

Morphology and Surface Structure of Eggs and First Instar Larvae of *Coccinella septempunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae)

Coccinella septempunctata (Linnaeus, 1758) (Coleoptera: Coccinellidae) Yumurtalarının ve Birinci Evre Larvalarının Morfolojisi ve Yüzey Yapısı

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ABSTRACT

The seven-spotted ladybug, *Coccinella septempunctata* (Coleoptera: Coccinellidae) L., is a polyphagous predatory species that feeds on many species of aphids and some soft-bodied insects in both larval and adult stages. For this reason, *C. septempunctata* L. (Coccinellidae) is very important in biological control. In this study, the egg structure and first larval period of *C. septempunctata* were examined using stereomicroscopy and scanning electron microscopy. *C. septempunctata* eggs are clear and oval, appearing elliptical dorsally and ventrally and slightly rounded at both ends. The first larvae that emerge from *C. septempunctata* eggs are dark brown and have 3 pairs of legs. The abdomen consists of 8 segments. In SEM photographs, star-shaped structures were observed on both sides of the strip on the dorsal surface of the thorax and abdomen of the larvae. Large stigma openings were found at equal intervals on the lateral of abdomen. The egg and larval structure of the invasive species *C. septempunctata* is described in detail.

Keywords: Eggs, Larvae, Stereomicroscope, Scanning electron microscope

Öz

Yedi noktalı uğur böceği, *Coccinella septempunctata* (Coleoptera takımı: Coccinellidae) L., hem larval hem de ergin dönemlerinde çok sayıda yaprak biti türü ve bazı yumuşak gövdeli böceklerle beslenen polifag bir predatör türdür. Bu nedenle *C. septempunctata* L. (Coccinellidae) biyolojik mücadelede oldukça önemlidir. Bu çalışmada, *C. septempunctata*'nın yumurta yapısı ve birinci larval dönemi stereomikroskop ve taramalı elektron mikroskobu kullanılarak incelenmiştir. *C. septempunctata* yumurtaları şeffaf ve ovaldir, dorsal ve ventral olarak eliptik ve her iki uçta hafif yuvarlaktır. *C. septempunctata* yumurtalarından çıkan ilk larvalar koyu kahverengi renktedir ve 3 çift bacağı sahiptir. Abdomen 8 segmentten oluşmaktadır. SEM fotoğraflarında larvaların toraks ve abdomeninin dorsal yüzeyinde şeridin her iki yanında yıldız şeklinde yapılar gözlenmiştir. Abdomenin lateralinde eşit aralıklarla büyük stigma açıklıkları bulunmuştur. İstilacı tür *C. septempunctata*'nın yumurta ve larval yapısı detaylı olarak tanımlanmıştır.

Anahtar Kelimeler: Yumurtalar, Larvalar, Stereomikroskop, Taramalı elektron mikroskobu

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Introduction

Coccinellids, members of the order Coleoptera, are well-known insects due to their bright colours and the fact that they are predators of some harmful insects. They are also popularly known as ladybirds and bridal bugs. Most ladybird beetle species are carnivorous. Their larvae and adults are natural enemies of many aphids and other pests (Chinery, 1993).

Coccinellids represent about 80% of the predators found in wheat fields and *C. septempunctata* (Coleoptera: Coccinellidae) L. accounts for 60% of the 3 existing coccinellid species (Özder & Sağlam, 1999). The seven-spotted ladybird beetle *C. septempunctata* L. is a polyphagous predator species that feeds on many aphid species and some soft-bodied insects in both larval and adult stages (Hodek, 1996; Agus et al., 2013). *C. septempunctata* L. (Coccinellidae) is an important biological control agent. This is because it preys on more than 20 aphid species of Coccoidea, as well as both Psylloidea and Tetranychidae species that infest crops in the area. It can be found in fields, greenhouses, and a wide variety of agricultural and/or natural habitats or crops all over the world (Hodek et al., 2012; Singh et al., 2004).

