Acta Entomologica Serbica, 2024, 29(1): 53-62

UDC: 595.762.1:591.465.3
DOI: 10.5281/zenodo.11487034

NOTES RELATED TO IMMATURE OVARY OF THE FOREST CATERPILLAR HUNTER ADULT *CALOSOMA SYCOPHANTA* (LINNAEUS, 1758) (COLEOPTERA: CARABIDAE) – LIGHT AND ELECTRON MICROSCOPY STUDIES

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Abstract

Calosoma sycophanta (Linnaeus, 1758) (Coleoptera: Carabidae) is one of the most important predators of pine processionary moth, *Thaumetopoea pityocampa* (Denis & Schiffermüller) (Lepidoptera: Thaumetopoeidae) larvae and pupae. Therefore, it is an important species in biological control. Relatively few studies have focused on the female reproductive system in Carabidae. We use light and electron microscopy to study the female reproductive anatomy and histology of *C. sycophanta*. The female reproductive organs of *C. sycophanta* consist of paired ovaries, lateral oviducts, a common oviduct, and a genital chamber. Each ovary is formed of about 12 polytrophic meroistic ovarioles. In the longitudinal section of the ovary, it was noticed that the vitellarium region located after the germarium contains egg chambers that develop in a linear order, and it is distinguished that each chamber consists of an oocyte and nurse cells formed by incomplete cytokinesis from the same germ cell. The ovary connects to the lateral canal through a pedicel, and within each chamber, there's an oocyte alongside 16 food cells, all encircled by follicular cells. The epithelium of the pedicel and lateral oviduct is formed of monolayered cells overlaid by a muscle layer. The intima layer is distinguished on the side of the epithelium facing the lumen. Spines are seen in the intima. The purpose of this study is to describe ovary histoanatomy in *C. sycophanta* and compare it with other coleopteran species, including Carabidae.

KEY WORDS: Carabidae, ovary, polytrophic meroistic ovariole, histology, anatomy

Introduction

The order Coleoptera is examined in four suborders: Archostemata, Myxophaga, Adephaga, and Polyphaga (Lodos, 1995; Beutel & Leschen, 2005; Gullan & Cranston, 2010). Adephaga is the second and largest insect suborder with over 45,000 species and many predatory species (Beutel *et al.*, 2020 ; Duran & Gough, 2020). The species in Adephaga generally belong to the terrestrial family Carabidae (ground beetles, >35,000 extant

species) (Crowson, 1960). Most of the Carabidae family feeds as predators. In addition to predators, there are also omnivorous and occasionally phytophagous species. The majority of those fed as phytophagous cause economic losses. Due to the increase in biological control, great economic importance will also be given (Lodos, 1989; Hurka, 1996; Lövei & Sunderland, 1996).
This insect is known to feed on many forest pests, Lepidoptera, such as the pine tree pendant moth *Dendrolimus pini* L. (Lepidoptera: Lasiocampidae), moth *Lymantria monacha* L. (Lepidoptera: Lymantridae), pine processionary moth *Thaumetopoea pityocampa* Dennis and Schiffermüller (Lepidoptera: Thaumetopoeidae) and the oak processionary moth *Thaumetopoea processionae* L. (Lepidoptera: Thaumetopoeidae) (Escherich, 1942). This insect was introduced to the United States at the beginning of the last century to control of *Lymantria dispar* (Lepidoptera: Lymantridae) and is now an established predator. *C. sycophanta* L. (Coleoptera: Carabidae), which is a naturally occurring and important predatory insect in some regions of our country, is widely used in biological control against pine processionary moth (*T. pityocampa* or *T. willkinsoni*) (Lepidoptera: Thaumetopoeidae) (Serttaş & Çetin 2014).

