Original Research Article
ISSN: **3041-5357**
Journal homepage: https://diversity.researchfloor.org/

Journal of Diversity Studies

Morphotaxonomy with SEM description and life history trait of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae)

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ABSTRACT

Morphotexonomy and life history traits of Coccinella septumpunctata developmental stages were investigated. The incubation period was 5.8 days, and the development duration of 1st,2nd,3rd and 4th instar was 2.8±0.84, 3.6±0.55, 3.8±0.84 and 4.4±1.34 respectively. The longevity of females and males was 42.65 ± 5.84 and 40.3 ± 4.91 days, respectively. By using Scanning electron microscopy, the analysis shows the pedicel, scape and nine flagellomeres comprise the antennal morphology of both males and females. However, the (hss) hook-shaped sensilla was confirmed by SEM, highlighting the antennae differences between male and female beetles.

Keywords: *Coccinella septumpunctata, Morphotexonomy, Scanning electron microscopy, life history traits.*

Citation: Lalit Kumar and Thakur [2025]. Morphotaxonomy with SEM description and life history trait of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). *Journal of Diversity Studies.* **DOI: https://doi.org/10.51470/JOD.2025.4.1.08**

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Article History: *Received 21 October 2024 | Revised 17 November 2024 | Accepted 23 December 2024 | Available Online January 14, 2025*

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Introduction

Coccinella septempunctata L. (Coleoptera: Coccinellidae), is a well-known predator and beneficial group of insects known to human beings. Coleoptera, is the largest order of class Insecta, consisting of about 40% insect populations and about 2,50,000 species known to mankind [1]. Family Coccinellidae comprises six subfamilies, of which five, Coccindulinae, Coccinellinae, Sticholotidinae, Chilocorinae, and Scymninae comprise predatory species, and the phytophagous species are included in subfamily Epilachninae [2]. More than 6000 species of coccinellids in the world and around 550 belonging to 90 different genera have been reported so far from India [3].

In Himachal Pradesh, about 65 coccinellid species have been known so far from different agro and forest ecosystems. Coccinellids inhabit two distinct types of habitats: ectotopic and eurytopic. Eurytopic species exhibit high ecological adaptability, enabling them to thrive across diverse environmental conditions. [4]. In contrast, ecotropic species live on exposed surfaces, such as plant leaves, stems, or other external environments, rather than within concealed or specialized microhabitats. [5]. Coccinellids have specific dirt for their survival and thus are found in a particular area of preference and may be associated with the suitability of the environment for females to oviposit and the development of subsequent larval instar.

Male and female both are brightly coloured mostly red or brown with black markings [6]. Coccinellids exhibit sexual dimorphism, and females typically have a greater body mass than male counterparts. The outward signs of sexual dimorphism are evident, male may have stripes on the femur and pale dots in front of the coxa. There was a notable distinction between the sexes in the seventh abdominal sternite [7]. After mating in favorable environmental conditions, females may lay up to 1000 eggs.

The females can store sperm in their spermatheca and fertilize the eggs according to their needs. The timing and quantity of eggs laid may be determined mostly by the environmental circumstances [8]. Coccinellids are holometabolous organisms that go through four distinct life stages viz. egg, larva, pupa and adult. This indicates that they undergo complete metamorphosis. The entire developmental process, from egg to adult, can take up to 45 days in unfavorable environments and 17 days in favorable ones [9]. Longevity of adults may vary from one to two years.

Normally, the eggs are laid in clusters of four to five, but occasionally, deposited single on selected oviposition substrate [10]. There is variation in egg size between species and occasionally even within species also thus received their nourishment before their dispersal. Larvae emerge within four to five days and newly emerged larvae destroy the unhatched eggs this is called sibling cannibalism or kin cannibalism. This sort of feeding gives them a nutritional supply before dispersal [11]. *C. septempunctata* a seven-spot ladybird beetle, is an entomophagous predator that plays a significant role in biological control by consuming a variety of aphid species. Aphids are one of the most damaging insect pests which suck the cell sap and affect the overall health of the plant by releasing honeydew, which promotes the growth of sooty mould and modifies the regular physiology of the leaves [12].