It is important to know the biology and characteristics of natural enemies to initiate a successful biological control program (Skouras & Stathas, 2015). It is also aimed to contribute to future larval and egg morphology studies. Larval studies on *C. septempunctata* have been found before. However, there is no detailed information about larvae and eggs. In this study, we examined the larvae and eggs of *C. septempunctata* using light and scanning electron microscopy.

Methods

Insect and stereomicroscope (SM)

Adult specimens of *C. septempunctata* were collected in May 2021 from an agricultural field in Ankara, Turkey. The development of eggs laid by adult females in a laboratory environment was followed and the hatched larvae were photographed using Olympus SZX7 stereomicroscope (SM).

Scanning electron microscope (SEM)

For SEM examinations, they were first washed with sodium phosphate buffered (pH 7.2) in 2.5% glutaraldehyde. They were then dehydrated in an increasing series of ethanol (from 50% to 99%). Subsequently, the samples were transferred to hexamethyldisilazane (HMDS) and air-dried. Finally, samples mounted on SEM stubs with double-sided tape were coated with a thin layer of gold in a Polaron SC 502 sputter coater. Observations were made and photographed using a JEOL JEM 6060 LV SEM.

Results and Discussion

Egg

C. septempunctata eggs are transparent and oval, appear elliptical dorsally and ventrally, and are slightly rounded at both ends (Figure 1). *C. septempunctata* eggs are approximately 0.6 mm wide and 1.20 mm long. The chorion thickness of the eggs examined under stereomicroscope is thin and the larvae inside the egg are visible (Figure 1).



Figure 1.
General view of *C. septempunctata* egg (SM).



Figure 2.
Hatching *C. septempunctata* larvae (SM).

The hatching larvae are visible and the eggshell is transparent (Figure 2). The chorion then appears as a transparent white empty shell after hatching (Mahyoub et al., 2013).

At the base of the egg structure examined by scanning electron microscopy, an adhesive surface was found to stabilize the egg. The adhesive surface is circular and has polygonal patterns (Figure 3). The egg opening line was photographed (Figure 4). When the outer surface of the egg was examined, micropylar processes were found in groups in certain places in the form of protrusions (Figures 5-6).

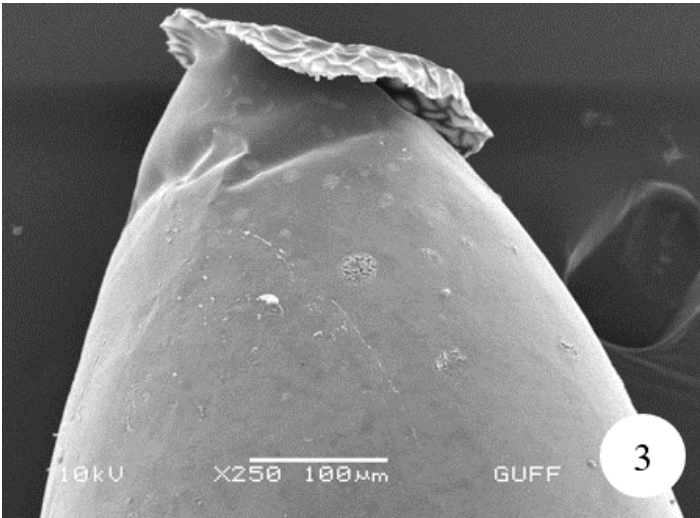


Figure 3.
C. septempunctata egg general view and adhesive surface (SEM)

