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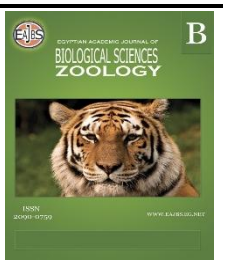

Morpho-Molecular Identification of Female Black Widow, *Latrodectus tredecimguttatus* (Rossi, 1790) Inhabiting Olive Orchard at Menoufia Governorate, Egypt

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ABSTRACT

The Mediterranean widow spider, or "Dolmak," *Latrodectus tredecimguttatus* (Rossi, 1790), family Theridiidae, is one of the medically significant spiders. Also, it is listed as a dangerous animal due to its extensive geographic range and its deadly or seriously problematic toxic bites. A holistic understanding of *L. tredecimguttatus* requires combining knowledge derived from both morphological and molecular investigations. Thus, this study aimed to offer researchers a framework for investigating these essential species and to educate farms and visitors about the importance of avoiding any behaviour that could antagonize the spiders. For this study, specimens of *L. tredecimguttatus* were manually collected randomly from some olive orchards between September 2023 and August 2024 at Sadat City, Menoufia Governorate, Egypt. The morphological parameters utilized for identification were the total body length, carapace width, abdomen length, and (1st,3rd, and 4th legs) length. Results indicated that the total length of females ranged from 8.92 to 10.15mm. The female dorsal pattern if present; consists of several orange spots (usually in young females). Adult females lack the dorsal pattern and ventral hourglass reduced to two transverse oranges to red Markings. The results of the mitochondrial *CO1* gene confirm that our tested species identified as *Latrodectus tredecimguttatus* under the accession number (PQ358290.1) in GenBank for the first time in Menoufia Governorate, Egypt. So, the identification of a female Black Widow according to morphological and molecular genetic methods is of great importance.

INTRODUCTION

The family Theridiidae Sundevall, 1833, includes 2544 species and 124genera of spiders (World Spider Catalog, 2023). Popularly referred to as 'black-widow spiders', the largest spiders in the family Theridiidae are found in the genus *Latrodectus* Walckenaer, 1805, They are well-known in both the medical and public sectors because their venom poses a neurological threat to humans (Holz and Habener, 1998). These spiders inhabit arid, warm regions across all continents (Lotz, 1994). Specifically, in Egypt, three Latrodectus species *L. tredecimguttatus* (Rossi, 1790), *L. geometricus* C.L. Koch, 1841, and *L. pallidus* O. Pickard-Cambridge, 1872 have been documented (El-Hennawy, 2002).

El-Hennawy (2006) provided insights into *L. pallidus* in Egypt and Jordan, while Mohafez (2015) studied the *L. geometricus* life cycle. Metwally *et al*. (2015) investigated the duration of *L. tredecimguttatus*, and El-Gendy (2020) researched its ecology and taxonomy specifically in Menoufia Governorate. Like every other *Latrodectus* species worldwide, *L. tredecimguttatus* "can bite humans." Bites frequently have serious consequences; in two-thirds of cases, the bite results in excruciating, chronic agony, and one-third of cases, the bite

keeps the patient from falling asleep. In almost half of the cases, the pain intensifies within the initial 60minutes and primarily spreads to the appendages or evolves in the abdomen. Around 70% of patients experience perspiration, and 20–30% experience additional systemic effects (less than 20% experience nausea and vomiting, 10% experience elevated body temperature and neuromuscular effects, and less than 10% experience hypertension). According to Nentwig *et al*. (2015), pain typically lasts 1-2days, whereas other symptoms last 1-4days. A comprehensive knowledge of this species demands an integration of its morphological traits and molecular structure.

Therefore, this study focuses on the morphological measurements and molecular characterization that were essential for identifying and classifying female *L. tredecimguttatus*.

MATERIALS AND METHODS

Studied Area and Method of Collection:

In the present investigation, specimens of *Latrodectus tredecimguttatus* (Rossi, 1790) were manually collected randomly from some olive orchards at Sadat City, Menoufia Governorate, Egypt. The individuals were found beneath a stone in olive trees between September 2023 and August 2024, were collected and placed in small plastic vials, and picked up to the laboratory.

Preservation and Examination:

The individuals were held in 10x7cm plastic vials within an incubator (27 ±1°C, 70–80% RH) and fed various prey like Mealworm *Tenebrio molitor* Linnaeus, 1758 (Coleoptera: Tenebrionidae), Egyptian cotton leaf worm *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuoidea) and larvae of the greater wax moth *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae). The examination was conducted in the Biological Control Lab, Plant Protection Department, Assiut University, with the aid of a trinocular stereomicroscope Leica DC150 and with the HDMI MULTI-OUTPUT HD (Toup Cam_120) camera.

