# The Ovarian Structure and Vitellogenesis, Spermatheca, and Egg in the Alfalfa Weevil, *Hypera postica* (Gyllenhal, 1813) (Coleoptera: Curculionidae)

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# The Ovarian Structure and Vitellogenesis, Spermatheca, and Egg in the Alfalfa Weevil, *Hypera postica* (Gyllenhal, 1813) (Coleoptera: Curculionidae)

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## Abstract

The female reproductive system, spermatheca, and eggs of the alfalfa weevil, *Hypera postica*, were analyzed using stereo, light, and scanning electron microscopy (SEM). The system consists of paired ovaries, lateral and common oviducts, a spermatheca, and a genital chamber. Each ovary contains two long, telotrophic-meroistic ovarioles, divided into the terminal filament, germarium, vitellarium, and pedicel. The germarium houses trophocytes and young oocytes, while the vitellarium contains oocytes at various developmental stages. Previtellogenic oocytes feature a cylindrical follicular epithelium without yolk. In contrast, vitellogenic oocytes possess a cuboidal epithelium and yolk-filled ooplasm; during the choriogenic stage, the follicular epithelium becomes squamous, and the ooplasm becomes densely packed with yolk. Histological differences in epithelium and muscle layers distinguish the lateral and common oviducts. Eggs, measuring approximately 0.2 mm by 0.3 mm, are laid on alfalfa stems. Initially whitish, they darken to black during embryonic development. SEM analysis revealed a porous endochorion and an exochorion characterized by transverse and longitudinal lamellar air spaces, likely facilitating gas exchange. These morphological and histological findings offer critical insights into the reproductive biology of *H. postica*, providing a structural basis for improving pest management and biological control interventions.

**Key words:** chorion, electron microscope, light microscope, ovariole, spermatheca

## Introduction

Alfalfa, or lucerne (*Medicago sativa* L.), is one of the most important forage crops cultivated worldwide due to its high adaptability to diverse environmental conditions. Despite its resilience, alfalfa is susceptible to a range of insect pests, among which the alfalfa weevil, *Hypera postica* (Gyllenhal), is considered the most destructive globally (Metcalf & Luckman, 1994). *H. postica* is a typical oligophagous herbivore, feeding primarily on *Medicago* species, though it occasionally consumes related genera such as *Melilotus*, *Trifolium*, and *Trigonella* in regions where alfalfa is cultivated (Fick, 1976). Both the larval and adult stages of *H. postica* are voracious feeders, causing extensive damage to terminals, foliage, and new crown shoots. Infested fields often appear silvery or whitish as leaves become skeletonized or entirely consumed, and severe infestations can result in substantial yield losses (Fick, 1976).

In insects, the female reproductive system consists of two ovaries, two lateral oviducts, a common oviduct, a spermatheca, and, in some species, an accessory gland (Gardner, 1986). Sperm are transferred into the spermatheca from the spermatophore deposited by the male in the female's bursa copulatrix (Qazi et al., 1996). The ovaries consist of a series of egg tubes called ovarioles, the number of which varies according to the type, size, and specific life history of the insect (Wheeler, 2009). Ovarioles, oviducts, and the spermatheca —parts of the female reproductive system—vary in number and shape among most insect species (Heming, 2018). The structure of the female reproductive system can be helpful not only for understanding species' life cycles and adaptations but also in taxonomic and phylogenetic studies (Ueno, 1994; Jaglarz & Bilinski, 1998; Maffei et al., 2001).

Numerous studies have been conducted on the biology, ecology, and management of *H. postica*. For instance, Lecato and Pienkowski (1973) studied the sperm storage process of *H. postica*. In Hower (1971), the presence of reproductive system abnormalities in natural populations of *H. postica* was investigated. In Tombes (1964), the relationship between gonadal development in *H. postica* and temperature and seasonal cycles was demonstrated. Roberts et al. (1995) reported that *H. postica* larvae can be infected by the fungal pathogen *Zoophthora phytonomi*, producing different types of spores. Zahiri et al. (2010) investigated the demographic parameters of the species, including life tables, reproduction, and longevity, under constant temperatures ranging from 11.5 to 31.5°C. Moradi-Vajargah et al. (2011) studied the population density and spatial distribution of *H. postica*. Sanaei and Seiedy (2016) examined developmental differences among populations under laboratory conditions. Baysal et al. (2018) evaluated the effects of specific *Beauveria bassiana* isolates on *H. postica* larvae in laboratory experiments. Irec et al. (2021) analyzed the impact of chemical control applied at different heights of alfalfa plants on *H. postica* and fresh forage yield. Özyurt Koçakoğlu et al. (2024) described the adult developmental morphology, pupae, larvae, and damage caused by *H. postica*. Collectively, these studies provide

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comprehensive information on the biology and management of *H. postica*. However, to date, no scientific research has directly addressed the anatomy and histology of the female reproductive system of *H. postica*. Considering the increasing species diversity and pest impact, the lack of studies on the internal morphology of the female reproductive system in the genus *Hypera* represents a significant knowledge gap. The present study aims to fill this gap by examining the structure of the female reproductive system. While highlighting the paucity of data on internal anatomy in *Hypera* species, this research provides the first detailed description of the anatomy and histology of the adult female reproductive system of *H. postica* using both light and scanning electron microscopy.