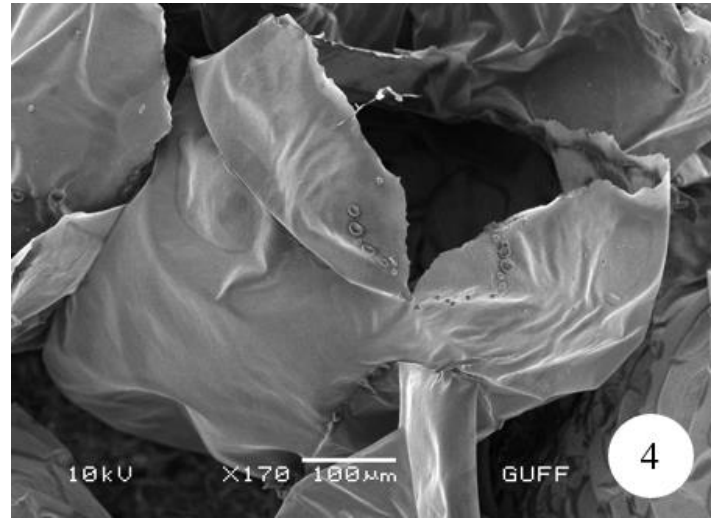


Figure 4.
General view of the egg opening line (SEM).

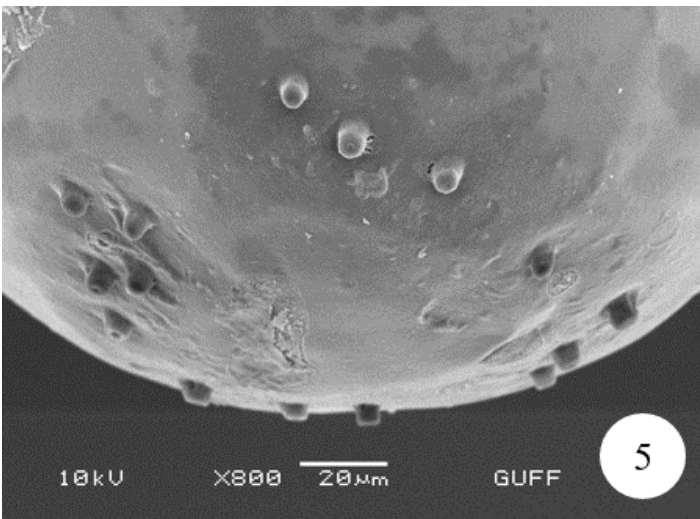


Figure 5.
General view of micropyle on the egg surface (SEM).

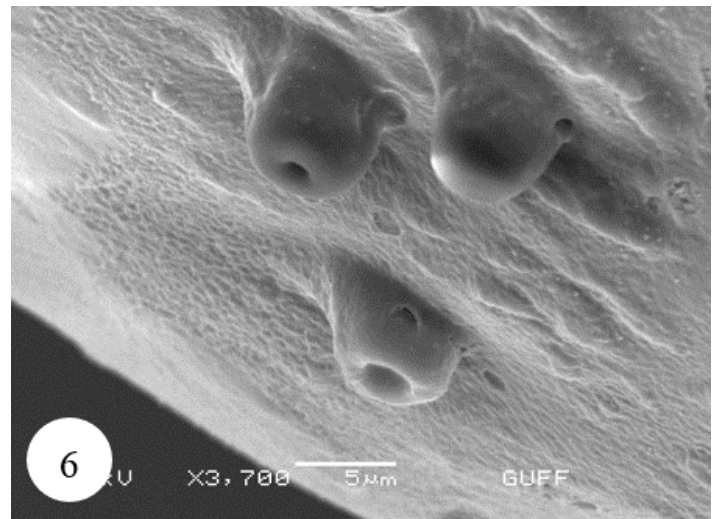


Figure 6.
Detailed view of micropyle on the egg surface (SEM).

Egg color and size differ in different families belonging to Coleoptera orders and are of systematic importance. *C. septempunctata* eggs are transparent and oval. *Adalia decempunctata* (Coccinellidae) is elongated oval and yellow (Uygun, 1981). *Harmonia axyridis* (Coccinellidae) eggs are yellow and oval (El-Sebaey & El-Gantiry, 1999). *Gabrius osseticus* (Staphylinidae) eggs are whitish and light brownish in color and oval (Staniec & Pietrykowska-Tudruj, 2007). *Tasgius melanarius* (Staphylinidae) eggs are light brown and have a large oval shape (Staniec & Pietrykowska-Tudruj, 2007). *Philonthus punctus* (Staphylinidae) eggs are white and oval (Pietrykowska-Truduj & Staniec, 2006). Similarly, eggs of some species have been reported to be white and oval. For example: *Bisnius nitidulus* (Staphylinidae), *Gabrius splendidulus* (Staphylinidae), *Gabrius trossulus* (Staphylinidae), *Neobisnius villosulus* (Staphylinidae), *Philonthus alpinus* (Staphylinidae) eggs are also white, *Philonthus atratus* (Staphylinidae), The eggs of *Philonthus corvinus* (Staphylinidae), *Philonthus lepidus* (Staphylinidae), *Philonthus micans* (Staphylinidae),

