Original Article

Laboratory Rearing and Biological Characteristics of *Latrodectus tredecimguttatus* in Bojnord County, North Khorasan Province, Iran

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Abstract

Background: This study presents a laboratory-based investigation into the morphology, biology and ecology of the Mediterranean black widow spider, *Latrodectus tredecimguttatus*, in Bojnord County, North Khorasan Province, Iran. Despite the species' medical importance across Southern Europe and the Middle East, information on its biological characteristics in northeastern Iran remains limited.

Methods: Adult spiders and egg sacs were collected from agricultural and peri-urban habitats in Bojnord County in 2014. Species identification was based on morphological characteristics using a stereomicroscope. Behavioral and developmental traits, including feeding preferences, reproductive output and responses to environmental factors, were evaluated under controlled laboratory conditions.

Results: The collected specimens were morphologically identified as *L. tredecimguttatus*. Under laboratory conditions, females produced an average of 10 egg sacs (approximately 300 eggs per sac) and completed up to three generations. Feeding experiments demonstrated an observed preference for soft-bodied prey with high moisture content, whereas hard-bodied or desiccated insects were generally rejected. Environmental observations suggested that temperature and relative humidity may be associated with developmental processes and reproductive performance under laboratory conditions; however, these relationships were not experimentally tested.

Conclusion: This study provides baseline information on the reproductive biology, development and feeding behavior of *L. tredecimguttatus* in northeastern Iran. The observations obtained under laboratory conditions provide a basis for future ecological and experimental studies on this medically important spider.

Keywords: Reproductive biology; Developmental biology; Feeding behavior; Egg sac production; Latrodectism

Introduction

Spiders are among the most diverse and ecologically significant groups of terrestrial predators, playing a pivotal role in regulating invertebrate populations and maintaining ecosystem stability (1, 2). Among them, the family Theridiidae commonly known as comb-footed or tangle-web spiders, comprises 2,584 species in 131 genera worldwide. This family is characterized by specialized webs used for prey capture and shelter construction (3–5). Within

the family Theridiidae, the genus *Latrodectus* Walckenaer, 1805, commonly known as widow spiders, comprises 35 species worldwide, nine of which occur in Europe and five in Iran (6–8). The Mediterranean black widow, *L. tredecimguttatus* (Rossi), is particularly notable because of its medical importance. Its venom contains the potent neurotoxin α-latrotoxin, which is responsible for latrodectism in humans (8, 9). Clinical manifestations include severe muscle pain, cramps, nausea, diaphoresis and, in rare cases, fatal outcomes (10). Beyond its medical importance, *L. tredecimguttatus* plays an important ecological role. The species inhabits a wide range of environments, including wheat fields, cultivated lands, uncultivated areas and human-modified habitats, reflecting its ecological adaptability (2). As an ambush predator, it constructs irregular webs that capture a wide variety of invertebrates, including Diptera, Coleoptera, Lepidoptera, Hemiptera and Hymenoptera, and occasionally small vertebrates such as lizards and amphibians (11, 12). In Iran, information on *Latrodectus* species remains limited and is based mainly on scattered faunistic records (13). Recent molecular investigations, including DNA barcoding analyses of Iranian populations of *L. tredecimguttatus*, have provided additional information on species identification and genetic variation, offering valuable phylogeographic context for interpreting biological variation within this species (13). Among these understudied regions, North Khorasan Province, particularly Bojnord County, represents a geographically and ecologically diverse area that may support populations of *Latrodectus* spiders (14). Consequently, information on the distribution, habitat preferences, biological characteristics and ecological interactions of *Latrodectus* species in this region is still incomplete (14).

North Khorasan Province, particularly Bojnord County, represents a geographically and ecologically diverse area that may support populations of *Latrodectus* spiders (14). However, little is known about their occurrence, habitat preferences, reproductive biology, feeding behavior and responses to environmental conditions. Therefore, the present study aimed to provide baseline information on the laboratory rearing and biological characteristics of *L. tredecimguttatus* collected from Bojnord County, North Khorasan Province, Iran. The study focused on morphological identification, developmental and reproductive characteristics, and qualitative observations of feeding behavior under laboratory conditions.

Materials and Methods

Study area

Bojnord City, Bojnord County, the capital of North Khorasan Province in northeastern Iran, is located at 37°28′ N and 57°20′ E. The region is characterized by diverse climatic conditions, varied topography and heterogeneous vegetation, providing suitable habitats for a wide range of arthropods, including spiders (Fig. 1).

Specimen collection

Spider specimens were collected in April, June and August 2014 by systematically searching typical microhabitats (15). These included cracks and crevices in clay or stone walls, abandoned rodent nests, tree trunks, wooden debris, construction waste and areas within or around agricultural fields (16). Each specimen was placed in a ventilated container immediately after collection, and relevant ecological data (collection date, location, microhabitat type) were recorded before sealing. The samples were transported to the Vector Biology Laboratory for further processing. The number of specimens from each site was recorded separately, and morphological identification was performed using established taxonomic keys (17–19).