Ovariole type of insects is an important distinguishing feature in order level. Histologically, there are two types of ovarioles in insects: panoistic and meroistic. In meroistic type ovarioles, oocytes are divided into two subtypes: telotrophic and polytrophic, depending on the location of the feeding cell mass (Bonhag, 1958). The polytrophic ovary type is the rule throughout the Adephaga group. In polytrophic ovaries, each follicle contains one oocyte and many trophocytes (Stein, 1847; Büning, 1994). Nurse cells are at the anterior end of the follicle, and oocytes at the posterior end. All together, they are surrounded by follicular epithelium. Nurse cells are connected to the oocyte and each other by cytoplasmic extensions (ring oviducts), thus forming a cystocyte complex. This type of ovariole is seen in Dermaptera, Lepidoptera, Diptera, Hymenoptera, and polyphagous Coleoptera. Except for Siphonaptera, polytrophic ovarioles are observed in most holometabolous insects (Wigglesworth, 1950; Bonhag, 1958; Chapman, 2004; Klowden, 2007).

Although the Carabidae family is very important economically, studies on the female reproductive system are very few. *Pterostichus melas italicus* (Carabidae) and *Apotomus rufus* (Carabidae), studied the female reproductive system (Vommaro *et al.*, 2022; Gomez *et al.*, 2023). Although comparative morphology of the reproductive system has been widely used in taxonomic and phylogenetic studies, the reproductive system of females has been little studied compared to males. The number of ovarioles and the morphology of the ovaries are of great importance to study the role of developmental processes (Church *et al.*, 2021). The purpose of this study is to describe the immature ovary of adult *C. sycophanta* (Carabidae), which is used in biological control and contributes to the description of the reproductive system of other coleopteran families, including Carabidae, and systematic studies.

Material and Methods

Specimens

We studied the immature ovary of adult *C. sycophanta* from Turkey. They prepared for light microscopy and scanning electron microscopy. The specimens of *C. sycophanta* were collected from *Pinus brutia* forest Kayadibi village, Bucak, Burdur region.

Light microscopy (LM)

For histological observations, Adult *C. sycophanta* were kept in ethyl acetate vapor in glass jars. The female reproductive system of *C. sycophanta* was dissected under a stereomicroscope (SM) in phosphate buffer (PB). The general structure of the extracted female reproductive system was photographed with a stereo-microscope

and then determined in formalin-fixing fluid for light microscopy. Samples were washed with tap water and dehydrated with an increasing series of ethyl alcohol and then embedded in paraffin blocks. Thin sections, approximately 5-7 µm thick, were taken from paraffin blocks with a microtome. The sections taken were stained with Hematoxylin-Eosin covered with Entellan and turned into a permanent preparation. Sections of the reproductive system were examined and photographed under an Olympus BX51 light microscope at different magnifications.

Scanning electron microscopy (SEM)

Fort he SEM observations, we dissected the female reproductive system from specimens. We rinsed the material in PB before cutting the organs. We fixed the organs onto 2,5% glutaraldehyde. We dehydrated them in ethanol prior to Hexamethyldisilazane (HMDS). Afterwards, the samples were dried in the air. We mounted the material on aluminium stubs and coated with gold. We examined the samples using a JEOL JSM 6060 LV.

Result and Discussion

In *C. sycophanta,* the female reproductive system consists of a pair of ovaries, a pair of lateral oviducts, and a common oviduct (Figs. 1A, 4A). Each ovary consists of 12 thin, long ovarioles (Fig. 1A). The ovariole numbers of each ovary varies interspecific (Jaglarz, 1998). In *C. sycophanta,* each ovary consists of 12 ovarioles. *Smicronyx fulvus* (Curculionidae), *Veturius sinuatus* (Eschscholtz) (Passalidae), *Rhynchophorus ferrugineus* (Oliver) (Curculionidae), *Rhyncophorus palmarum* (Curculionidae) and *Trypophloeus klimeschi* (Curculionidae), each ovary has two ovarioles (Korman & Oseto, 1989; El Naggar *et al.*, 2010; Wan *et al.*, 2018; Camargo Mathias *et al.*, 2011; Salazar *et al.*, 2017; Gao *et al.*, 2021). Each ovary of *A. rufus* (Carabidae) contains 3-4 ovarioles (Gomez *et al.*, 2023). Each ovary in *Eriopis connexa* (Coccinellidae) consists of 4 ovarioles (Maffei *et al.*, 2001). Each ovary of *P. melas* (Carabidae) consists of 6 ovarioles (Vommaro *et al.*, 2022). There are 14 ovarioles in each ovary of *Chrysomela populi* (Chrysomelidae) (Özyurt Koçakoğlu *et al.*, 2021). In *Chrysolina herbacea* (Duftschmid 1825) (Chrysomelidae), there are 18 ovarioles in per ovary (Özyurt Koçakoğlu *et al.*, 2022).