Material and Methods

Study area

The study area is Himachal Pradesh, a hilly state of India situated in the western Himalayas. The state occupies an area of 55673 km between 30º 22' 40'' to 33º 12' 40'' N latitude and 75º 45' 55'' to 79º 04' 20'' E longitude.

Collections of specimens
Collection of beetles has been carried out from fruit orchards, vegetable fields, agricultural fields, and the entire vegetation of forests including herbs, shrubs and trees, the Methods for collecting beetles were as, hand picking, directly in the insect collection tubes, containers, forceps, beating sheets and jars, depending upon the habitat and species available in that area. The data has been recorded on different parameters like time, date, location, weather conditions, and characteristics of each collection site.

Identification
The morphological features such as antennae, pronotum and spots on elytra, were used to identify the specimen. All identifications were made using checklists and keys developed by Poorani [13].

Biology
The traits development and morphology of coccinellids vary with their food. Biology of *C. septumpunctata* was carried out under controlled studies in laboratory conditions in the Department of Biosciences Himachal Pradesh University Shimla, at 24±1ºC temperature, 65±2% R.H., and 16:8 L: D photoperiod, by providing nymphs of *Rhophalosiphum padi* (Linneaus) as food as per the laboratory protocol developed by [14].
Adult of *C. septempunctata* collected from various field crops, were cultured on wheat aphids. For mass rearing, the collected adults were kept in paired Petri plates measuring 75 mm in diameter. Every day the eggs laid on the leaves or surrounding the Petri plate by the female coccinellids were collected and stored in separate Petri plates after being gently brushed with a soft camel hair brush. Freshly hatched grubs were cultured separately in Petri plates. The nymphs were placed in these petri dishes and monitored daily. The duration of incubation period of egg hatching was also noted. Each newly hatched grub was given 15–20 aphids nymph but as the grubs matured, nymph number increased. Exuviae released by the grubs were used to calculate each life stage's number of instars and length the Pupa developed in Petri dishes were maintained apart and undisturbed until they emerged as adults. The statistical parameters were derived by computing the length of time for each instar and the total life span. Laboratory-reared adults were sexed as male and female based on their body size. Longevity of males and females were studied separately. For this purpose, ten pairs of *C. septempunctata* were kept individually and data was recorded. The time after the emergence of adults from the pupa and the starting of oviposition was considered the pre-oviposition period [15].

Morphotaxonomic Studies
Morphological characteristics such as the pronotum, forelegs, middle legs, hindlegs, and elytra of adults were used to identify the specimens. The preserved specimen were dissected and their antenna, head, pronotum, elytra, abdominal segment and genitalia were extracted and photographed with a Digital camera model Nikon SMZ1270 by using NIS elements imaging software, which was installed in the Department of Biosciences at Himachal Pradesh University Shimla.

Genitalia Extraction
Species identification in coccinellids is generally based on comparing male and female genital characteristics. Gently lifting the last abdomen segment with fine forceps may determine the sex of the specimen. The following procedure will be followed to prepare male and female genital slides. For genitalia extraction, the last abdominal segments of the insect specimen were detached and submerged in 10% KOH solution at a high temperature until all the soft tissue was dissolved completely and the material was washed in distilled water. After proper washing, the material was dissected and the genitalia removed with the help of fine needles, and forceps and immersed in cold 10% KOH solution to dissolve away the remaining debris. Thereafter genitalia was transferred to distilled water and washed repeatedly and dehydrated in different grades of alcohols (30, 50, 70, 90, 100%) and finally mounted in DPX to make permanent slides for observation under a microscope.