Methods Used for Identification:

Specimens were identified using taxonomical knowledge that is currently accessible. Numerous catalogs, publications, and keys were utilized to identify the recorded species. The following description and literature were used to identify the obtained specimens at the species level to Levi (1959), Kakhki (2005), Peterson and McNalley (2013), El-Hennawy (2017), and EL-Gendy (2020).

Methods Used for Morphological Measurements:

The morphological parameters utilized for identification were total body length, carapace width, abdomen length, and leg length. The scale measurements and all morphological measures on photomicrographs are in millimeters (mm).

Molecular Genetic Identification:

1-DNA Isolation: According to Reineke *et al.* (1998), the genomic DNA was isolated from Mediterranean black widow leg specimens using acetyltrimethylammonium bromide procedure combined with an extra polyethylene glycol precipitation.

2-Polymerase Chain Reaction (PCR) Conditions: The partial mitochondrial *COI* gene was amplified using primers LCO1490 (forward) and HCO2198 (reverse) in a 50µL PCR reaction (Folmer *et al*., 1994). Each reaction contained genomic DNA, 1µL of each primer, and 25µL of PCR master mix. The amplification protocol began with a 240-second denaturation at 94°C, followed by 35 cycles of 60-second denaturation (94°C), annealing (49°C), and extension (72°C), culminating in a 10-minute final extension at 72°C. To separate the amplified products, 1.5% agarose gel stained with ethidium bromide was utilized. The amplified PCR fragments were sized using GeneDireX's 100bp DNA Ladder RTU (Ready-to-Use).

3-The Sequencing of PCR: Macrogen company (Seoul, South Korea) achieved all DNA sequencing. Accession numbers were obtained for the sequences by submitting them to the National Center for Biotechnology Information (GenBank/NCBI). Sequence divergence was quantified using the Kimura two-parameter model (Kimura, 1980). To determine the similarity of our sequence to those already found in the database, we used BLAST searches of the GenBank NCBI database.

4-GenBank Accession Number: The partial sequence of the *CO1* area described in this study can be found in the GenBank nucleotide sequence databases (http://www.ncbi.nlm.nih.gov) under accession number PQ358290.1.

Data Analysis:

Image J 1.48V was used for morphometric measurement data (Schneider *et al*., 2012). Microsoft Excel 2016 was used to calculate the means ± standard deviation (SD).

Using MUSCLE (Edgar, 2004) with its default settings, sequence alignment was carried out. With 1000 bootstrap iterations, phylogenetic tree analyses were conducted using MEGA version 7.0 18 (Kumar *et al*., 2016), and the Maximum Likelihood (ML), Neighbour Joining (NJ), and Minimum Evolution (ME) tree construction techniques (Felsenstein, 1985).

RESULTS

Morphological Features Description:

The common nickname "black widow" for the genus *Latrodectus* comes from its morphological traits. Interestingly, this spider is well known for the phenomenon of the female devouring the male spider after mating, thereby making her a Widow. The distinctive shiny black body makes it easy to identify. They are araneomorph spiders, large Theridiid spiders, ecribellate; entelegyne; and females with remarkable sexual dimorphism. The dorsal and ventral view of the female illustrated in (Fig.1a, b)

Carapace: More elongated than wide, sometimes about as long as wide**.** It is pear-shaped, and the thoracic region of the carapace is wider than the cephalic region (Fig.1e).

Chelicerae: Without teeth (Fig.2e).

Eyes: Eight eyes and the lateral eyes are widely separated (Fig.2d).

Legs: Are relatively thick and moderately long, the first pair longer than the fourth and the third are the shortest (Fig.1g, h, I, and j). A distinct theridiid tarsal comb is present on the ventral surface of the fourth tarsus. The legs are characterized by an absence or near absence of spines, and tarsi IV features a row of lightly curved, serrated bristles, and three tarsal claws.

Abdomen: Globose and usually black **(**Fig.1d).

Silk glands: modified and aggregated, and sticky silk is used to wrap prey.

Colulus: Large **(**Fig.2c).

Epigynum: wider than long, and the internal female genitalia possesses a single pair of dumb-bell-shaped seminal receptacles, and exhibits coiled spermathecal ducts **(**Fig.2a).

