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DESCRIPTION OF SURFACE STRUCTURE OF EGGS AND FIRST INSTAR LARVAE OF *AGAPANTHIA OSMANLIS* REICHE & SULCY (COLEOPTERA: CERAMBYCIDAE): BASED ON SCANNING ELECTRON MICROSCOPY

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Abstract

Morphology of eggs and first instar larvae of *Agapanthia osmanlis* Reiche & Saulcy, 1858 (Coleoptera: Cerambycidae) are described for the first time, with illustrations of the structural features obtained using light and scanning electron microscopy (SEM). *A. osmanlis* feeds only on hosts in the Dipsacaceae family and collected on *Cephalaria procera* Fish & Lall. (Dipsacaceae) from Bayburt (Kop Mountain Pass, Turkey). The female lays single eggs inside the stalks of *C. procera*, where the larva feeds internally. The egg is cylindrical, white, dorsally wide, narrower toward the front and back, and rounded anteriorly and posteriorly. The egg is 3.10-3.80 (3.35) mm long and 0.45-0.85 (0.58) mm wide. Under the dissection microscope, the chorion appears to be smooth, but in scanning electron microscope (SEM) magnifications, the chorion is covered with chorionic spines except for the anterior micropylar region. Each egg possesses a knob-like micropylar process at one end. Newly emerged larvae of *A. osmanlis* are cylindrical, C-shaped after leaving their galleries, whitish-grey, and legless.

KEY WORDS: *Agapanthia osmanlis,* Cerambycidae, *Cephalaria procera*, egg morphology, Türkiye

Introduction

The family Cerambycidae encompasses a vast and diverse array of beetles, with over 36,000 species identified worldwide. Adult cerambycids exhibit a wide range of body lengths, varying from as short as 1.5 mm, such as in the Caribbean twig-boring Lamiinae *Decarthria stephensi* Hope (Villiers, 1980; Peck 2011), to as long as 167 mm in the Prioninae species *Titanus giganteus* (L.) (Bleuzen, 1994). Nearly all cerambycids are phytophagous, feeding on plants during their adult stage and as larvae (Haack *et al.*, 2017).

Agapanthia osmanlis Reiche & Saulcy, 1858, a species of longhorn beetle belonging to the Cerambycidae family, is known from Bulgaria, Greece, Hungary, Montenegro, Romania, Serbia, Slovakia, and Turkey (Danilevsky, 2020). Limited information is available regarding the biology and host plants of this species. The preferred host of *A. osmanlis* is *Cephalaria procera* Fisch & Lall., which belongs to the Dipsacaceae family and is distributed in northern Iran, Armenia, and Turkey (Davis, 1972; Özdikmen, 2011). In Turkish, *C. procera* is referred to as "Gevrek." It is a perennial weed with deep roots, which poses challenges for land cultivation and farming. The feeding activity of the beetles significantly slows down the growth of this plant. Given its potential impact on controlling the growth of *C. procera*, *A. osmanlis* could be considered a potential biological control agent (Tozlu, 2010). Therefore, it is crucial to establish a comprehensive understanding of the egg and larval structure of the beetle, as well as its biology.

Extensive studies have highlighted the taxonomic and phylogenetic importance of the eggshell structure in pterygote insects, encompassing diverse orders and taxonomic levels (Hinton, 1981; Salkeld, 1983, 1984; Margaritis, 1985). The pattern and sculpturing of the egg surface serve as valuable taxonomic features, particularly in cases where adult specimens are not readily available for identification. The egg surface structure and larval stage of Coleoptera species, including Cerambycidae, have been reported by many authors (Lawson, 1976; Ellsbury & Baker, 1988; Hernandez 1990, 1991, Rector *et al.*, 2006, Rowley & Peters, 1972; Luff, 1981; Donaldson & Tonder, 1992; Arbogast & Byrd, 1982; Hu & Frank, 1995; Baker & Ma, 1987; Morell, *et al.*, 2002, 2005; Caron *et al.*, 2004; Kucerova & Stejskal, 2002), however, accurate knowledge of the egg morphology and larva is still lacking in many taxonomic groups.