Electron Microscopy (SEM)
Freshly anesthetized male and female antennae were removed, dried, and fixed in 2.5% v/v glutaraldehyde with phosphate buffer (pH 7.4) for 12 hours at 40°C. The fixed antennas were dipped for 20 minutes each in phosphate buffer (pH 7.4) three times. After being re-washed three to four times with distilled water, the antennae were dehydrated by going through different grades of alcohol, 30, 50, 70 and 90%. They underwent two changes of 20 minutes each to dehydrate them in 100% ethanol further. After dehydration, the materials were cleaned and treated with acetone for 20 minutes each. After allowing the specimens to air dry, they were mounted on bronze stubs with the desired antennal face facing upward using carbon adhesive tape. The attached antennae were left to dry for three hours. Ion sputtering was used to cover the antennae in gold. Scanning electron microscopy was done to describe the morphology of the antenna of a species. At higher magnifications, it is possible to distinguish several different types of sensillae and a few less common forms according to their gross structure. The morphological features and length of antennae in both sexes were recorded by scanning electron micrographs. The sensory structures found will be named following the terminology of Faucheus (1985) and Jourdon *et al.* (1995). The number of each type of sensilla was counted along the length of the antennae. Each sensilla's average length and basal diameter were obtained from micrographs.

Results and Discussion
The biology of *C. septempunctata* was carried out under laboratory conditions and results were obtained during the investigation. The female deposited clusters of bright yellow eggs, which became dark yellow before hatching. The average incubation period was 5.8 days. The larvae underwent four distinct instars by moulting three times and their average development duration was 2.8±0.84, 3.6±0.55, 3.8±0.84 and 4.4±1.34 days respectively. The prepupal and pupal stages lasted for 2.6±0.55 and 6.2±0.84 days respectively. The average longevity of males and females was 17.6±2.97 and 18.6±3.85 respectively, as shown in Table 1. In a similar study [15], Gurung and all found that the average incubation period was 5.2±1.28 days and larvae passed four distinct instars, with three moulting. Their results show that adult males and females' longevity was 16.7 ± 2.83 and 18.2 ± 3.22 days and the entire life spans of females and males were 42.65 ± 5.84 and 40.3 ± 4.91 days, respectively. Similarly [16], Sattar reveals that the pupal period was 4.9±0.58 days and the mean duration of the first, second, third and fourth larval instars was 2.9±0.42, 4.69±0.47, 5.4±0.66 and 7.5±0.72 days, respectively.

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Table 1. The developmental period of different life stages of C. septumpunctata.

Developmental stages	Duration (in days)
Egg hatching	5.8±0.84
1st instar	2.8±0.84
2nd instar	3.6±0.55
3rd instar	3.8±0.84
4th instar	4.4±1.34
Pre-pupa	2.6±0.55
Pupa	6.2±0.84
Adult male	17.6±2.97
Adult female	18.6±3.85

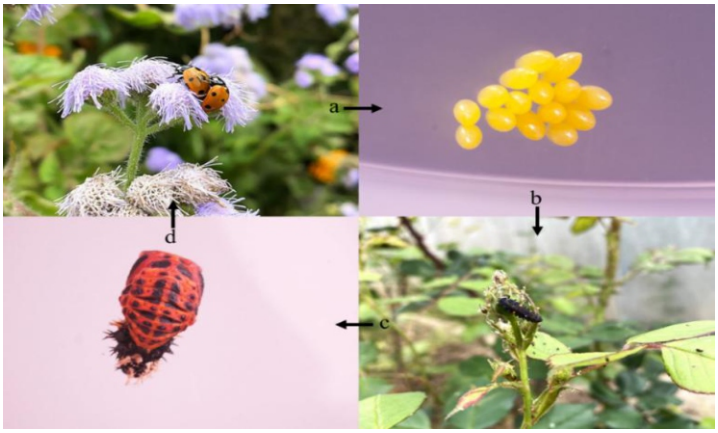

Fig.1. Life cycle trait (a) adult male and female (b) eggs (c) larva (d) pupa

Morphotaxonomic Studies

Body and Head

Bilateral symmetry and slightly longer oval-shaped structure. Head with black eyes. Two dark brown club-shaped antennae with a big flagellum, pedicle, and scape are divided into eleven segments. The mouthparts are specialized for the consumption of soft-bodied prey, the mandibles are adapted for biting and mastication. The maxillae and labium facilitate food manipulation, while the labial palps contribute to sensory perception. It has been reported that the antenna of coccinellids is very variable, with 7- 11 segments. The eyes are large and occupy a large portion of the head. In a similar study [17], Majerus describes the specialized mouthparts of predatory beetles, Ladybirds also possess mouthparts specialized for biting, with large mandibles adapted to their specific diets. The inner biting surfaces of the mandibles feature two distinct sections: the incisor region and the molar region.