## Material and Methods

### Specimen Collection

Adult female *H. postica* beetles ($n = 10$) were collected from alfalfa (*Medicago sativa* L.) plants in Bingöl, Turkey (38°52′59″N, 40°30′03″E; altitude ~1000 m) on 31 May 2024. Live beetles were transported to the Microtechnique Laboratory, Department of Biology, Faculty of Science, Gazi University (Ankara, Turkey), where they were maintained for several days prior to microscopic analyses. *H. postica* specimens collected from the field were reared in the laboratory at 24°C in perforated boxes with alfalfa plants.

### Stereomicroscope (SM)

Adult insects were euthanized using ethyl acetate vapor for 10 min and immediately dissected in sodium phosphate buffer (pH 7.2). Dissections were performed under an Olympus SZX7 stereomicroscope (Tokyo, Japan) following these steps: (i) removal of elytra, (ii) removal of pronotum, and (iii) extraction of the female reproductive system from the abdomen.

### Light Microscope (LM)

Five specimens were fixed in 10% neutral formalin for 24 h, rinsed in tap water for 24 h, and dehydrated through a graded ethanol series (50%, 70%, 80%, 90%, 96%, and 99%; each step lasted 1 h). Samples were cleared in a xylene-paraffin mixture (3 × 15 min) and embedded in paraffin at 65° C for 24 h. Paraffin blocks were sectioned at 6–7 µm using a Microm HM310 microtome (Walldorf, Germany). Transverse and longitudinal sections were stained with Mallory's trichrome, Hematoxylin-Eosin (H&E), and bromophenol blue. Mallory's trichrome stains connective tissue blue, muscle fibers red, and nuclei dark red; H&E stains nuclei blue-violet and the cytoplasm and connective tissue pink. Bromophenol blue, on the other hand, stains proteins in shades of blue, highlighting areas with high protein density. Stained sections were examined under an Olympus BX51 light microscope (Tokyo, Japan).

### Scanning Electron Microscope (SEM)

For SEM, five specimens were fixed in 2.5% glutaraldehyde (pH 7.2, sodium phosphate buffer, +4° C), rinsed three times in the buffer, and then dehydrated in a graded ethanol series (70%, 80%, 90%, 99%; 15 min each). Samples were transferred to hexamethyldisilazane for 15 min and air-dried (2 min). Dried specimens were mounted on SEM stubs using double-sided tape, coated with gold using a Polaron SC502 sputter coater (Uckfield, UK), and examined in a JEOL JSM 6060 LV SEM (Tokyo, Japan). Digital micrographs were captured for detailed analysis (Özyurt Koçakoğlu et al., 2026).

## Results

The female reproductive system of *H. postica* consists of a pair of ovaries, lateral oviducts, a common oviduct, a spermatheca, and a genital chamber. Each ovary contains a pair of long, linear ovarioles extending throughout the entire abdomen and even into the thorax region. This remarkable length results from the high number of oocytes within the vitellarium ($n \approx 20$) (Fig. 1a). Dense trachea networks intertwine among the ovarioles (Fig. 1b). Each ovariole is composed of a terminal filament, germarium, vitellarium, and pedicel (Fig. 1a). The terminal filament is a thin, elongated thread connecting the ovarioles to each other and to the body wall (Fig. 2a). The germarium is pear-shaped, being broader distally and narrower proximally (Fig. 2a). Toward its distal region, trophocytes are present, each with a large, round nucleus occupying most of the cell (Figs. 2b, 2c). Wide intercellular spaces occur among trophocytes (Fig. 2b), and a distinct nucleolus can be observed adjacent to one side of the nucleus (Fig. 2c). Proximal to the trophocytes are smaller prefollicular cells (Fig. 2d). In this section, additionally, young oocytes are observed (Fig. 2d). The vitellarium is the longest region of the ovariole, where oocytes are arranged linearly. Oocytes nearer the germarium are whitish,