Ovarioles of *C. sycophanta* are tubular and elongate (Fig. 1A). Each ovary meets at its tip and is attached to the body wall by a suspensory ligament (Fig. 1B). In SM photographs, quite large tracheal networks are visible on the surface of the ovaries (Fig. 1A). Each ovary expands in the middle and narrows towards both ends (Fig. 1A). The ovaries are placed dorsolateral to the alimentary canal, enclosed in a peritoneal sheath. Each ovary is covered with a network of tracheoles (Fig. 1A). The peritoneal sheath has a mesh-shaped perforated appearance (Fig. 1C, D).

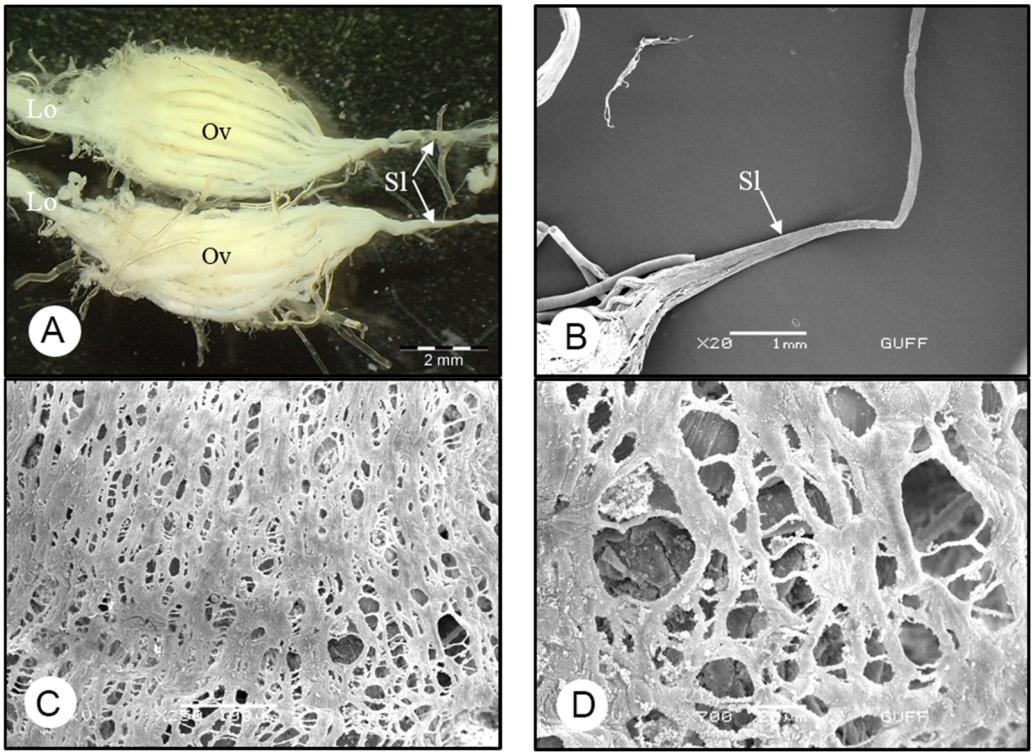

Figure 1. A) The general view of immature ovary in *C. sycophanta* (SM). B) Suspensory ligament extending from the ovary (SEM). C, D) SEM photograph of sheath surrounding the ovary. Lo-lateral oviduct, Ov-ovary, Sl-suspensory ligament.