The egg morphology and first instar larvae of *A. osmanlis* have been examined for the first time by scanning electron microscope (SEM). The samples are preserved at Atatürk (Faculty of Agriculture, Department of Plant Protection, Insect Systematics Laboratory) and Gazi (Prof. Dr. Zekiye Suludere, Electron Microscopy Center) Universities.

Materials and Methods

The beetles were collected from the Kop Mountain pass of Bayburt province in Türkiye, at an elevation of 1,750-2,000 m. The female beetles lay individual eggs inside the stalks of *C. procera*, from which fresh eggs and larvae were obtained. The hatched eggs and first instar larvae were preserved in 70% ethanol for subsequent scanning electron microscopy analysis. Initial observations and examinations were conducted using an Olympus SZX7 Stereomicroscope, and photographs were captured using an Olympus Digital Camera mounted on the microscope.

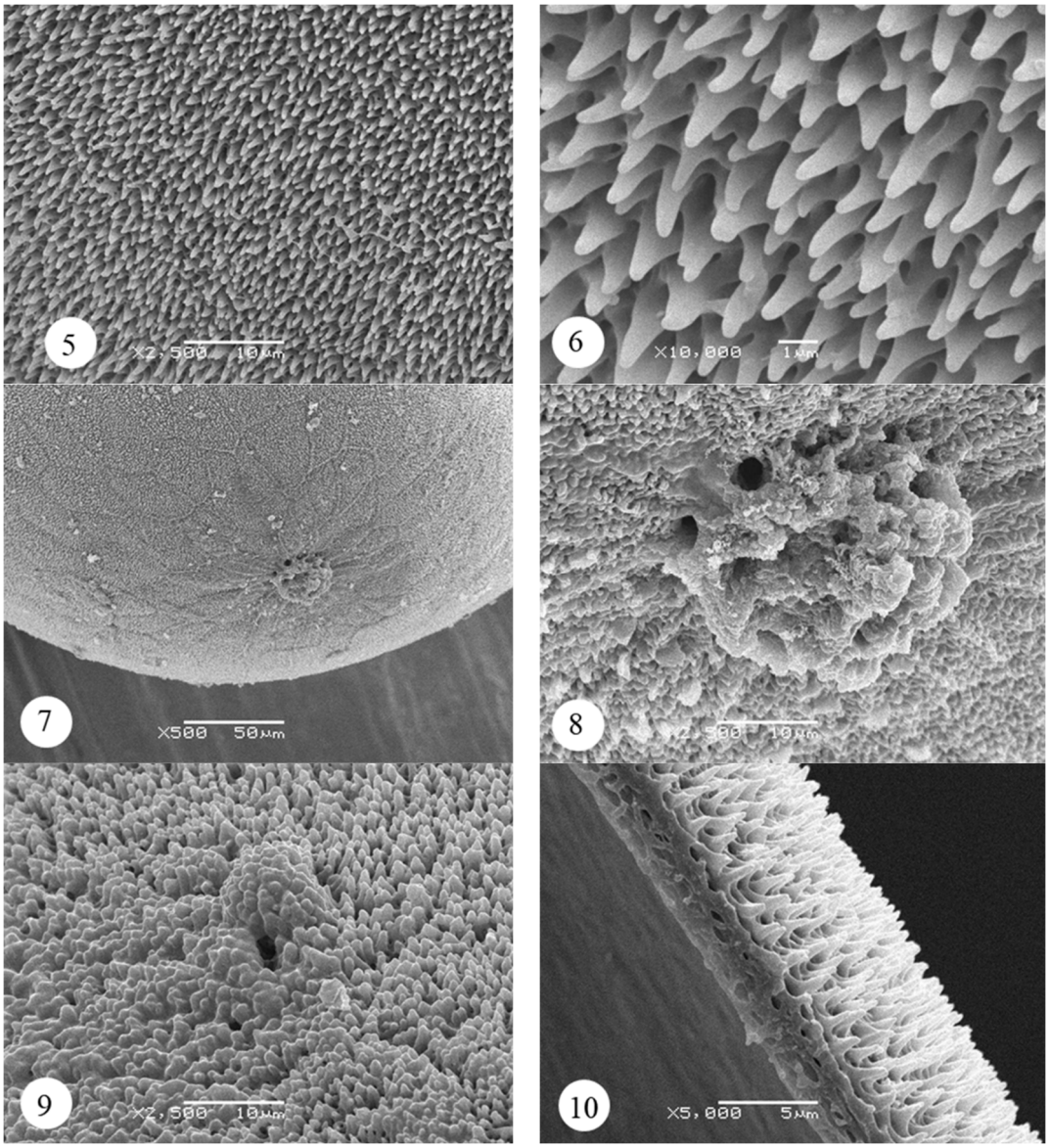
The specimens of eggs and larvae were first cleaned and air-dried to prepare them for examination under SEM. Subsequently, they were securely mounted onto SEM stubs using double-sided tape. A thin layer of gold was deposited on the specimens using a Polaron SC502 Sputter Coater to enhance their conductivity and imaging quality. The prepared samples were then examined using a JEOL JSM 6060 LV SEM, operating at 10 and 15 kV. Measurements were conducted on ten selected specimens, including eggs (length 3.10-3.80 (3.35) mm, width 0.45-0.85 (0.58) mm) and first instar larvae (3.90-6.10 (4.96) mm).