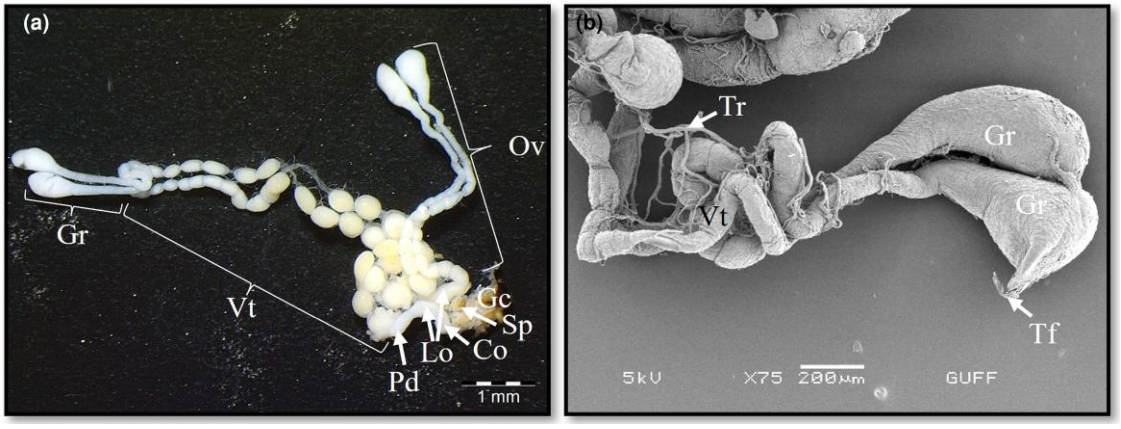



**Fig. 1.** (**a**) General view of the female reproductive system of *Hypera postica* (SM). (**b**) SEM micrograph showing the ovarioles. Co, common oviduct; Gc, genital chamber; Gr, germarium; Lo, lateral oviduct; Ov, ovariole; Pd, pedicel; Sp, spermatheca; Tf, terminal filament; Tr, trachea; Vt, vitellarium.

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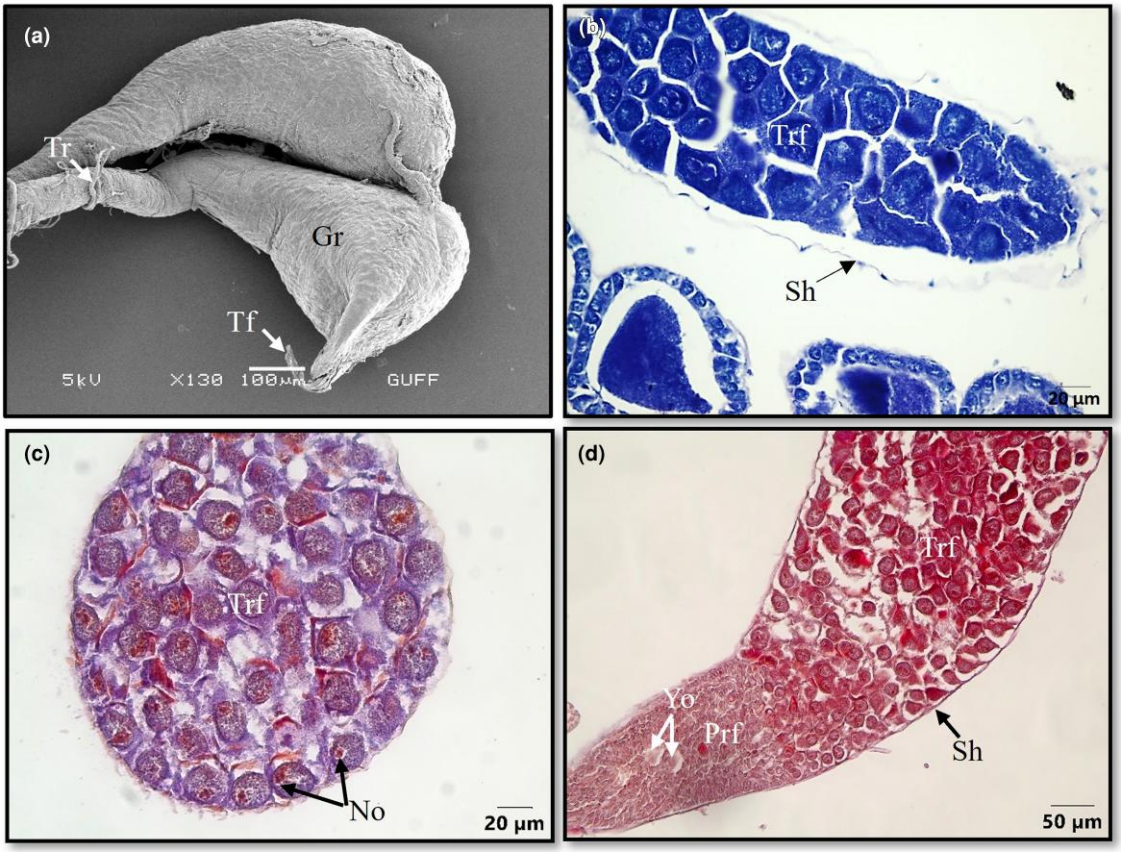