Thorax

There are seven spots, including one sutural spot directly behind the middle of the thorax and three elytra spots extending to each side. The elytra are reddish-orange to bright red, with two yellowish patches on either side of the black mark on the scutellum. The scutellum is typically triangular with the apex toward the back is always visible. The posterior has a tiny ridge, while the lateral side has a discernible ridge. The black elytra have no hairs on it. Along with the basal subquadrate tooth, the middle and rear legs are charcoal black and have two spurs. A similar observation was revealed by Slipinski [18].

Abdomen

Both sexes have six abdominal sterna that cover the rear margin; In males, it is slightly notched, while in females, it is narrowly rounded or distinctly divided in the center. This feature differentiates them from one another. The present study is in close agreement with the findings of [5]. The structural differences in abdominal sterna and tergites between male and female ladybirds align with previous studies on sexual dimorphism in beetles.

Both sexes have six abdominal sterna, with the posterior margin being convex in females and blunt in males, as shown in **figure 2.** Additionally, the six abdominal tergites curve over the posterior margin in females, while they are more strongly convex in males, [19]. These morphological variations may play a role in reproductive or behavioral adaptations, warranting further investigation.

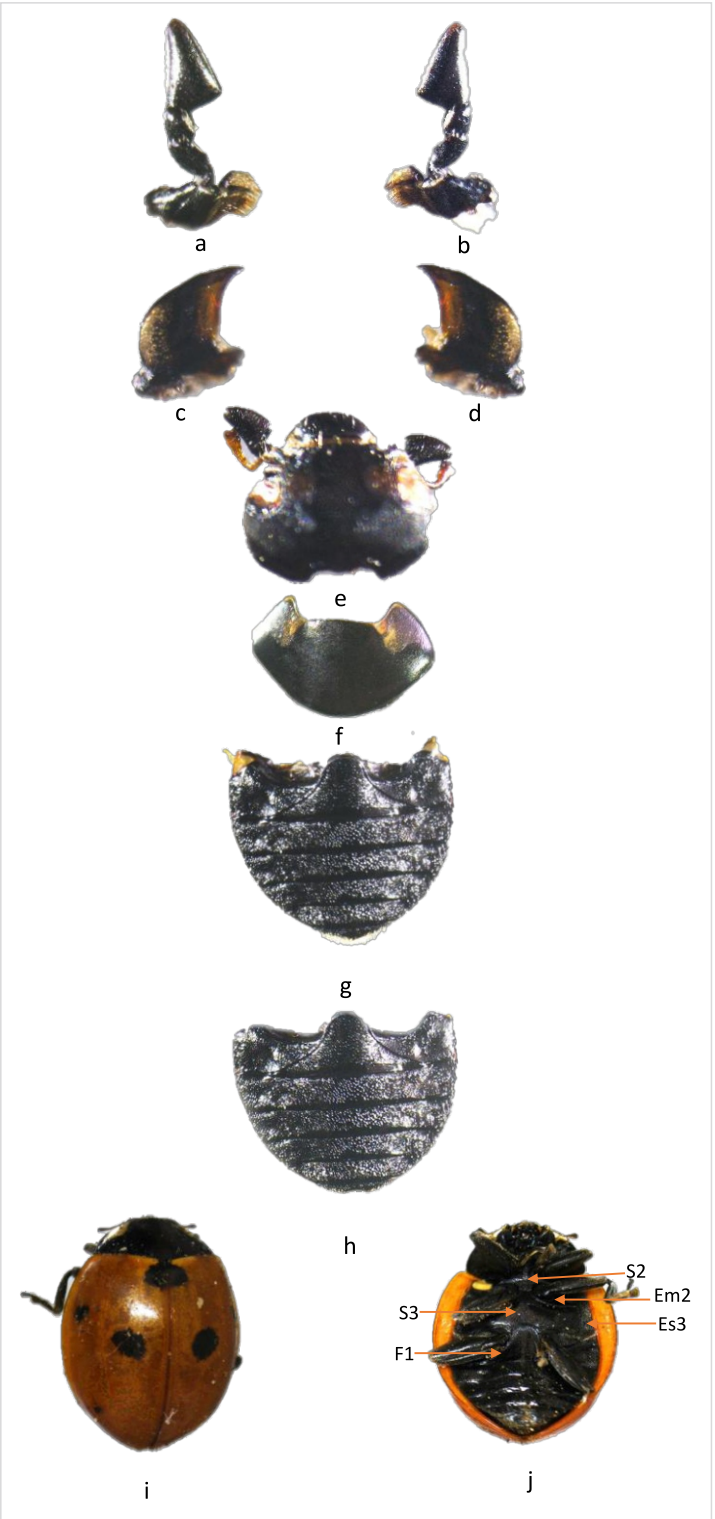

Fig 2. maxillae (a,b) Mandible (c,d) head capsule (e) prothorax (f) female abdomen (g) male abdomen (h) C. septumpunctata (i) adult ventral view (j) (F1-femoral line, S2, S3-meso and metasternum, Em2-mesometaepimeron, Es3-mataepisternum)