Heterothops niger (Staphylinidae) are oval and white (Staniec & Pietrykowska-Tudruj, 2007).

C. septempunctata eggs are about 0.6 mm wide and about 1.20 mm long. The eggs of *H. axyridis* are about 1.2 mm long (El-Sebaey & El-Gantiry, 1999). The eggs of *P. punctus* (Staphylinidae) are about 1.57 mm long (Pietrykowska-Truduj & Staniec, 2006). *N. villosulus* (Staphylinidae) eggs are 0.66-0.82 mm long. *B. nitidulus* (Staphylinidae) eggs are 0.92-1.08 mm long. *P. alpinus* (Staphylinidae) eggs are 1.00-1.20 mm long. *P. micans* (Staphylinidae) eggs are 1.05-1.19 mm long. *P. corvinus* (Staphylinidae) eggs are 1.08-1.22 mm long. *G. osseticus* (Staphylinidae), 1.12-1.29 mm in length. *P. lepidus* (Staphylinidae) eggs are 1.22-1.43 mm long. *H. niger* (Staphylinidae) eggs are 1.35-1.62 mm long. *P. atratus* (Staphylinidae) eggs are 1.54-1.89 mm long. *G. splendidulus* (Staphylinidae) eggs are 1.62-1.78 mm long. *G. trossulus* (Staphylinidae) eggs are 1.62-1.81 mm long. *T. melanarius* (Staphylinidae) eggs are 2.56-2.91 mm long (Staniec & Pietrykowska-Tudruj, 2007).



Figure 7.
Dorsal and ventral general view of *C. septempunctata* first larval stage.

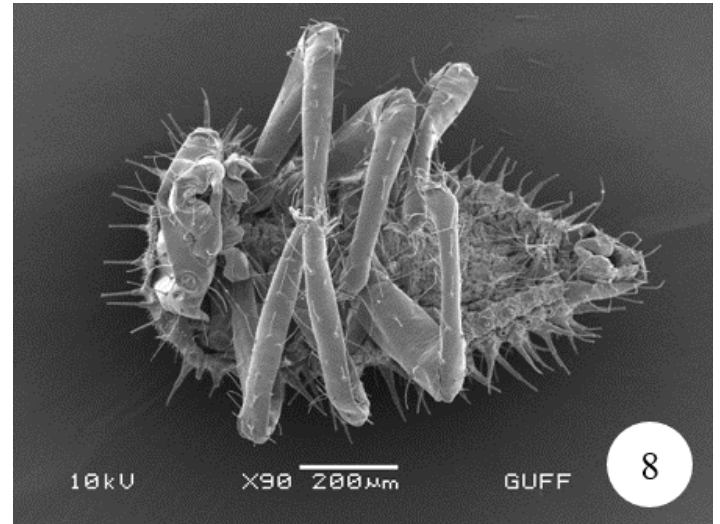


Figure 8.
Ventral general view of *C. septempunctata* first larval stage.