Morphometrics Measurements:

The morphological parameters utilized for identification were total body length, carapace width, abdomen length, and (1^{st}, 3^{rd}, and 4^{th}legs) length. All morphological measurements are given in millimeters (mm). Scale measurements on photomicrographs are in millimeters (mm). Each collection (n=5) refers to female specimens measured.

a. Total Body Length (TBL): The total body length of adult females (8.92-10.15mm) with an average of 9.47±0.55mm.

b. Carapace Width (CW): The carapace width of adult females (3.05-3.51mm) with an average of 3.28±0.19mm.

c. Abdomen length (ALF): The female measurements of abdomen length min = 6.06mm, max = 6.92mm with an average of (6.52±0.32mm).

d. legs length (LLF): The female measurements of first length min= 22.06mm, max= 22.50mm with an average of (22.72±0.64mm). The female measurements of third length min= 7.88mm, max= 9.41mm with an average of (8.51±0.64mm). The female measurements of fourth length min= 21.32mm, max = 24.03mm with an average of (22.63±0.97mm).

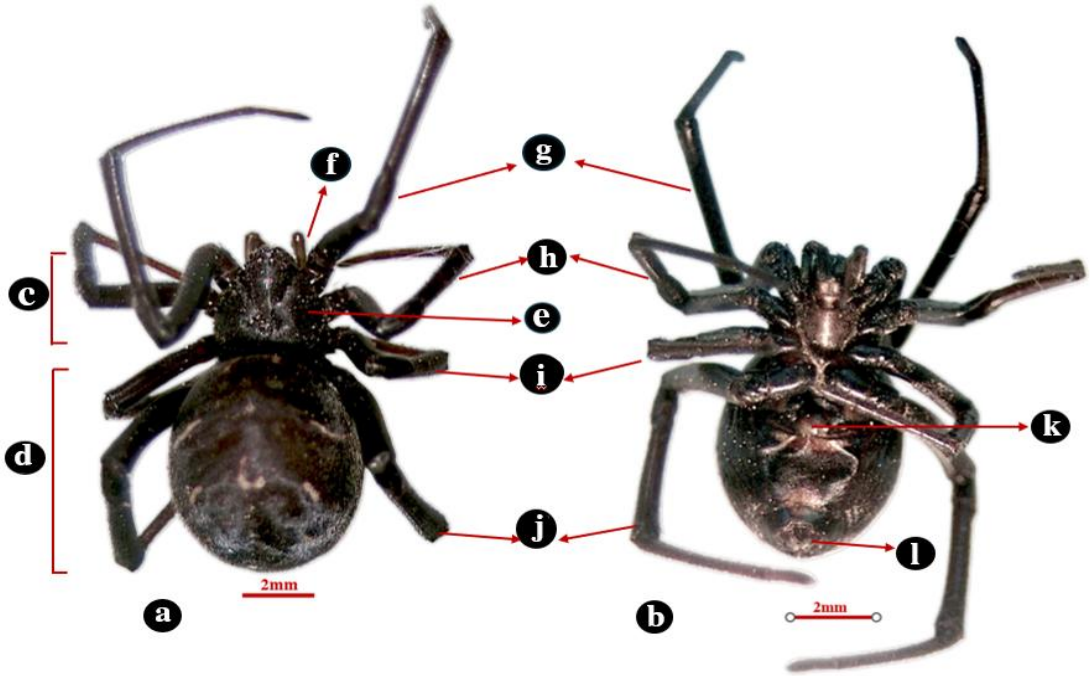

Fig. (1): Monograph of female *Latrodectus tredecimguttatus* (Rossi, 1790):
a) dorsal view; **b)** ventral view; **c)** cephalothorax; **d)** Abdomen; **e:** carapace; **f)** palp; **g)** leg I; **h)** leg II; **i)** leg III; **j)** leg IV; **K)** epigynum; **l)** spinnerets.