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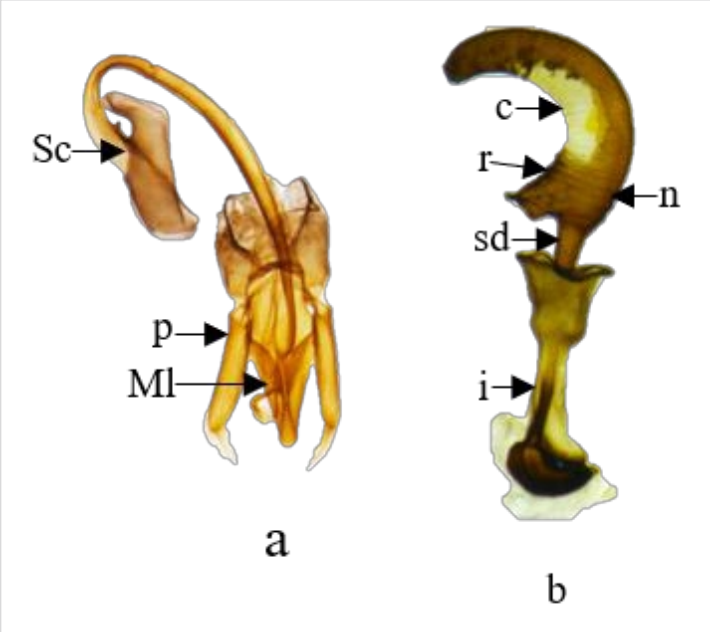

Fig 3. C. septumpunctata genitilia: (a) male, (b) female.Male- (SC-siponal capsule, P-paramera, ML-median lobus) Female- (c-cornus, r-ramus, n-nodulus, sd-sperm duct, i-infundibulum).

Genitalia

The tegmen have asymmetrical short trabes, the lateral lobes are slightly smaller than the basal lobes, and the male sternum apodeme 9 is rod-like and chitinized. The median lobe was comparatively longer than the parameres. The siphonal capsule was thick and protruded, with the outer arm relatively thicker than the inner arm. Siphonal tubes are long and have a sac-like structure at the end. Broad genital plates and almost semicircular spermatheca are characteristics of females. Almost similar characterstics of genitalia of male and female insect were noticed by [5]. The tegmen and sipho are the two components of the male genitalia proper. The tegmen consists of a basal component and paired para mesa (lateral lobes) and can vary in thickness, symmetry, or asymmetry. The structural characteristics of the siphonal tube observed in this study are consistent with previous descriptions of similar species. The tube is notably long, with a basal bend and an almost straight form along its length, ending in a sac-like structure at the distal end. Furthermore, the apex displays three distinct points of distortion, and the siphonal capsule appears swollen and dense [20]. These morphological traits may be significant for species identification and functional adaptations, meriting further investigation."