**Fig. 2.** (**a**) Terminal filament extending from the distal end of the germarium (SEM). (**b**) Longitudinal section of trophocytes in the germarium (H&E) (LM). (**c**) Cross-section of trophocytes in the germarium (Mallory) (LM). (**d**) Longitudinal section of trophocytes and prefollicular cells in the germarium (Mallory) (LM). Gr, germarium; No, nucleolus; Prf, prefollicular cells; Sh, sheath; Tf, terminal filament; Tr, trachea; Trf, trophocytes; Yo, young oocyte.

while those toward the pedicel appear light yellow, reflecting progressive oocyte maturation (Fig. 1a). Oocyte size increases correspondingly with this color gradient. Numerous previtellogenic oocytes occur in the distal part of the vitellarium (Figs. 3a–3d), interspersed with interfollicular tissue (Fig. 3c). These oocytes are surrounded by a single layer of cylindrical follicular epithelium containing oval nuclei with heterochromatin clumps. No yolk accumulation is yet visible in the ooplasm, and the germinal vesicle is centrally located (Figs. 3b, c). In the middle of the vitellarium, vitellogenic oocytes predominate, with no interfollicular tissue between them (Figs. 4b, 4c). Their surfaces are smooth and densely enveloped by tracheal networks (Fig. 4a). Each vitellogenic oocyte is surrounded by a single layer of cuboidal follicular epithelium with round nuclei (Figs. 4b–4d). The germinal vesicle shifts toward the ooplasmic periphery, and the ooplasm becomes filled with yolk granules (Figs. 4b, 4c). The vitelline membrane becomes distinguishable at this stage (Fig. 4b). Bromophenol blue staining revealed partial positive reactions in the yolk granules of vitellogenic oocytes (Fig. 5a). At the posterior end of the vitellarium, choriogenic oocytes are observed, enclosed by a single layer of flattened follicular epithelium and the chorion (Figs. 5b–5d). In these oocytes, bromophenol blue staining showed that almost all yolk granules in the ooplasm reacted positively (Fig. 5b). Each ovariole connects to the lateral oviduct via a pedicel, and the lateral oviducts join to form the common oviduct (Figs. 1a, 6d). Histological sections of the pedicel reveal a single-layered cylindrical epithelium with oval nuclei and heterochromatin clumps, surrounded by a muscle layer and forming deep folds toward the lumen (Figs. 6a, 6b). The lateral oviduct consists of a single-layered squamous epithelium with flattened nuclei, also forming deep luminal folds and surrounded by a muscle layer (Fig. 6c). The common oviduct exhibits a thick cuticular intima layer with deep invaginations, a single-layered cuboidal epithelium with round nuclei, and a distinctly thick muscular coat displaying striations and numerous nuclei (Fig. 6e). In Figure 6f, hair-like, spine-shaped projections extending from the intima of the common oviduct are observed. The eggs are fertilized within the common oviduct by sperm originating from the spermatheca. Light and scanning electron micrographs reveal that the spermatheca lumen is densely packed with sperm masses (Figs. 7a, 7b). The spermatheca is surrounded by a thick cuticular intima and a glandular epithelial layer with granular cytoplasm and oval nuclei (Fig. 7a). Fertilized eggs are deposited through the genital chamber onto the stems of alfalfa leaves, the host plant of the species (Fig. 8a). The eggs are initially whitish but turn black during embryonic development (Fig. 8b). They measure approximately 0.2 mm in width and 0.3 mm in length and possess small tubercular and conical projections on the surface (Figs. 8c, 8d). In eggs, the endochorion and exochorion layers are distinguishable (Figs. 8e, 8f), with pores in the endochorion and transverse and longitudinal lamellar air spaces in the exochorion.

## Discussion

In *H. postica* the female reproductive system consists of a pair of ovaries, a pair of lateral oviducts, a common oviduct, a