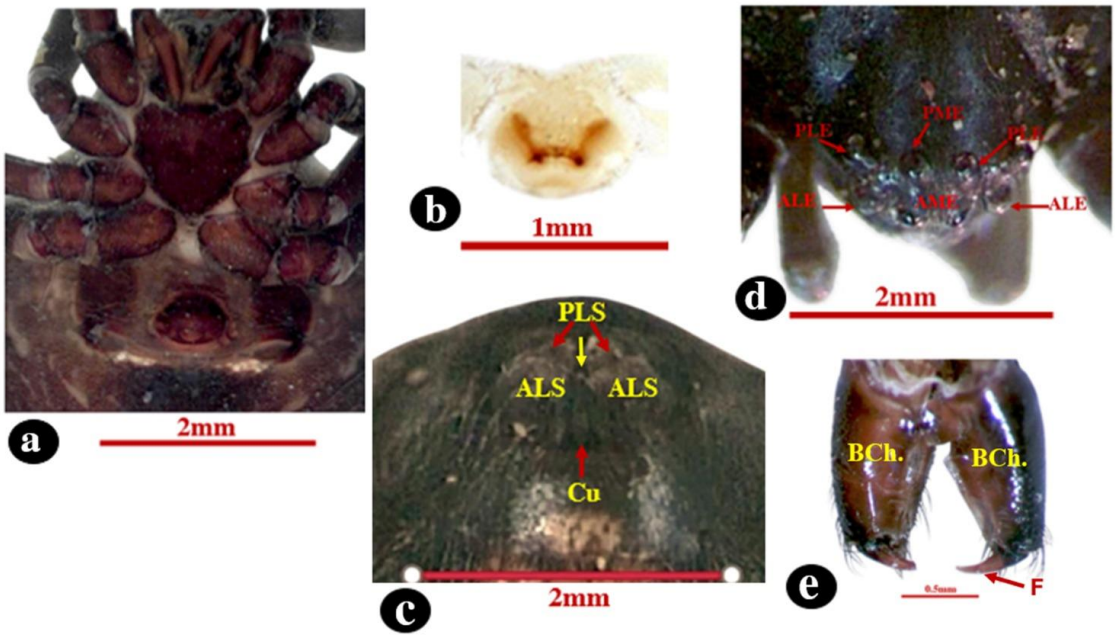

Fig. (2): Monograph of female *L. tredecimguttatus*: **a)** Female epigynum ventral view; **b)** Female vulvae dorsal view; **c)** Ventral-apical view of the spinnerets. The yellow arrow refers to PMS; **d)** Frontal view of carapace shows different eyes; **e)** Detail of the chelicerae. Abbreviations: ALE= anterior lateral eye; ALS= anterior lateral spinneret; AME= anterior median eye; BCh.= basal chelicera; Cu= colulus; F= fang; PLE= posterior lateral eye; PLS= posterior lateral spinneret; PME= posterior median eye; PMS= posterior median spinneret.

Molecular Genetic Identification:

The nucleotide length of 603bp obtained from the sequencing of *COI* was entered into GenBank with accession number PQ358290.1. Adenine (A), cytosine (C), guanine (G), and thymine (T) had nucleotide frequencies of 33.8, 14, 10.8, and 41.4%, respectively. The C+G content was lower than the A+T content, which was 75.2%. Following BLAST/N analysis of the *COI* sequence at NCBI, 17species of the *Latrodectus* family were identified, with three species of the Braconidae family acting as an out-group (Table 1).

The sequences of the studied sample *L. tredecimguttatus*, along with those of 17related species and three out-group species, were submitted for phylogenetic tree analysis using *COI* sequencing. We used two phylogenetic methods for more explanatory phylogenetic relations: Minimum Evolution based on (*COI*) sequence and Neighbour Joining. The understudied sample containing *L. tredecimguttatus* established a sister clade with *L. tredecimguttatus* (KC414084.1) from the GenBank/NCBI, according to the techniques, which demonstrated a high degree of relational similarity, differing only in support values (Figs. 3, 4).

The understudied *L. tredecimguttatus* and 17 members of the family Theridiidae had pairwise genetic distances ranging from 0.0040 to 0.0220. The *L. tredecimguttatus* (KC414084.1) from the GenBank was the species most closely related to our sample, with a genetic distance of 0.0040, while *Crustulina sticta* (KX537455.1) had the most distant genetic distance, at 0.0220 (Table 2).

Table 1: The understudied *Latrodectus tredecimguttatus* with their related species and out-group from the GenBank/ NCBI based on (*COI*) sequences.

No.	Species	Accession number
1	*Latrodectus_tredecimguttatus*	PQ358290.1
2	*Latrodectus_tredecimguttatus*	KC414084.1
3	*Latrodectus_garbae*	PP204850.1
4	*Latrodectus_thoracicus*	GU112105.1
5	*Latrodectus_diaguita*	PP204869.1
6	*Latrodectus_corallinus*	PP204871.1
7	*Latrodectus_katipo*	EU305449.1
8	*Steatoda_borealis*	JN309819.1
9	*Steatoda_cingulata*	JN817103.1
10	*Latrodectus_pallidus*	KC414082.1
11	*Latrodectus_umbukwane*	MN094889.1
12	*Steatoda_washona*	KP652859.1
13	*Asagena_phalerata*	KY269302.1
14	*Latrodectus_mirabilis*	PP204863.1
15	*Steatoda_hespera*	JF886079.1
16	*Robertus_scoticus*	KY269468.1
17	*Theridion_rabuni*	KP649575.1
18	*Crustulina_sticta*	KX537455.1
19	*Aphidiinae*_sp.	FJ413759.1
20	*Aphidius_uzbekistanicus*	OP133190.1
21	*Aphidius_gifuensis*	OP002856.1