Electron Microscopy (SEM)

Antenna

The male and female ladybeetles had identical general morphologies, with their antennae situated at the dorsolateral corners of the frons in front of the eyes. The (hss) hook-shaped sensilla was recorded on the antenna of a male, and this is not found in females, it reveals the sexual dimorphism in the antennae of male and female *C. septumpunctata*. Every segment is cylindrical, and the thickness of each flagellomere increases progressively from base to tip. They have eleven annuli total, the pedicel, the scape, and a flagellum with nine annuli in each sex. The total length of the male antennae measures 100 µm in both, with a width of 21.0 mm and 15mm as observed under a magnification of 275X. and 190X. The annuli had a cylindrical shape, and they typically got shorter toward the distal end of the antennae. In similar observations, Srivastava [21].

A study on the antennal morphology of *C. septempunctata* aligns with previous research on coccinellid species. Scanning electron microscopy revealed that both males and females possess eleven-segmented, scaly antennae, with lengths measuring 950 µm in males and 980 µm in females. The presence of eight distinct sensilla types- chaetica, trichoidea, basiconica, campaniformia, ampucellaceous, scolopalia, placoidea, and hook-shaped sensilla, found in male but not on female antenna. The longest portion of the antennae for both sexes is the scape. The length of each segment does not significantly differ between males and females. However, all of the male antennae's segments are comparatively wider than the females. Antenal length of *C. septempunctata* was noticeably longer than that of females. The male *C. septempunctata* flagellomeres F1 through F8 were noticeably shorter than the female conspecifics whereas F9 was noticeably longer. The results are similar to the observation of [22].

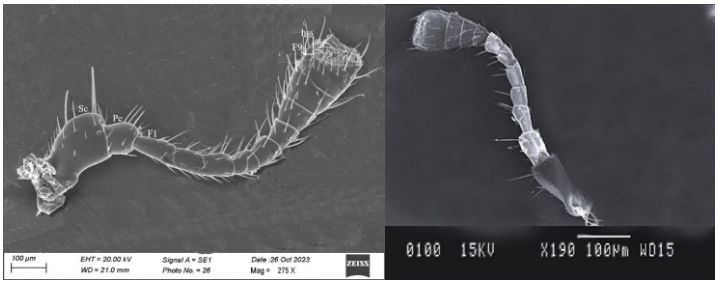

Figure 4. (a) A Male antenna, (b) A Female antenna, Sc- scape, Pe-pedicel, F1-F9- the first to the ninth flagellomere, hss- hook-shaped sensilla.

Conclusion

The study of *C. septempunctata* reveals distinct morphological and developmental characteristics. The larvae undergo four instars with varying life spans, and adult females tend to live longer than males. The species displays bilateral symmetry and an elongated oval body, with the head featuring black compound eyes and 11-segmented, club-shaped antennae. The thorax has seven characteristic spots, and the red elytra with yellow patches are consistent with previous descriptions. Males and females both possess six abdominal sterna. The abdomen in males is slightly notched, while in females, it is narrowly rounded or distinctly divided in the center, confirming earlier research. Male genitalia show asymmetry, with shorter lateral lobes, a chitinized sternum apodeme 9, and a prominent siphonal capsule, aiding in taxonomic classification. Sexual dimorphism is evident in the antennae, with males exhibiting hook-shaped sensilla (hss) but absent in females. Both sexes share similar antennal structures, but male antennae are slightly broader. These findings align with previous studies and contribute to a deeper understanding of the morphology, sexual differences and taxonomic placement of *C. septempunctata*.

Acknowledgment

The authors are grateful to the Chairperson, Department of Biosciences, Himachal Pradesh University, Shimla, for encouragement, and for providing necessary facilities. The authors also truly appreciate the faculty and staff for their guidance and the funding agency CSIR for their support, I am grateful for the trust CSIR has placed in my work.

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