Results and Discussion

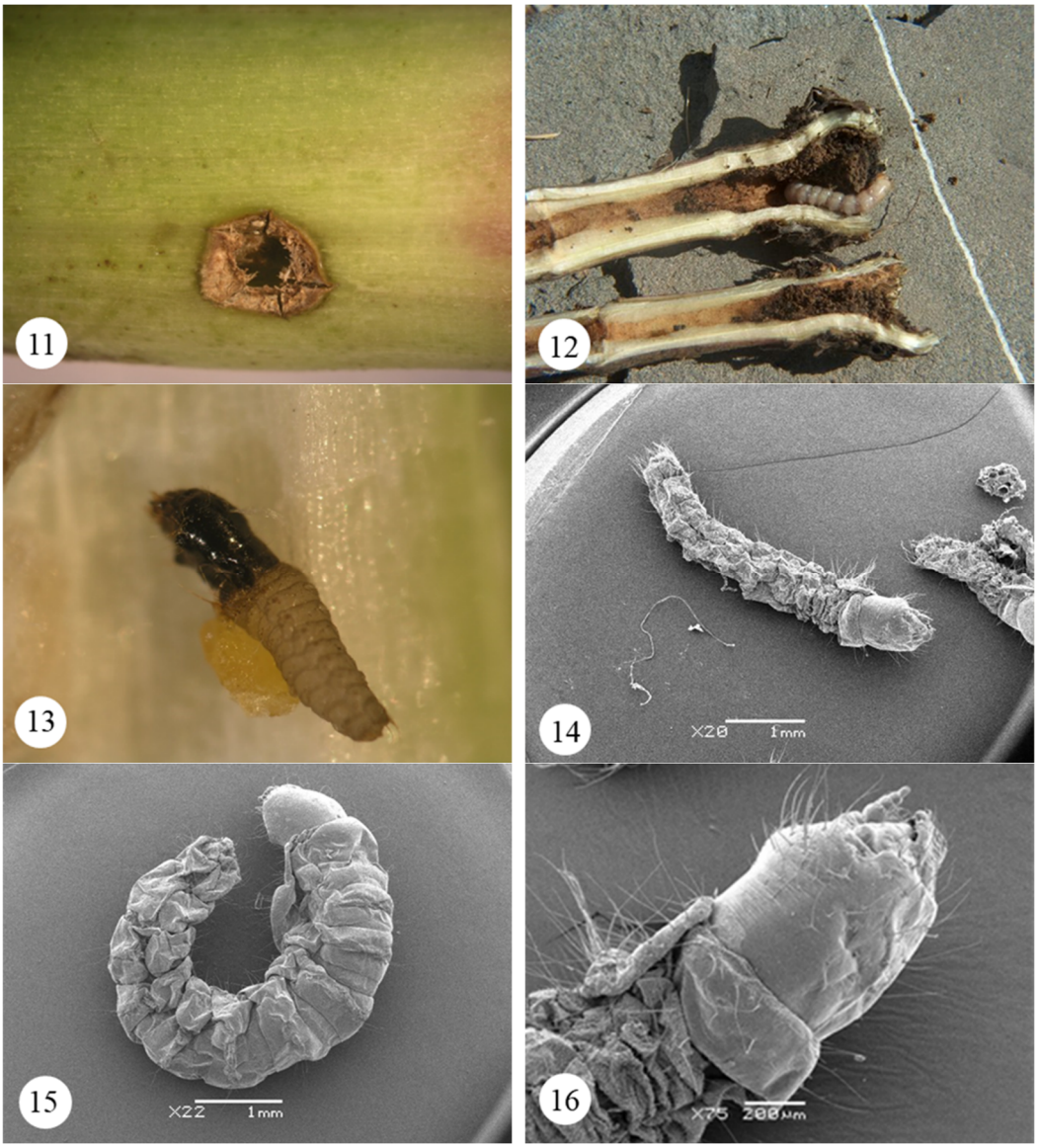
Description of *A. osmanlis* eggs

A. osmanlis adults drill into stalks of *C. procera* (Fig. 1), and eggs are laid singly. The eggs are always located on the inner wall of the *C. procera* stalks (Figs. 2-3), where the larvae feed internally. Female *A. osmanlis* beetles lay their eggs parallel to the stem, in the stem base (Figs. 2-3). The eggs are cylindrical, wider dorsally and narrower towards the front and back, with rounded anterior and posterior ends (Fig. 4). Initially, the eggs have a creamy white color, but as they mature and approach hatching, they gradually turn yellowish-brown. Typically, around 2-8 eggs (with an average of 6) are deposited in the section of the plant that is approximately 35-50 cm long. The eggs measure about 3.10-3.80 mm (average 3.35 mm) in length and 0.45-0.85 mm (average 0.58 mm) in width. Under the dissecting microscope, the chorion appears to be smooth and dull (Figs. 2-4), but under high SEM magnifications, the egg surface is covered with dense, sharp chorionic spines except for the anterior micropylar region. Chorionic spines extend from an egg in an upright position and are connected (Figs. 5-6). Each egg possesses a knob-like micropylar process at one end (Figs. 7-8). The dome-shaped micropylar region of the eggshell, referred to as the anterior end of the egg, is coarsely textured and sponge-shaped. The basal periphery of the micropylar knob has 10-12 holes. The micropylar region is surrounded by petal-shaped primary cells. These primary cells are surrounded by a series of polygonal-shaped cells (Figs. 7-8). There is an opening at the opposite end of the micropylar process. (Fig. 9). The chorion of *A. osmanlis* is composed of two layers, the endochorion and the exochorion (Fig.10).

Figures 1-4. 1-Ovipositional perforation (Arrow) on the stalk of *C. procera*. 2-3. The egg lay singly per dissected inside of the stalk of *C. procera.* 4. SEM photo of the egg of *A. osmanlis*.