In histological sections of *C. sycophanta*, it was determined that the ovariole was of polytrophic meroistic type (Fig. 2A, C, D). Polytrophic meroistic ovarioles have been found in Coleoptera Archostemata and Adephaga species (Stys & Bilinski, 1990; El Naggar *et al.*, 2010; Wan *et al.*, 2018; Vommaro *et al.*, 2022). However, Coleoptera polyphaga has telotrophic meroistic ovarioles (Korman & Oseto, 1989; Maffei *et al.*, 2001; Camargo Mathias *et al.*, 2011; Szklarzewicz *et al.*, 2014; Gao *et al.*, 2021; Özyurt Koçakoğlu *et al.*, 2021-2023). Polytrophic meroistic type ovarioles are also seen in some other orders (Diptera, Lepidoptera, Mecoptera, Psocodea etc.) (Parks & Larsen, 1965; Lee *et al.*, 1985; Yel & Eren, 2000; Żelazowska, 2005; Ma & Hua, 2010; Amir, 2013; Zhang *et al.*, 2017; Shirai *et al.*, 2023).

In the germarium of *C. sycophanta*, germ cells and young oocytes are distinguished (Fig. 2B). Mitotic divisions of germ cells occur in the germarium (Figs. 2A, B). In the longitudinal section of the ovary, it was noticed that the vitellarium region located after the germarium contains egg chambers that develop in a linear order, and it is distinguished that each chamber consists of an oocyte and nurse cells formed by incomplete cytokinesis from the same germ cell (Fig. 2A, C, D). Each chamber consists of an oocyte and 16 food cells and is surrounded by follicular cells (Fig. 2D). There are large round nuclei in the center of the nurse cells (Fig. 2D). The oocyte ooplasm and nurse cells appear basophilic. A germinal vesicle is around the ooplasm (Fig. 2C, D).