Laboratory conditions for spider rearing

Egg sacs obtained from field-collected gravid females were transferred to glass aquaria equipped with adequate ventilation and secure

lids. To simulate natural environmental conditions, aquarium floors were lined with plant leaves, small branches and moist soil. After hatching, the spiderlings were reared until they could be distinguished by sex. Four large aquaria were then prepared following the protocols described below, and one male-female pair was placed in each aquarium. All four pairs were offspring derived from the same field-collected female. For feeding experiments, live insect prey was individually introduced into the aquaria, including *Anopheles* and *Culex* mosquitoes, to assess prey capture behavior and feeding preferences under standardized laboratory conditions. Observations included mating behavior, egg sac production, feeding behavior, offspring development, lifespan and number of generations. The insects used for feeding experiments included *Culex* mosquitoes, *Anopheles* mosquitoes, horseflies, dragonflies and damselflies, honeybees, butterflies, ants, true bugs, cockroaches and grasshoppers. Prey acceptance and feeding responses were observed qualitatively during routine laboratory rearing. These observations were not conducted as a standardized quantitative preference assay.

Experimental setup and rearing conditions

To observe biological performance under different laboratory rearing conditions, three types of laboratory aquaria were designed and constructed. The small aquarium (15× 20× 30 cm) served as a molting chamber, where molting events, inter-molt intervals and the total number of molts until maturity were recorded using external labels. The medium aquarium (20× 20× 30 cm) was used for mating trials, individual rearing and temporary isolation when required. The large aquarium (70× 70× 100 cm) was used for continuous rearing and behavioral observations, including web construction, prey capture behavior, and general activity patterns. This unit was equipped with a hygrometer and thermometer for continuous environmental monitoring. All aquaria were fitted with a 15 cm diameter top opening covered with fine mesh to prevent the escape of spiderlings. The base of each aquarium was lined with soil, and branches were provided to facilitate shelter construction and web-building behavior. Temperature and humidity conditions were maintained according to two previously described laboratory rearing protocols. Aquaria 1, 3 and 4 were maintained under the Satpathi protocol (20), with temperatures ranging from 25–30 °C and relative humidity of 40–70%. Aquarium 2 was maintained under the Duma protocol (21), with temperatures ranging from 15–25 °C and relative humidity of 35–40%. Spider activity was monitored daily, including molting events, egg sac production, mortality, and activity level. Minimum, maximum and mean daily temperature and humidity were also recorded throughout the study period. To observe the potential influence of photoperiod under laboratory conditions, an 8 h light: 16 h dark (L8) photoperiod was used during the corresponding observations. The effects of this photoperiod on egg sac production were recorded descriptively. Their effects were assessed in terms of development rate, mating behavior, reproductive output and survival. All experiments were conducted under controlled laboratory conditions.

Statistical analysis

Data from the laboratory experiments were analyzed descriptively. Mean ± SD and ranges were calculated for biological parameters, including egg sac production, developmental duration and longevity. Observations related to photoperiod were also summarized descriptively. Descriptive analyses were performed using SPSS version 26.

Results

Sampling locations and schedule

Latrodectus specimens were collected from three localities in North Khorasan Province, Iran: Mahnan (37°21′55″N, 57°18′23″E), Besh Qardash (37°24′00″N, 57°17′00″E) and Baba Aman (37°28′38″N, 57°26′23″E). Sampling was conducted in April, June and August.

Life history and reproductive traits

A total of one adult female and two egg sacs of *L. tredecimguttatus* were collected from the field and transferred to the laboratory. The two field-collected egg sacs were initially maintained in a primary aquarium and subsequently yielded a total of 234 spiderlings. Following morphological identification of the laboratory-reared spiders as *L. tredecimguttatus*, four male–female pairs were established, with one pair maintained in each of four separate aquaria. All four laboratory-reared pairs originated from the offspring of the same field-collected female. The four laboratory-reared females produced 13, 5, 11 and 10 egg sacs, respectively, corresponding to a total of 39 egg sacs and a mean of 9.75±3.30 egg sacs per female. The female maintained in Aquarium No. 3 produced 11 egg sacs. Spiderlings required 5–8 instars to reach adulthood, with females completing eight instars and males five instars. Sexual maturity was reached approximately 59 days after hatching. The first egg sac was produced 62–75 days after hatching, whereas the last egg sac was produced at 141–147 days after hatching. For detailed assessment of egg-sac morphology and egg content, a subset of 10 egg sacs was examined. Three egg sacs were selected from each of Aquaria 1 and 2, and two egg sacs from each of Aquaria 3 and 4. The mean longitudinal and transverse diameters of these egg sacs were 17.45±1.46 mm and 13.64±1.67 mm, respectively and the mean number of eggs per sac was 298.80±62.36. No sexual cannibalism was observed during the laboratory observations, whereas multiple mating events were recorded after oviposition.

