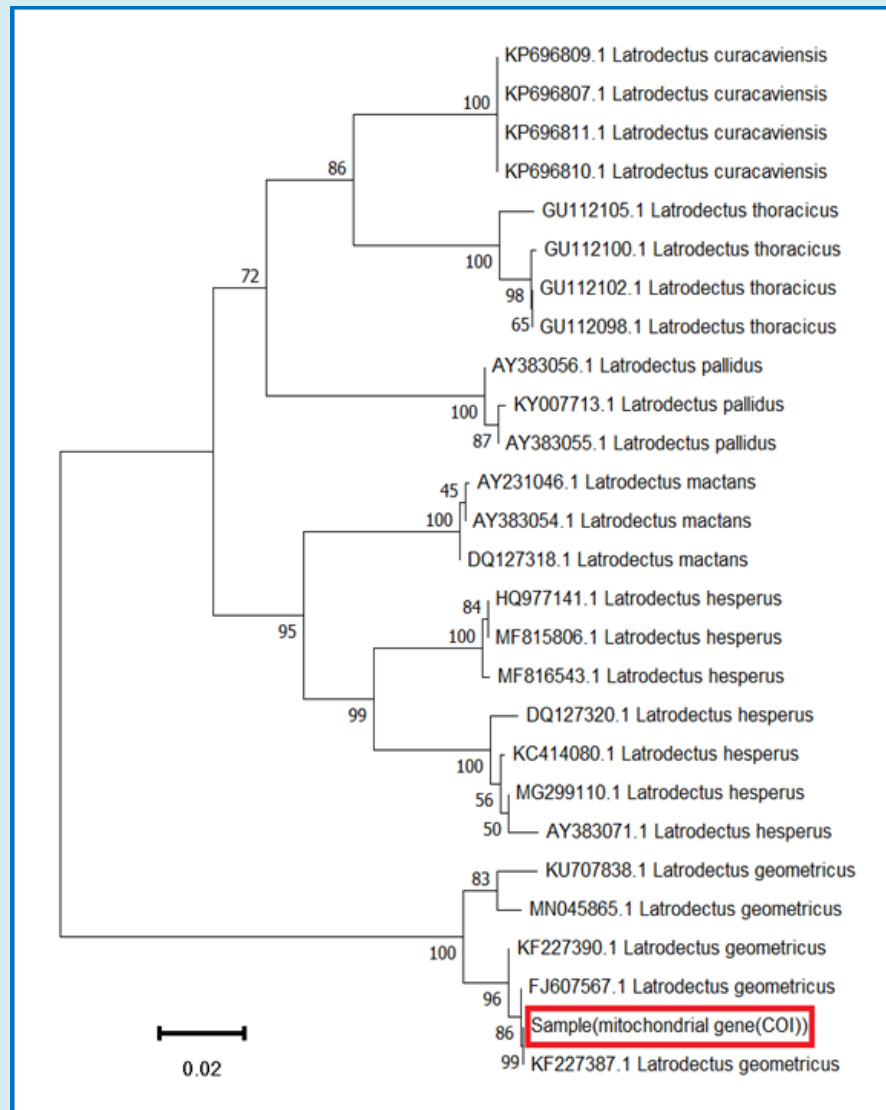


Figure 3: A neighbor-joining tree using DNA sequence of different species of *Latrodectus* genus and the Sample sequence of *L. geometricus* obtained by DNA bar coding.

	Source	Query Coverage %	Sequence identity %	Country/Region	Accession Number
Query Sequence of widow brown spider	Mitochondrion <i>L. geometricus</i>	100	100	Kuala Lumpur Malaysia	KF22738.1
	Mitochondrion <i>L. geometricus</i>	100	100	East Mill Street, Akron	FJ607567.1
	Mitochondrion <i>L. geometricus</i>	100	97.72	USA California	KU707838.1
	Mitochondrion <i>L. geometricus</i>	100	97.11	Rancho Penasquitos, USA, California	MN045865.1

Table 1: The result of BLASTn search of sample COI sequences found in Hail region of Saudi Arabia.

For many decades, the brown spider was solely discovered in State of Florida (USA), where it is still rather prevalent [22]. Since the first decade of the 21st century, the brown spider has been progressively increasing its range

from Texas (USA) to the Gulf of Mexico and along the Atlantic coast in South Carolina [6]. In 2001, it was discovered as far north as Beaufort, South Carolina, indicating that it has now spread to every county in Florida. In addition to southern

California and Hawaii, the brown widow spider has been reported [16]. As long as the habitat is ideal, brown spiders will continue to spread across North America and other places where they may thrive. There is a good chance that the brown spider *L. geometricus* will spread to New Mexico. The brown widow spider was later discovered in Israel, Turkey and the Philippines, as well as South Asia (Pakistan and India) and East Asia (Japan) [23].

Malaysian Peninsular is the most recent place to be infested with brown widows according to reports from South and North America. This is the first time that the brown spider has been found in the Hail area of Saudi Arabia, according to the findings of this research. The physical qualities of the specimen made it easy to identify. DNA barcoding was used to confirm the species of the material.

As with black widow bites, brown widow bites tend to be less severe, and the pain is typically localized to the region where the bite occurred [24]. The bites may also be dry, meaning no venom is administered, in roughly 15% of cases [25]. However, some black widow bites might produce more significant systemic symptoms. The more severe, systemic signs that black widows are renowned for do occur in certain cases [26-36].

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