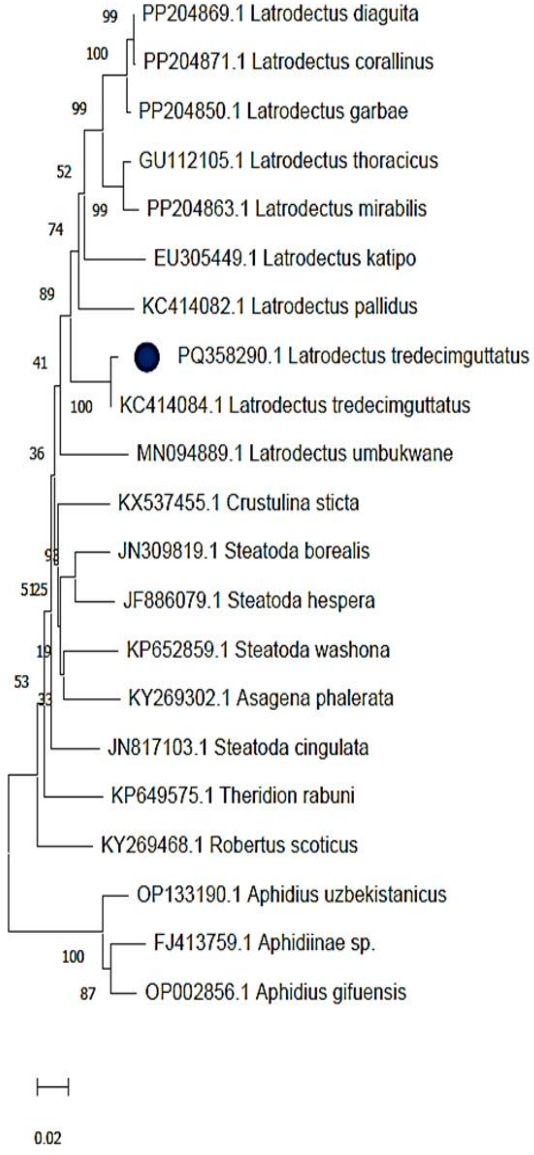

Fig. (3) Phylogenetic tree using the Neighbour Joining method among *Latrodectus tredecimguttatus* with their related species and out-group from the GenBank/ NCBI based on (*COI*) sequences.

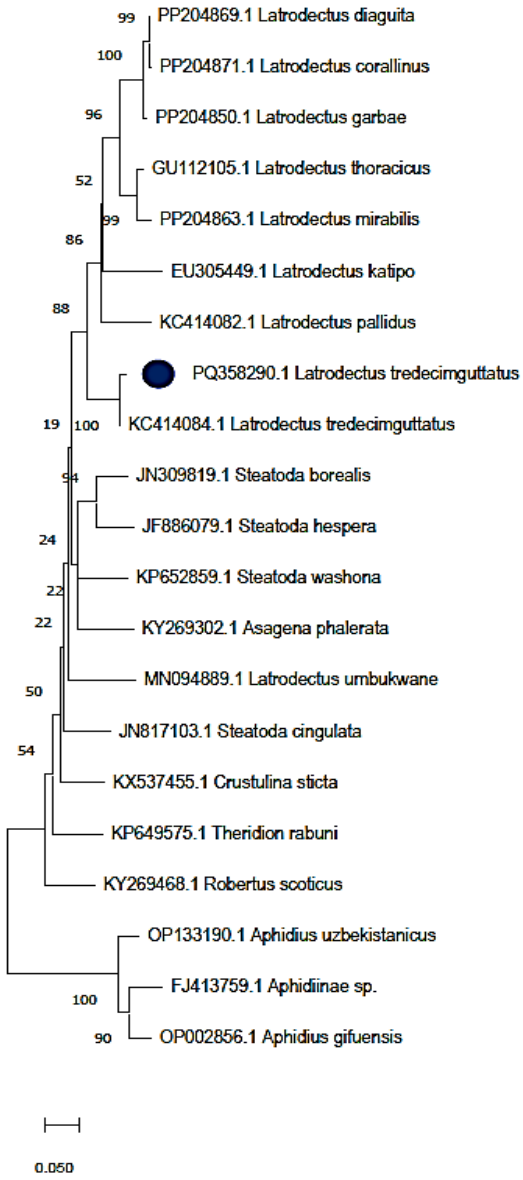

Fig. (4) Phylogenetic tree using the Minimum Evolution method among *Latrodectus tredecimguttatus* with their related species and out-group from the GenBank/ NCBI based on (*COI*) sequences.