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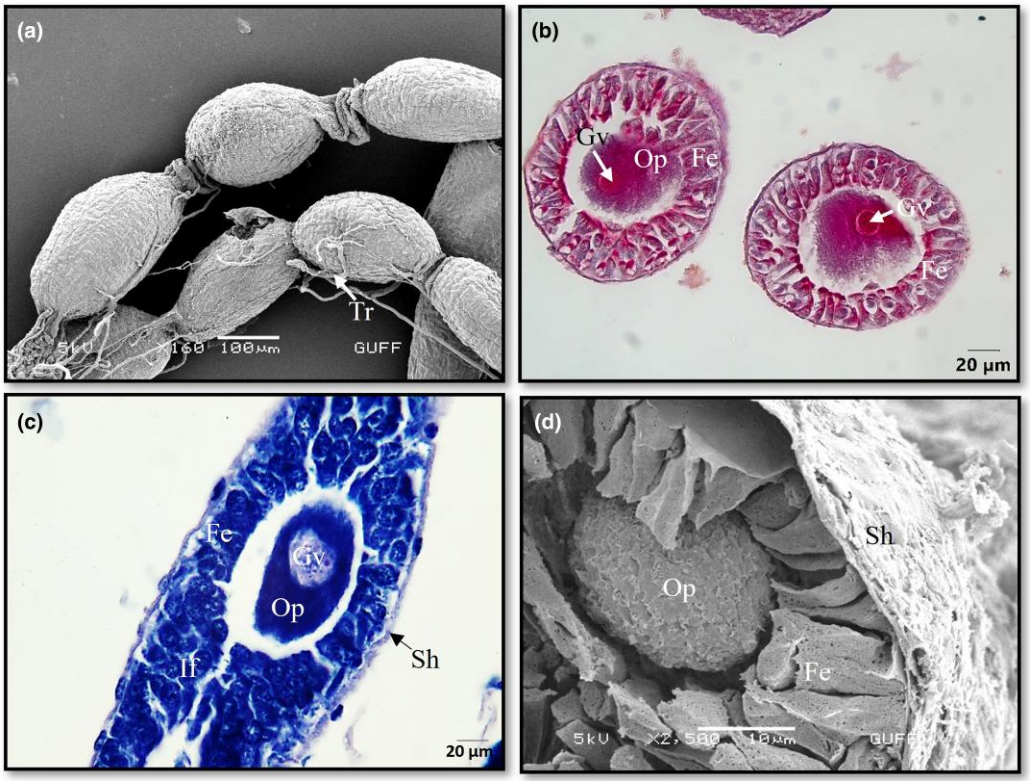



**Fig. 3.** (**a**) SEM micrograph of previtellogenic oocytes. (**b**) Cross-section of previtellogenic oocytes (Mallory) (LM). (**c,d**) Light and scanning electron micrographs of the follicular epithelium surrounding a previtellogenic oocyte (H&E) (LM) (SEM). Fe, follicular epithelium; Gv, germinal vesicle; If, interfollicular cells; Op, ooplasma; Sh, sheath; Tr, trachea.