Predatory behavior observations

Predatory behavior was observed following prey ensnarement, which generally induced escape attempts; however, large or loosely entangled prey were sometimes able to escape. Prey-generated vibrations were detected by the spiders, which approached the prey and immobilized it by applying silk using the serrated bristles of the fourth pair of legs. A paralytic bite was occasionally observed. Spiders were offered a range of prey items, including *Culex pipiens*, *Anopheles stephensi*, cockroaches, dragonflies, damselflies, true bugs, butterflies, ants, horseflies, flies, honeybees and small grasshoppers. Feeding behavior varied with hunger state; satiated spiders wrapped prey in silk and stored it near the retreat for later consumption, whereas hungry individuals generally fed soon after immobilization. Qualitative observations indicated that soft-bodied and relatively moist prey were generally more readily accepted than hard-bodied or desiccated prey. Ants were generally avoided, and in one observation, an ant was followed by an immediate retreat after a defensive bite. *Culex* and *Anopheles* mosquitoes appeared to contribute less frequently to feeding observations. Horseflies, flies, honeybees and small grasshoppers were also observed to be readily accepted. These observations were preliminary and qualitative and were not based on a standardized quantitative assay; therefore, no quantitative ranking of prey preference was established (Tables 1–3).

Effects of light-dark cycles on egg sac production

Under the 8 h light: ١٦ h dark (L٨) photoperiod, females produced 7–8 egg sacs, with limited variation among individuals. Because only the L8 photoperiod was evaluated, no comparative assessment of different photoperiod regimes was performed. The lower number of egg sacs observed in this observation compared with the initial study may be related to seasonal differences, as the observations were conducted during autumn and winter.

Table 1. Reproductive parameters and longevity of *Latrodectus tredecimguttatus* females/males under laboratory conditions

Parameter	Aquarium				
	No. 1	No. 2	No. 3	No. 4	Mean± SD
Age at first mating (days)	53	61	60	64	59.50±5.51
Age at first egg sac production (days)	62	73	73	75	70.75±5.85
Age at last egg sac production (days)	141	147	145	144	144.25±2.22
Total number of egg sacs produced	13	5	11	10	9.75±3.30
Shortest interval between successive egg sacs (days)	4	11	4	6	6.25±3.77
Longest interval between successive egg sacs (days)	13	37	13	12	18.75±11.87
Female longevity (days)	164	243	270	186	215.75±41.06
Male longevity (days)	92	78	146	121	109.25±28.89

Table 2. Morphometric parameters and egg content of egg sacs produced by *Latrodectus tredecimguttatus* females under laboratory conditions

Egg sac number	Length(mm)	Width(mm)	Number of eggs
1	20	16	230
2	19	15	312
3	16	12	258
4	16	13	234
5	16	14	406
6	17	12	315
7	16	15	245
8	17	16	401
9	17	15	318
10	20	12	269
Mean ± SD	17.45 ± 1.46	13.64 ± 1.67	298.80 ± 62.36