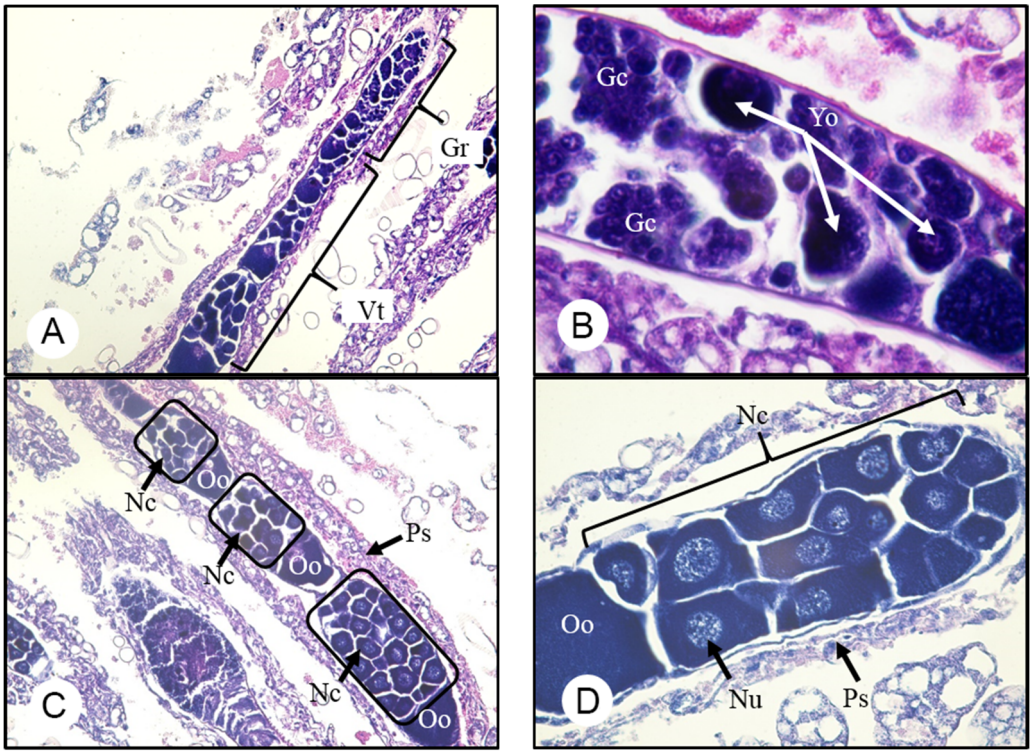

Figure 2. A) Longitudinal section of germarium and vitellarium (X100). B) Longitudinal section of germarium (X400). C, D) Nurse cell and oocyte in vitellarium (x200, x400). Gc-germ cell, Gr-germarium, Nc-nurse cell, Nu-nucleus, Oo-oocyte, Vt-vitellarium, Yo-young oocyte.

The pedicel is the last part of the ovary and has a thin, long oviduct-shaped structure (Fig. 3A, B). In Fig. 3B, the epithelial plaque structure removed from the pedicel is distinguished. In histological sections of the pedicel, it is seen that the single-layered cylindrical epithelial structure is surrounded by thick, striated muscles (Fig. 3C, D). The lumen is quite relatively. On the side of the pedicel facing the lumen, groups of finger-shaped spiny structures are distinguished (Fig. 3E, F). In *C. sycophanta*, numerous spines were observed in the intima of the pedicel and lateral oviduct. The common oviduct intima in *C. populi* has spines (Özyurt Koçakoğlu *et al.*, 2021). The vagina outer wall of *Longitarsus nigripennis* (Motschulsky 1866) (Chrysomelidae) has a chitinous spine (Devasahayam *et al.*, 1998). In *Chrysomela scripta* F., 1801 (Chrysomelidae), there are cteniform spines on the lateral oviduct intima (Cheetham, 1992).