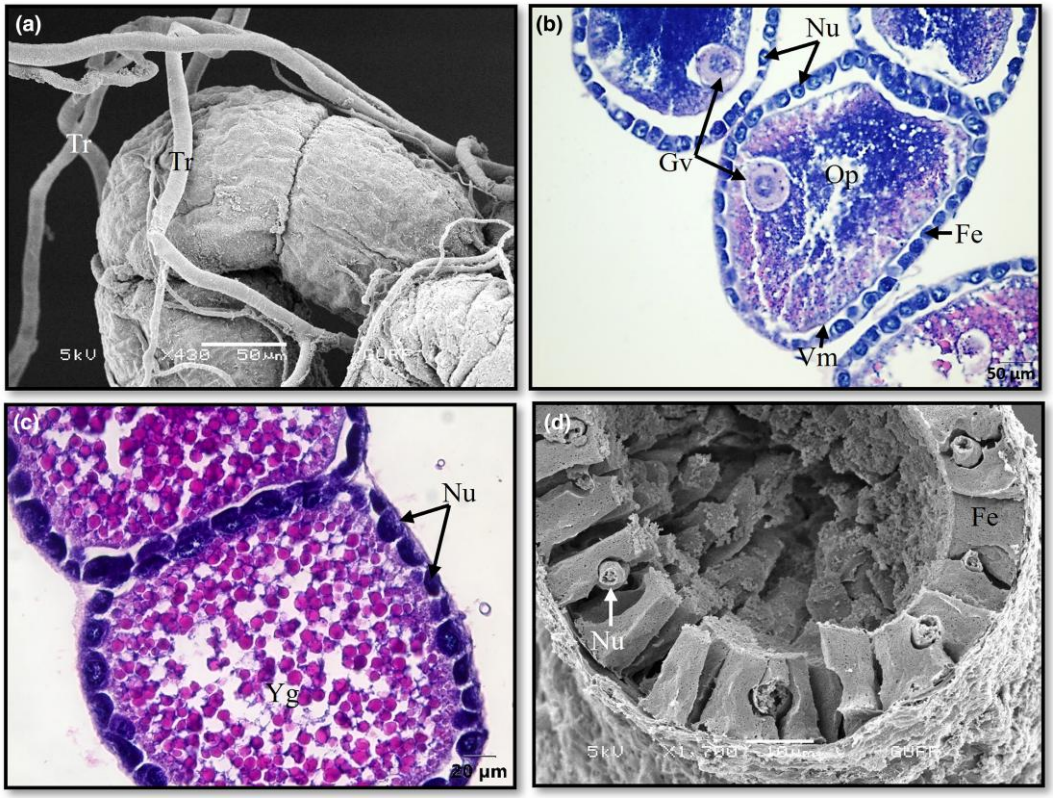



**Fig. 4.** (**a**) SEM micrograph of vitellogenic oocytes. (**b**) Histological section of vitellogenic oocytes (H&E) (LM). (**c**) Yolk granules accumulated in the ooplasm of vitellogenic oocytes (H&E) (LM). (**d**) SEM micrograph of the follicular epithelium surrounding a vitellogenic oocyte. Fe, follicular epithelium; Gv, germinal vesicle; Nu, nucleus; Op, ooplasma; Tr, trachea; Yg, yolk granules; Vm, vitelline membrane.

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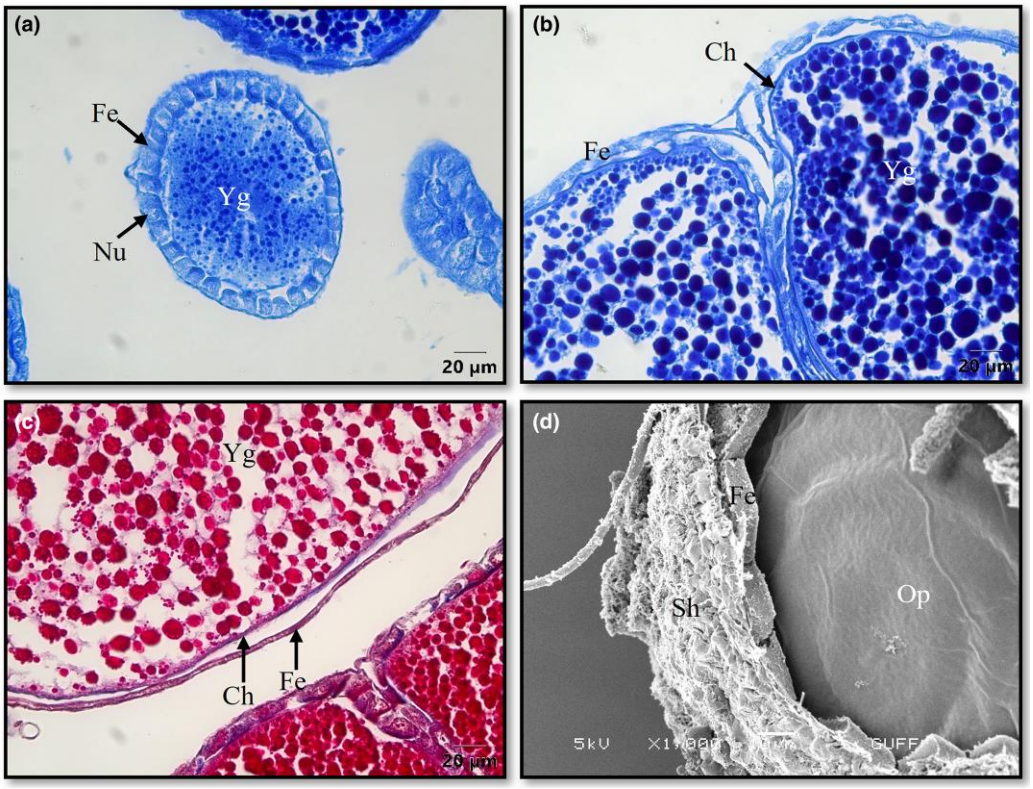



**Fig. 5.** (**a**) Positive bromophenol blue reaction of nutrient granules in the ooplasm of a vitellogenic oocyte. (**b**) Positive bromophenol blue reaction of nutrient granules in the ooplasm of a choriogenic oocyte. (**c**) Histological section of the follicular epithelium and chorion surrounding a choriogenic oocyte (Mallory) (LM). (**d**) SEM micrograph of the follicular epithelium surrounding a choriogenic oocyte. Ch, chorion; Fe, follicular epithelium; Nu, nucleus; Op, ooplasm; Sh, sheath; Yg, yolk granules.

spermatheca, and a genital chamber. Similar structures have been observed in other Curculionidae species (Goldson & Emberson, 1981; Barker, 1989; Korman & Oseto, 1989; Rubio et al., 2008; El Naggar et al., 2010; Gao et al., 2021). But, in addition to these structures, there are an accessory gland in *Hypothenemus hampei* (Curculionidae) and two accessory glands in *Trypophloeus klimeschi* and in *Dendroctonus* sp. (Curculionidae) (Rubio et al., 2008; Gao et al., 2021; García-Román et al., 2022). However, the type of ovariole, the number of ovarioles, and the parts that form the ovaries may vary between species and are important characters from a systematic perspective.