Table 2: Pairwise distances among *Latrodectus tredecimguttatus* with their related species and out-group from the GenBank/NCBI based on (*COI*) sequences.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
PQ358290.1_Latrodectus_tredecimguttatus		0.0040	0.0192	0.0183	0.0199	0.0202	0.0207	0.0208	0.0197	0.0219	0.0202	0.0214	0.0203	0.0211	0.0216	0.0205	0.0204	0.0220	0.0369	0.0346	0.0360
KC414084.1_Latrodectus_tredecimguttatus	0.0084		0.0176	0.0181	0.0184	0.0187	0.0200	0.0195	0.0187	0.0207	0.0197	0.0205	0.0194	0.0197	0.0208	0.0202	0.0194	0.0211	0.0372	0.0346	0.0363
PP204850.1_Latrodectus_garbae	0.1422	0.1302		0.0143	0.0050	0.0053	0.0199	0.0229	0.0212	0.0203	0.0234	0.0218	0.0223	0.0141	0.0231	0.0245	0.0209	0.0225	0.0402	0.0363	0.0379
GU112105.1_Latrodectus_thoracicus	0.1354	0.1329	0.0862		0.0142	0.0146	0.0214	0.0248	0.0225	0.0188	0.0237	0.0225	0.0220	0.0072	0.0239	0.0248	0.0214	0.0226	0.0393	0.0364	0.0373
PP204869.1_Latrodectus_diaguita	0.1498	0.1376	0.0136	0.0840		0.0017	0.0196	0.0231	0.0218	0.0204	0.0243	0.0212	0.0226	0.0139	0.0231	0.0248	0.0224	0.0235	0.0412	0.0362	0.0384
PP204871.1_Latrodectus_corallinus	0.1524	0.1402	0.0154	0.0862	0.0017		0.0192	0.0236	0.0223	0.0206	0.0246	0.0215	0.0230	0.0141	0.0236	0.0253	0.0227	0.0239	0.0408	0.0366	0.0388
EU305449.1_Latrodectus_katipo	0.1544	0.1472	0.1493	0.1549	0.1467	0.1442		0.0243	0.0235	0.0228	0.0235	0.0249	0.0255	0.0217	0.0270	0.0246	0.0265	0.0254	0.0417	0.0378	0.0419
JN309819.1_Steatoda_borealis	0.1594	0.1470	0.1808	0.1972	0.1808	0.1836	0.2057		0.0181	0.0230	0.0215	0.0194	0.0200	0.0242	0.0155	0.0208	0.0216	0.0191	0.0396	0.0359	0.0351
JN817103.1_Steatoda_cingulata	0.1544	0.1448	0.1740	0.1814	0.1819	0.1846	0.1900	0.1312		0.0234	0.0207	0.0200	0.0194	0.0245	0.0201	0.0185	0.0187	0.0182	0.0353	0.0346	0.0337
KC414082.1_Latrodectus_pallidus	0.1653	0.1552	0.1472	0.1325	0.1472	0.1498	0.1816	0.1854	0.1863		0.0239	0.0229	0.0219	0.0199	0.0243	0.0274	0.0232	0.0232	0.0401	0.0403	0.0417
MN094889.1_Latrodectus_umbukwane	0.1610	0.1559	0.1976	0.2011	0.2062	0.2091	0.1976	0.1749	0.1680	0.1993		0.0239	0.0237	0.0263	0.0199	0.0224	0.0211	0.0214	0.0369	0.0375	0.0352
KP652859.1_Steatoda_washona	0.1657	0.1558	0.1705	0.1768	0.1653	0.1679	0.2028	0.1396	0.1530	0.1777	0.1905		0.0192	0.0230	0.0206	0.0211	0.0215	0.0206	0.0392	0.0367	0.0372
KY269302.1_Asagena_phalerata	0.1653	0.1553	0.1808	0.1768	0.1836	0.1863	0.2156	0.1545	0.1488	0.1716	0.1927	0.1488		0.0249	0.0195	0.0228	0.0220	0.0185	0.0409	0.0386	0.0382
PP204863.1_Latrodectus_mirabilis	0.1650	0.1524	0.0807	0.0283	0.0785	0.0807	0.1645	0.1926	0.2016	0.1401	0.2218	0.1808	0.2028		0.0241	0.0270	0.0229	0.0237	0.0423	0.0388	0.0411
JF886079.1_Steatoda_hespera	0.1740	0.1639	0.1906	0.1985	0.1906	0.1934	0.2225	0.0974	0.1584	0.2016	0.1493	0.1527	0.1460	0.1990		0.0222	0.0206	0.0196	0.0385	0.0365	0.0353
KY269468.1_Robertus_scoticus	0.1645	0.1620	0.2084	0.2115	0.2141	0.2170	0.2063	0.1607	0.1399	0.2260	0.1887	0.1669	0.1926	0.2300	0.1831		0.0205	0.0212	0.0340	0.0328	0.0314
KP649575.1_Theridion_rabuni	0.1692	0.1593	0.1788	0.1807	0.1947	0.1974	0.2310	0.1777	0.1468	0.1879	0.1753	0.1671	0.1853	0.1926	0.1655	0.1640		0.0207	0.0337	0.0326	0.0326
KX537455.1_Crustulina_sticta	0.1748	0.1647	0.1801	0.1723	0.1856	0.1883	0.2014	0.1361	0.1380	0.1866	0.1633	0.1528	0.1411	0.1844	0.1433	0.1687	0.1641		0.0344	0.0333	0.0309
FJ413759.1_Aphidiinae_sp.	0.3297	0.3332	0.3761	0.3572	0.3877	0.3838	0.3846	0.3587	0.3126	0.3699	0.3404	0.3583	0.3697	0.3858	0.3595	0.3026	0.2993	0.3059		0.0152	0.0128
OP133190.1_Aphidius_uzbekistanicus	0.3040	0.3040	0.3280	0.3211	0.3280	0.3317	0.3288	0.3141	0.2968	0.3667	0.3281	0.3318	0.3395	0.3468	0.3321	0.2832	0.2833	0.2832	0.0943		0.0125
OP002856.1_Aphidius_gifuensis	0.3299	0.3335	0.3578	0.3430	0.3615	0.3653	0.3858	0.3193	0.3029	0.3818	0.3225	0.3434	0.3475	0.3785	0.3339	0.2771	0.2961	0.2710	0.0771	0.0729	