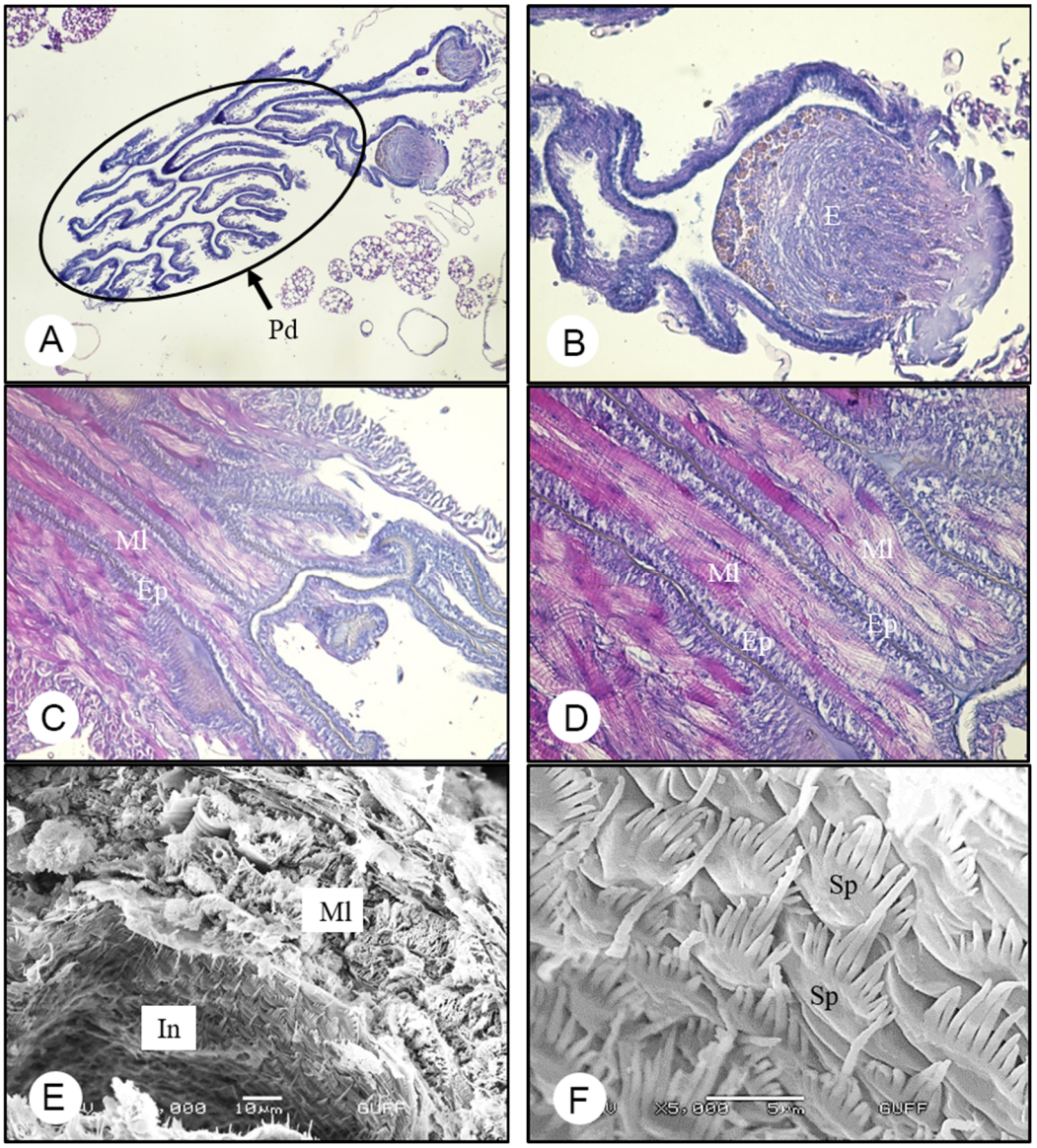

Figure 3. A-D) Histological section of pedicel (x40, 200, 200, 400). E, F) The spines extend from the pedicel intima (SEM). E-epithelial plaque, Ep-epithelium, In-intima, Ml-muscle layer, Pd-pedicel, Sp-spine.

In *C. sycophanta*, the pedicel connects to the common oviduct via a pair of lateral oviducts (Fig. 4A, B). In the SEM photo, it can be seen that the lateral oviducts are in the form of thin oviducts (Fig. 4B). Tracheas were found on its surface (Fig. 4C). In the histological section of the lateral oviduct, intima, single-layer epithelium and muscle layers are distinguished. Spines are seen in the intima (Fig. 4D). The spines extending from the intima in the pedicel and lateral oviduct help push the egg into the common oviduct. The muscles surrounding these oviducts are responsible for contraction.