Ovarioles are divided into two types: panoistic and meroistic; the meroistic type also includes telotrophic (nutrient cells remain in the germarium) and polytrophic (nutrient cells develop together with oocytes) subtypes (Buning, 1993, 1994). Polyphaga (Coleoptera) has telotrophic meroistic ovarioles (Korman & Oseto, 1989; Gao et al., 2021). Such as *H. postica*, *Hyperodes bonariensis* (Curculionidae) (Goldson & Emberson, 1981), *Listronotus bonariensis* (Curculionidae) (Barker, 1989), *Sitophilus granarius* (Curculionidae) (Gaino & Fava, 1995), and *T. klimeschi* (Curculionidae) (Gao et al., 2021).

In Curculionoidea, the ovarioles are generally quite short; however, in some Entiminae species (e.g., Polyphrades species) and in Gonipterinae, they are extremely elongated and coiled within the body cavity (Calder, 1990). In *H. postica*, the ovarioles are also linear and quite long. The number of ovarioles in insects is a variable depending on the reproductive strategy of the species (Chapman, 2013; Klowden, 2013; Heming, 2018). In the Curculionidae family, each ovary has two ovarioles; rarely, each ovary has four or six ovarioles (Calder, 1990). This finding suggests that the low ovariole number observed in *H. postica* is consistent with the general reproductive organization of the Curculionidae family and that the species' reproductive strategy may depend more on ovariole development and elongation than on ovariole number alone. Table 1 shows the distribution by Curculionidae species.

In *H. postica*, the ovariole are divided into sections starting from the tip, namely the terminal filament, germarium, vitellarium, and pedicel. In vitellarium, oocytes are in successive stages of oogenesis and connected by follicular cells. Similar structures have been reported in *H. bonariensis*, *L. bonariensis* and *T. klimeschi* (Curculionidae) (Goldson and Emberson, 1981; Barker, 1989; Gao et al., 2021).

Lecato and Pienkowski (1973) examined the microanatomy of the spermatheca, spermathecal duct, and spermathecal gland in *H. postica*. Similar structures were also observed in *S. granarius*, *H. bonariensis* and *L. bonariensis* (Curculionidae) (Tombes & Roppel, 1972; Goldson & Emberson, 1981). In *H. postica*, the spermathecal lumen was observed to be filled with sperm. Similar structures are found in *T. klimeschi* (Curculionidae) (Gao et al., 2021) and *S. granarius* (Curculionidae) and *Smicronyx fulvus* (Curculionidae) (Tombes & Roppel, 1972; Korman & Oseto, 1989). The spermathecal morphology of *H. postica* is consistent with the conserved and functionally specialized sperm-storage system characteristic of many Curculionidae species.

The shape, color, surface patterns, and size of insect eggs are useful systematic characters for identifying species (Wightman & Southgate, 1982; Cheetham, 1992; Caron et al., 2004).