Figures 5-10. SEM photos of the egg of *A. osmanlis*. 5-6. Chorion covered with dense, sharp chorionic spines. 7-8. Knob-like micropylar process at one end of the egg. 9. Opening at the opposite end of the micropylar process. 10. SEM photo of the section of egg chorions of *A. osmanlis*

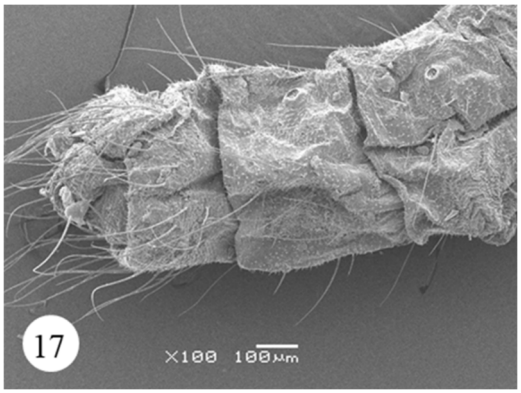


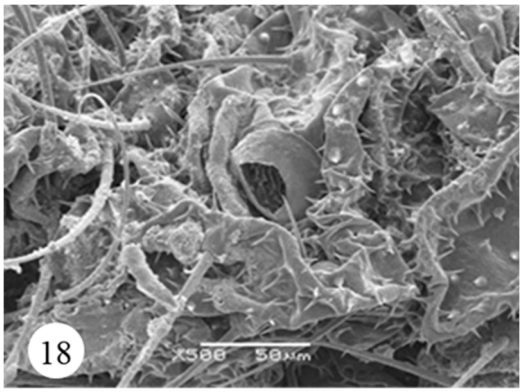
Figures 11-16. 11. Hole on stem *C. procera*. 12. Hibernating mature larva near the stem base of *C. procera.* 13-15. First instar larvae, general view. 16. Head of larva.

Description of first instar larva of *A. osmanlis*

The early-stage larvae of *A. osmanlis* construct galleries within the stem of their host plant that can extend up to 35-40 cm in length, depending on the height of the plant (Fig. 11). As mature larvae, *A. osmanlis* hibernate within the stems of *C. procera* near the base of the stem (Figure 12). Under normal ecological conditions, *A. osmanlis* completes one generation per year. The larval body is cylindrical and takes on a C-shaped form after

emerging from the galleries. The larvae appear legless, light brown in color, with the area touching the thorax slightly darker (Figs. 13-15). The anterior end of the larvae is somewhat prolonged and oval-shaped. The labrum, maxilla, and labium are white, and the mandible is black. The thoracic segments are wider than the head and abdomen. The prothorax has a brown spot divided by a white stripe dorsally and two very light spots ventrally. Long and strong setae are arranged between the prosternum and metasternum, while the ventrolateral parts of other segments have sparser setae. The abdomen has a blunt apex and is surrounded by a ring of dense and strong setae in a crown-like pattern (Figs. 14-16). The first instar larva is 3.90-6.10 mm (average 4.96 mm) in length, and the mature larva reaches a length of 22.50-25.00 mm (average 23.60 mm). The total duration of the larval stage lasts approximately 8-12 days (Tozlu, 2010).





Figures 17-18. Setae and last abdominal segments of larva 18. Stigma opening on abdominal segments.

Cerambycids lay their eggs on, in, or near the host plants where their larvae will develop (Trägårdh, 1930; Butovitsch, 1939; Duffy, 1953; Linsley, 1959, 1961). The oviposition behaviors exhibited by cerambycids vary significantly among species. For instance, in many Lepturinae and Prioninae, females insert their ovipositor into the substrate during egg laying, which often consists of soft, partially decayed wood or soil near the base of the larval host plant. Some cerambycids deposit their eggs on the outer surface of their host plants (e.g., certain Cerambycinae and Spondylidae), while most cerambycids, excluding Lamiinae, infest woody plants by laying their eggs beneath bark scales or in bark crevices (Linsley, 1959; Haack *et al.*, 2017).