DISCUSSION

Based on the World Spider Catalog (2023), the Theridiidae ranks as the fifth largest spider family, with 2544 species spread across 124genera. These spider species are categorized as extremely poisonous, and capable of causing illness and even death (Afshari *et al*., 2009). The scientists were interested in learning more about them because of the dangerous bites (Jäger and Gromov, 2011)**.**

The *Latrodectus tredecimguttatus* is the most medically dangerous spider species that can bite humans, causing health issues and in rare cases, death. Knowledge about the black widow spider especially *L. tredecimguttatus* in Egypt is still very limited and is confined to two records Metwally *et al*. (2015) studied the duration of *L. tredecimguttatus* and El-Gendy (2020) studied its ecology and taxonomy at Menoufia Governorate. However, the observations on the white widow *L. pallidu*s were published by El-Hennawy (2006) in Jordan and Egypt. Also, Mohafez (2015) studied the life cycle of *L.geometricus*.

Discussing the morphological features and measurements of the present study results in agreement with Shafaie *et al*. (2021) who described *L. tredecimguttatus* from Iran. The morphological female measurements of *L. tredecimguttatus* by El-Hennawy *et al.* (2022) from Jordan including TBL= 8.5mm, and Cw =3.4mm agreed with the present study in some specimens that were TBL=9.47±0.55mm, and Cw =3.28±0.19mm.

Concerning the taxonomy of the present species, under investigation, all previous studies used traditional taxonomic methods that have relied heavily on morphology for species identification, but the advent of molecular techniques has provided a more precise and accurate classification, especially within complex genera such as *Latrodectus*. When traditional morphology fails to distinguish species, systematic studies using multiple lines of evidence including morphological, molecular, ecological, and biogeographic data yield more reliable hypotheses (DeSalle *et al.*, 2005; Zhang *et al.*, 2013; Valdez-Mondragón *et al.*, 2019; Navarro-Rodríguez and Valdez-Mondragón, 2020; Hazzi and Hormiga, 2021).