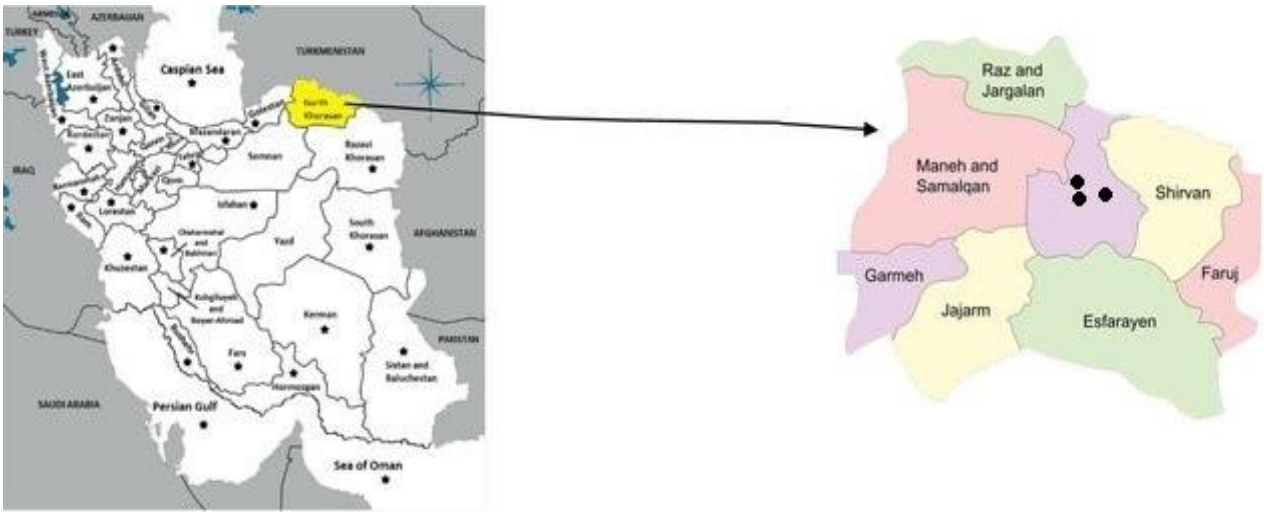

Fig. 1. Map of North Khorasan Province, Iran, showing the location of Bojnord County and the sampling sites (black circles, ●) (22)

Table 3. Egg sac deposition intervals of *Latrodectus tredecimguttatus* females across individual aquaria

Parameter	Aquarium				Mean± SD
	No. 1	No. 2	No. 3	No. 4	
Age at first egg sac deposition (days)	62	73	73	75	70.75± 5.51
Age at last egg sac deposition (days)	141	147	145	144	144.25± 2.22
Number of egg sacs deposited	13	5	11	10	9.75± 3.30
Minimum interval between successive egg sacs	4	11	4	6	6.25± 3.77
Maximum interval between successive egg sacs	13	37	13	12	18.75± 11.81



Fig. 2. Third-instar widow spider (*Latrodectus tredecimguttatus*) after the first molt (Original photo)



Fig. 3. Aquarium number 3 used for rearing *Latrodectus tredecimguttatus*, illustrating the individual housing system and territorial behavior of the spiders (Original photo)

Discussion

The present study expands current knowledge on the biology, reproductive traits and feeding behavior of *L. tredecimguttatus* in northeastern Iran. Morphological examination confirmed the identity of the specimens, consistent with previous faunistic records of *L. tredecimguttatus* from Iran and neighboring regions (23–25). Although the present study focused on laboratory-based ecological and physiological observations, previous molecular studies on Iranian populations of *L. tredecimguttatus* provide valuable phylogeographic context for interpreting

biological variation within this species. Integrative studies combining genetic information with ecological and physiological traits are needed to better understand population-level variation in this medically important spider (13). Field collection records indicated the occurrence of this species in both natural and human-modified environments, including agricultural and peri-urban areas such as Mahnan, Besh Qardash and Baba Aman. These observations represent collection sites and should not be interpreted as definitive evidence of habitat preference at the population level. Laboratory observations provided baseline information on reproductive and developmental characteristics. Females produced multiple egg sacs under controlled conditions, which was generally consistent with previous reports for the genus *Latrodectus* (26, 27). No sexual cannibalism was observed during laboratory observations, in contrast to some previous reports describing this behavior in widow spiders (26–28), However, differences in sample size, environmental conditions and experimental design may influence the occurrence of such behaviors. Feeding experiments indicated a higher acceptance of soft-bodied prey, whereas hard-bodied insects were generally less frequently consumed. These findings represent preliminary observations that require further evaluation using larger sample sizes and controlled feeding experiments. Similar feeding preferences have been reported in previous studies on widow spiders (24, 29). The low acceptance of ants, dragonflies and mosquitoes may be associated with prey-specific defensive mechanisms, handling difficulty, body size, or reduced capture efficiency. Differences in feeding responses may also reflect individual behavioral variation and laboratory conditions. In the present study, temperature and relative humidity were monitored under different laboratory rearing protocols; however, these factors were not independently manipulated. Therefore, the individual effects of temperature, humidity, or their interaction on developmental and reproductive traits could not be determined. In addition, the limited replication of environmental protocols, particularly the single aquarium under the Duma protocol, restricts statistical interpretation of differences among conditions. Future studies using replicated factorial experimental designs with independent temperature and humidity treatments are required to clarify the role of environmental factors in the biology of *L. tredecimguttatus*.

Conclusion

This study provides baseline biological information on *L. tredecimguttatus* collected from North Khorasan Province, Iran. Laboratory observations revealed multiple egg sac production, distinct developmental stages and no evidence of sexual cannibalism under controlled conditions. Feeding assays indicated a preference for soft-bodied prey, whereas hard-bodied insects were generally less accepted. Given the limited sampling design, the findings of this study are restricted to laboratory conditions and should not be extrapolated directly to natural populations. Further investigations with larger sample sizes and field-based observations are required to better understand the biology, ecology and population dynamics of this species.

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Ethical considerations

This research has been registered with the ethics code IR.TUMS.SPH.REC.1392.309 in the Ethics Committee of the School of Public Health, Tehran University of Medical Sciences.

Conflict of interest statement

The authors declare there is no conflict of interest.

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