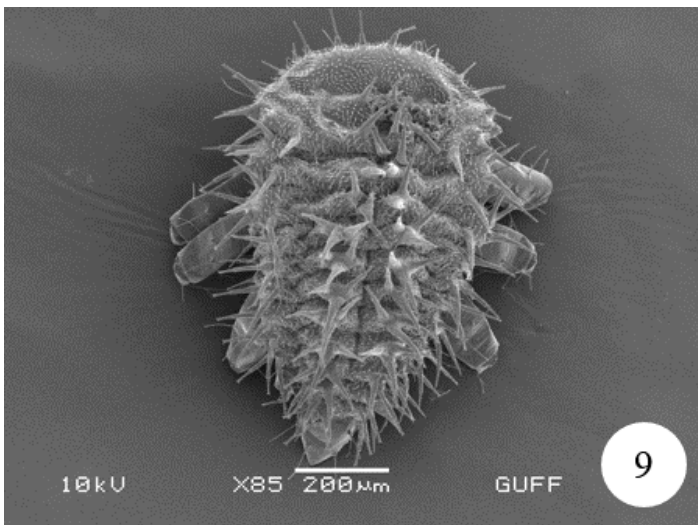


Figure 9.
Dorsal general view of *C. septempunctata* first larval stage.

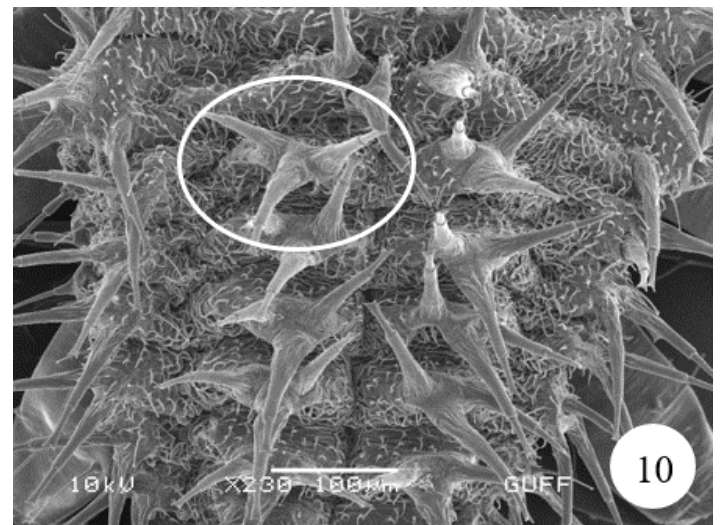


Figure 10.
Star-shaped appendages on the larvae.

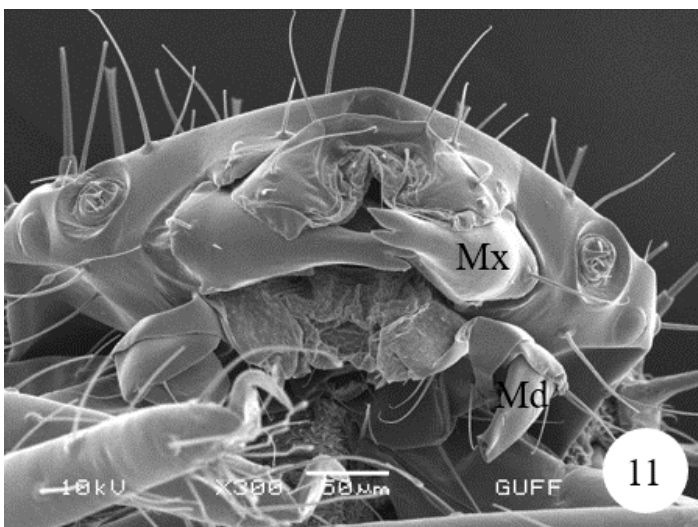


Figure 11.
General view of larval mouth structure (Mx: Maxilla, Md: Mandibular).