Certain subfamilies in the Cerambycidae family display specific oviposition behaviors, laying their eggs in pre-existing galleries such as entrance holes, exit holes, and other structures created by bark- and wood-boring insects (Linsley, 1959). A notable example is the Lepturinae species *Anthophylax attenuatus* (Haldeman), as observed in a study by Youngs (1897), which oviposited in galleries of the ptinid beetle *Ptilinus ruficornis* Say by inserting their ovipositor into the exit holes. Similarly, some members of the Lamiinae genus *Acanthocinus* exhibit a unique oviposition strategy by constructing pits directly over entrance holes and ventilation holes created by bark beetles belonging to the Scolytinae subfamily. These ventilation holes are openings formed by bark beetles along their egg galleries, extending into the outer bark (Linsley, 1959).

Agapanthia osmanlis, commonly known as a stalk borer cerambycid, exclusively feeds on hosts belonging to the Dipsacaceae family (Kovaks, 1998; Rejzek *et al.*, 2003). This species has been found in Bulgaria and Turkey on *Dipsacus* ssp. During spring and summer, adult *A. osmanlis* are active and feed on the foliage of flowering plants (Rector *et al.*, 2006). Their distribution ranges across Bulgaria, Greece, Hungary, Montenegro, Romania, Serbia, Slovakia, and Turkey (Danilevsky, 2020). In Turkey, *A. osmanlis* has been documented in

various locations (Rejzek *et al.*, 2001; Tozlu *et al.*, 2003; Rapuzzi & Sama, 2012; Sama *et al.*, 2012; Özdikmen, 2013; Tatar & Tozlu, 2023). As mature larvae, *A. osmanlis* hibernate in the stems of *Cirsium procera* near the stem base. Upon emergence, the adults feed on the leaves of *C. procera*, starting from the leaf edges and moving toward the center (Tozlu, 2010).

A literature review reveals that Cerambycid eggs generally have an elongated shape, often being more than twice as long as they are wide (Butovitsch, 1939). For instance, *Molorchus minor* (L.) eggs have an average length of 1.1 mm and a width of 0.3 mm at their widest point. Similarly, the eggs of the Parandrini *Neandra* (= *Parandra*) *brunnea* measure around 1.5 mm in length and 0.5 mm in width. In the Prioninae subfamily, the eggs of *P. reticularis* are approximately 2.4 mm long and 0.4 mm wide, while those of *Ergates spiculatus* (LeConte) measure 3.1 mm in length and 1.5 mm in width. The eggs of *Prionus coriarius* (L.) in the Prioninae subfamily are about 4.1 mm long and 0.5 mm wide (Duffy, 1953; Rogers *et al.*, 2002). On the other hand, the eggs of *A. osmanlis* have a cylindrical shape, appearing white in color and wider dorsally, gradually narrowing towards the front and back, with rounded anterior and posterior ends. They measure approximately 3.10-3.80 mm in length and 0.45-0.85 mm in width (average 3.35 mm in length and 0.58 mm in width) (Tozlu, 2010).

Usually, cerambycid eggs are laid individually, although they may occasionally be found in small clusters. For instance, Walsh & Linit (1985) investigated 652 oviposition pits created by the Lamiinae species *Monochamus carolinensis* and discovered that out of those pits, 89 were empty, 559 contained a single egg, and 4 contained two eggs. Similarly, the branch-borer, cerambycine species *Osphranteria coerulescens* Redtenbacher predominantly lays eggs individually but occasionally in groups of two to three (Shari *et al.*, 1970). In contrast, *Phoracantha semipunctata* (F.), a member of the Cerambycinae subfamily, generally deposits eggs in groups of 3 to 30 under the bark (Scriven *et al.* 1986).

According to various studies (Butovitsch, 1939; Duffy, 1953; Linsley, 1961), it is commonly observed that cerambycid eggs typically hatch within a few days or sometimes take up to four to five weeks, the average being two weeks. When the larvae emerge from the eggs (eclosion), they break the egg chorion either by utilizing specialized spines located on their head, thorax, or abdomen (referred to as egg burster spines) or by using their mandibles (Linsley, 1961; Gardiner, 1966). Once hatched, most cerambycid larvae burrow into the host plant's tissues or the soil, where they undergo feeding and development for several months to years. Due to their lengthy life cycle and the extensive feeding within the host plant, the larval stage of cerambycids is considered the most destructive phase for the host plant.