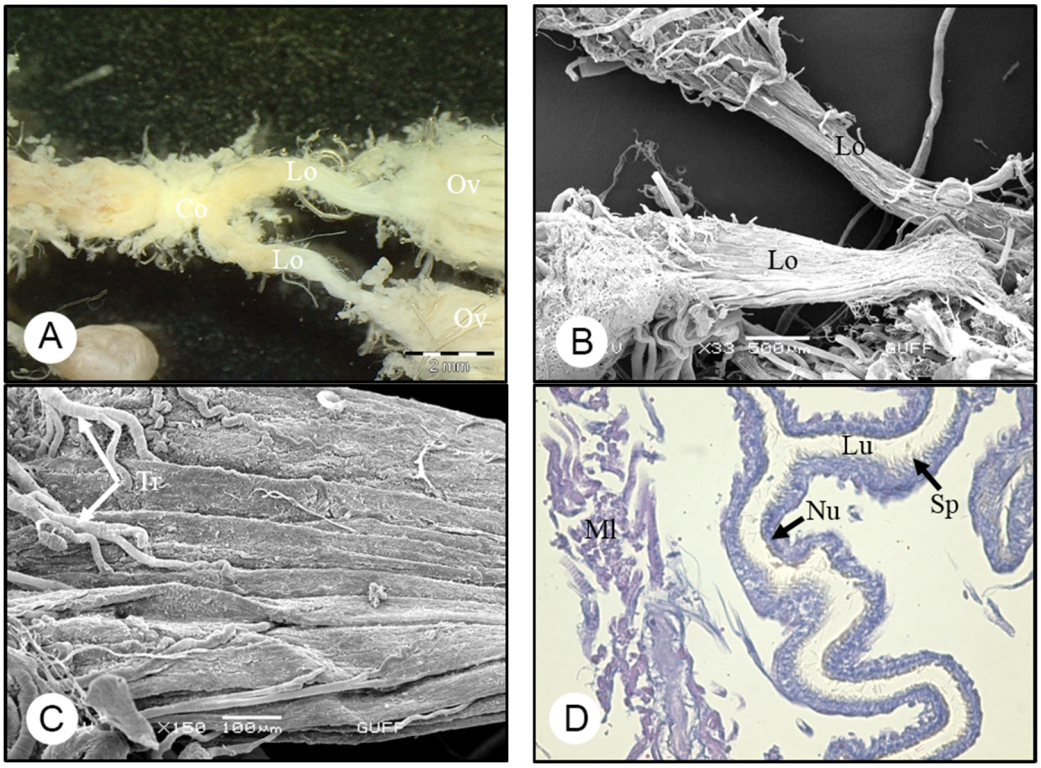

Figure 4. A, B) The general view of lateral oviduct (SM, SEM). C) SEM photograph of lateral oviduct surface. D) The histological section of lateral oviduct (x100). Lo-lateral oviduct, Lu-lumen, Ml-muscle layer, Nu-nucleus, Ov-ovary, Tr-trachea.

This study, which examines the histological structures of the female reproductive system of *C. sycophanta* (Carabidae) and compares it with other insect groups, will help future systematic studies on female insect reproductive histoanatomy and contribute to agricultural control studies.

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ЗАПАЖАЊА О НЕЗРЕЛИМ ЈАЈНИЦИМА ОДРАСЛИХ *CALOSOMA SYCOPHANTA* (LINNAEUS, 1758) (COLEOPTERA: CARABIDAE) – УПОТРЕБОМ СВЕТЛОСНЕ И ЕЛЕКТРОНСКЕ МИКРОСКОПИЈЕ

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Извод

Calosoma sycophanta (Linnaeus, 1758) (Coleoptera: Carabidae) је један од најважнијих предатора гусеница и лутака борового четника *Thaumetopoea pityocampa* (Denis & Schiffermüller) (Lepidoptera: Thaumetopoeidae). Стога је веома важна врста у биолошкој контроли. Релативно мало студија се фокусирало на женски репродуктивни систем код *C. sycophanta*. Код ове врсте, метода је заснована на употреби светлосне и електронске микроскопије за проучавање репродуктивне анатомије и хистологије. Женски репродуктивни органи *C. sycophanta* састоје се од парних јајника, бочних јајовода, заједничког јајовода и генитална коморе. Сваки од јајника чини око 12 политрофних мероистичких овариола. На уздужном пресеку јајника примећено је да вителарни регион, који се налази после гермаријума, садржи јајне коморе које се развијају у линеарном редоследу. Разликује се по томе, да се свака комора састоји од ооцита и ћелија хранилица насталих непотпуном цитокинезом из исте примарне ћелије. Јајник се повезује са бочним каналом кроз педицел, а унутар сваке коморе налази се ооцита уз 16 ћелија хранилица. Целокупна структура је окружене фоликуларним ћелијама. Епител педицела и бочног јајовода је формиран од једнослојних ћелија прекривених мишићним слојем. Унутрашњи - интима слој се издваја на страни епитела окренутом ка лумену. У интима слоју виде се шиљати наставци. Сврха ове студије је да опише хистоанатомију јајника код *C. sycophanta* и упореди је са другим врстама Coleoptera, укључујући и фамилију Carabidae.

Received: February 12th, 2024
Accepted: May 20th, 2024