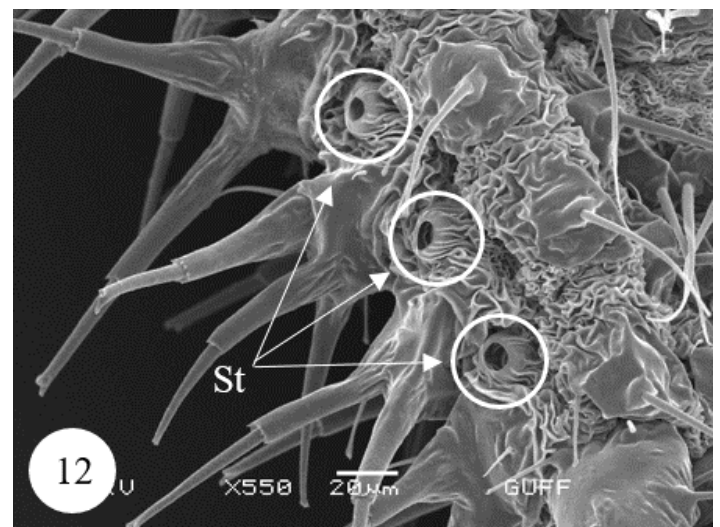


Figure 12.
Stigma openings on the lateral of abdomen of the larvae (St: Stigma).

Larvae

The larvae of *C. septempunctata* pass through four instars (Mahyoub et al., 2013). *H. axyridis* (Coccinellidae) has four larval stages, prepupa, pupa, and adult (Hodek, 1973). But, the larvae of *P. punctus* (Staphylinidae) were reported to undergo three larval stages (Pietrykowska-Truduj & Staniec, 2006).

The larvae of *C. septempunctata* range in size from 1 mm to 4-7 mm and complete their development between 10 and 30 days, depending on the quantity and quality of food (Katsarou et al., 2005; Cantrell, 2011).

The first instar larvae to hatch from *C. septempunctata* eggs are dark brown with 3 pairs of legs (Figures 7-8). In SEM photographs, thin, long spiny structures were observed on the leg segments (Figure 8). The abdomen has 8 segments (Figure 7). On the body's dorsal side, an orangish band extends from the back of the head to the end of the abdomen in the midline (Figure 7). In SEM photographs, star-shaped appendages were observed on both sides of the stripe on the dorsal surface of the thorax and abdomen of the larvae (Figure 9-10). Each of the stellate appendages has two segments. Thin, long spines of different lengths were found on the head. Thin, short spines were found on the dorsal surface of the body from the head. Mouthparts (maxilla, mandible) are prominent (Figure 11). Large stigma openings were found at equal intervals lateral to the abdomen (Figure 12).

The larvae of *A. decempunctata* (Coccinellidae) are pale gray. Fourth instar larvae have three yellow spots forming a triangle dorsal to the first and fourth abdominal segments. There are pale-colored tubercles with fine hairs on the dorsal side of the fifth to eighth segments of the abdomen (Anonim, 2022).

We observed that the abdominal segments of *C. septempunctata* consist of eight segments. Similarly, it was reported that the abdomens of *P. punctus* (Staphylinidae) larvae consist of eight segments (Pietrykowska-Truduj & Staniec, 2006). In *Hypera arator* (Curculionidae) (Linné, 1758), *Hypera denominanda* (Capiomont, 1868), *Hypera jucunda* (Capiomont, 1868), *Hypera nigrirostris* (Fabricius, 1775), *Hypera plantaginis* (De Geer, 1775), *Hypera suspiciosa* (Herbst, 1795), *Hypera venusta* (Fabricius, 1781) and *Hypera viciae* (Gyllenhal, 1813), abdominal segments were reported to consist of eight segments (Skuhrovec, 2004).

The larval and egg structure of *C. septempunctata* an invasive species, was described in detail, and its similarities and differences with other species were determined and contributed to taxonomic studies. It is also aimed to contribute to biological control efforts against this species.

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Yazar Katkıları: Konsept - NÖK, HA, SC; Tasarım - NÖK, HA, SC; Denetim - NÖK, HA, SC; Kaynaklar - NÖK, HA, SC; Malzemeler - NÖK, HA, SC; Veri Toplama ve/veya İşleme - NÖK, HA, SC; Analiz ve/veya Yorum - NÖK, HA, SC; Literatür Taraması - NÖK, HA, SC; Yazma - NÖK, HA, SC; Eleştirel İnceleme - NÖK, HA, SC; Diğer – NÖK, HA, SC

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