Cerambycid larvae typically have an elongated and cylindrical body shape, with extended mouthparts and underdeveloped thoracic legs. After emerging from their galleries, *A. osmanlis* larvae exhibit a whitish-grey, legless form that is cylindrical and C-shaped. The head and abdomen are oval-shaped, with a slightly darker area near the thorax. The labrum, maxilla, and labium appear white, while the mandible is black. The thoracic segments are wider than the head and abdomen. The prothorax features a brown spot divided by a white stripe dorsally and two light spots ventrally. Long and sturdy setae are observed between the prosternum and metasternum, with sparser setae on the ventrolateral parts of other segments. The abdomen has a blunt apex with a ring of dense and strong setae arranged in a crown-like pattern. The body length of first-stage larvae ranges from 3.90 to 6.10 mm, with an average of 4.96 mm, while mature larvae measure between 22.50 and 25.00 mm, with an average of 23.60 mm (Tozlu, 2010).

Cerambycid larval galleries typically exhibit an oval cross-section with a winding pattern when they tunnel through the cambial region. However, when tunneling occurs in the wood, the galleries are usually round in cross-section and relatively straight (Craighead, 1923; Duffy, 1953). The frass produced by cerambycid larvae, especially when they feed on woody tissue, often consists of granular particles mixed with coarse shreds or fibrous fragments of the wood (Hay, 1968; Solomon, 1977). The granular portion of the frass is composed of

material that has passed through the larva's digestive tract, while the wood shreds are pieces that have been torn off by the larva's mouthparts but not consumed (Craighead, 1923; Solomon, 1977).

Typically, it takes one to three years for most cerambycids to complete a single generation. However, certain species of cerambycids can complete two generations per year, known as bivoltine species (Matsumoto *et al.*, 2000; Pershing & Linit, 1986; Watari *et al.*, 2002; Logarzo & Gandolfo, 2005). Most cerambycids undergo the larval stage during winter (Haack *et al.*, 2017).

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ОПИС ПОВРШИНСКЕ СТРУКТУРЕ ЈАЈА И ЛАРВЕ ПРВОГ СТУПЊА *AGAPANTHIA OSMANLIS* REICHE & SULCY (COLEOPTERA: CERAMBYCIDAE): ПОМОЋУ СКЕНИРАЈУЋЕ ЕЛЕКТРОНСКЕ МИКРОСКОПИЈЕ

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Извод

Морфологија јаја и ларве првог ступња *Agapanthia osmanlis* Reiche & Saulcy, 1858 (Coleoptera: Cerambycidae) први пут је описана уз илустрације структурних карактеристика добијених коришћењем светлосне и скенирајуће електронске микроскопије (SEM). *A. osmanlis* се храни само биљкама из породице Dipsacacae и налази се на *Cephalaria procera* Fish & Lall. (Dipsacacae) из Бајбурта (Коп планински пролаз, Турска). Женка полаже појединачна јаја унутар стабљика *C. procera*, где се ларва храни изнутра. Јаје је цилиндрично, бело, дорзално широко, са предње и задње стране уже, а антериорно и постериорно заобљено. Јаје је дуго 3,10-3,80 (3,35) mm и широко 0,45-0,85 (0,58) mm. Под светлосним микроскопом, чини се да је хорион гладак, али на увећањима скенирајућег електронског микроскопа (SEM), хорион је прекривен бодљама, изузев предњег микропиларног региона. Свако јаје на једном крају има микропиларни наставак. Након излегања, ларве *A. osmanlis* су цилиндричног тела облика слова C, беличастосиве боје и без ногу.

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