Recently, molecular techniques for species delimitation have emerged as a novel solution to species complicated issues and underestimated diversity. Offering infra-specific genealogical information from DNA markers has proven particularly helpful in morphologically conservative groups. This enables the objective application of contemporary species ideas (e.g., biological, phylogenetic, genotypic cluster, cladistics, etc.). Two key aspects of the integrative taxonomy approach to species delimitation are the use of multiple delimitation methods and the limitation of lineages to those consistently supported across results (DeSalle *et al.,* 2005; Carstens *et al.,* 2013; Luo *et al.,* 2018; Valdez-Mondragón, 2020)

CONCLUSION

Knowledge about the black widow spider especially *Latrodectus tredecimguttatus* in Egypt is still very limited. While traditional taxonomic methods have relied heavily on morphology for species identification, the advent of molecular techniques has provided a more precise and accurate means of classification, especially within complex genera such as *Latrodectus.* Based on the obtained results, robust features at the species level cannot be obtained by traditional morphology alone. It is of interest to point herein that the results of the mitochondrial *CO1* gene confirm that our tested species identified as *Latrodectus tredecimguttatus* under the accession number (PQ358290.1) in GenBank for the first time in Menoufia Governorate, Egypt. However, the combination of morphological characteristics including female epigyna, along with certain morphological measurements, as well as molecular techniques, proved to be instructive characteristics for the identification of the Black widow spider, *L. tredecimguttatus*.

List of Abbreviation

Abbreviation	Full name
A	Adenine
AER	Anterior Eye Row
ALE	Anterior Lateral Eye
ALS	Anterior Lateral Spinneret
AME	Anterior Median Eyes
BCh.	Basal Chelicerae
C	Cytochrome c Oxidase Subunit I
CO1	Cytosine
DNA	Deoxyribonucleic Acid
F	Fang
G	Guanine
ME	Maximum Likelihood
ML	Minimum Evolution
NCBI	National Centre for Biotechnology Information
NJ	Neighbour Joining
PCR	Polymerase Chain Reaction
PER	Posterior Eye Row
PLE	Posterior Lateral Eyes
PLS	Posterior Lateral Spinneret
PME	Posterior Median Eyes
T	Thymine

Declarations

Ethical Approval: This study has been granted by the Research Ethics Committee of Faculty of Agriculture at Assiut University in accordance with Egyptian laws and university guidelines for the care of animals (approval no. 03-2025-0020).

Competing interests: The authors declare that there is no conflict of interest.

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ARABIC SUMMARY

التعريف المورفوجزيئي للأرملة السوداء *Latrodectus tredecimguttatus* (Rossi, 1790) القاطنة بساتين الزيتون في محافظة المنوفية، مصر

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عنكبوت أرملة البحر الأبيض المتوسط السوداء، أو"Dolmak" (Rossi, 1790) *Latrodectus tredecimguttatus*، التابعة لعائلةTheridiidae ، هي واحدة من العناكب ذات الأهمية الطبية. وتم إدراجه كحيوان خطير نظرا لنطاقه الجغرافي الواسع ولدغاته السامة المميتة والتي تشكل خطورة كبيرة. يتطلب الفهم الشامل لأفراد هذا العنكبوت الجمع بين المعرفة المستمدة من كل من الدراسات المورفولوجية والجزيئية. لذلك هدفت هذه الدراسة إلى توجيه الباحثين المهتمين بدراسة هذه الأنواع الخطيرة وتحذير المزارعين والزوار في هذه المناطق من ضرورة تجنب أي نشاط يزعج العناكب ويجعلها عدوانية.
شملت هذه الدراسة جمع عينات من عنكبوت (أرملة البحر الأبيض المتوسط السوداء) يدويا بشكل عشوائي من بعض بساتين الزيتون بين سبتمبر 2023 وأغسطس 2024 في مدينة السادات، محافظة المنوفية، مصر .
الصفات المورفولوجية المستخدمة لتعريف شملت الطول الكلي للجسم، وعرض الدرع، وطول البطن، وطول الساقين (1 و3 و4) .
أظهرت النتائج أن الطول الإجمالي للإناث يتراوح بين 9.0-17.0 ملم. النمط الظهري الأنثوي إن وجد. يتكون من عدة بقع برتقالية (عادة في صغار الإناث) بينما تفتقر الإناث البالغات إلى النمط الظهري والساعة الرملية البطنية التي تم اختزالها إلى اثنين من الخطوط البرتقالية المستعرضة إلي بقع حمراء. أكدت نتائج الوراثة الجزيئية باستخدام جين *CO1* للميتوكوندريا أن الأنواع التي تم اختبارها تم تعريفها على أنها *L. tredecimguttatus* وسجلت في بنك الجينات العالمي GenBankتحت رقم (PQ358290.1) لأول مرة في محافظة المنوفية، مصر. لذا، فإن توصيف أنثى الأرملة السوداء وفقا للطرق الوراثية المورفولوجية والجزيئية له أهمية كبيرة.

الكلمات المفتاحية: العناكب، Theridiidae، *Latrodectus tredecimguttatus*، مورفوجزيئي، *CO1* الجين.