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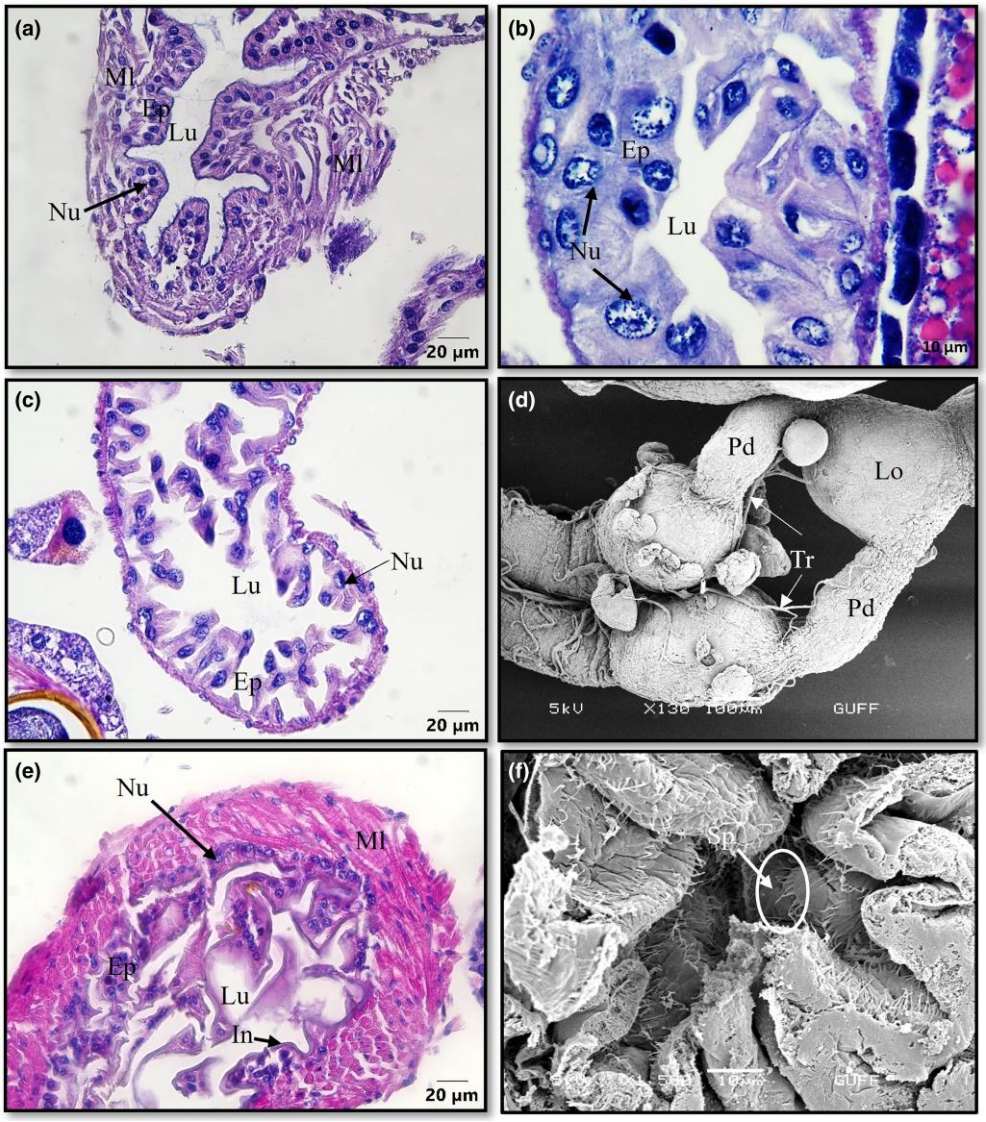



**Fig. 6.** (**a, b**) Histological sections of the pedicel, the terminal part of the ovariole (H&E) (LM). (**c**) Longitudinal section of the lateral oviduct (H&E) (LM). (**d**) Connection of ovarioles to the lateral oviduct via the pedicel (SEM). (**e**) Histological section of the common oviduct (H&E) (LM). (**f**) Hair-like, spine-shaped projections extending from the intima of the common oviduct. Ep, epithelium; Lo, lateral oviduct; Lu, lumen; Ml, muscle layer; Nu, nucleus; Pd, pedicel; Tr, trachea; Sp, spines; In, intima.

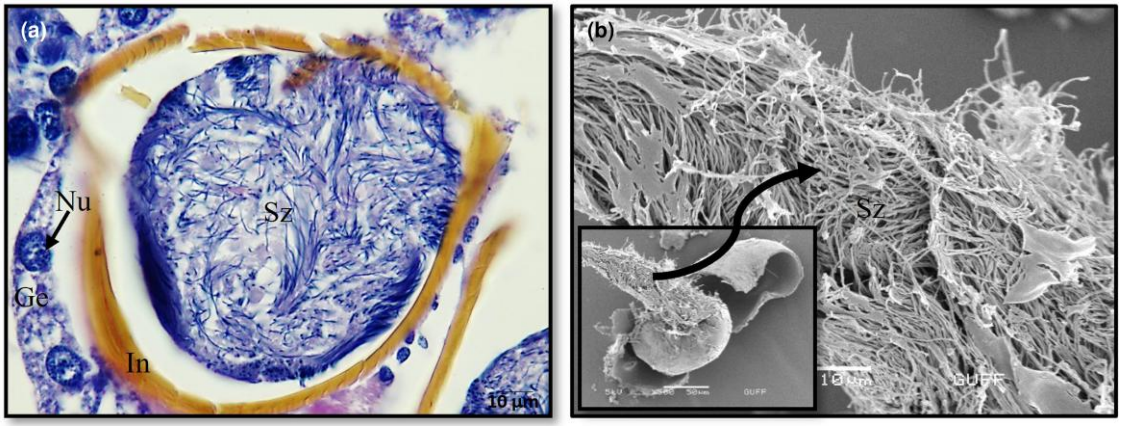



**Fig. 7.** (**a**) Histological section of the spermatheca showing the cuticular intima and surrounding epithelium (H&E) (LM). (**b**) Mature sperm bundles within the lumen of the spermatheca. Ge, glandular epithelium; In, intima; Nu, nucleus; Sz, spermatozoa.

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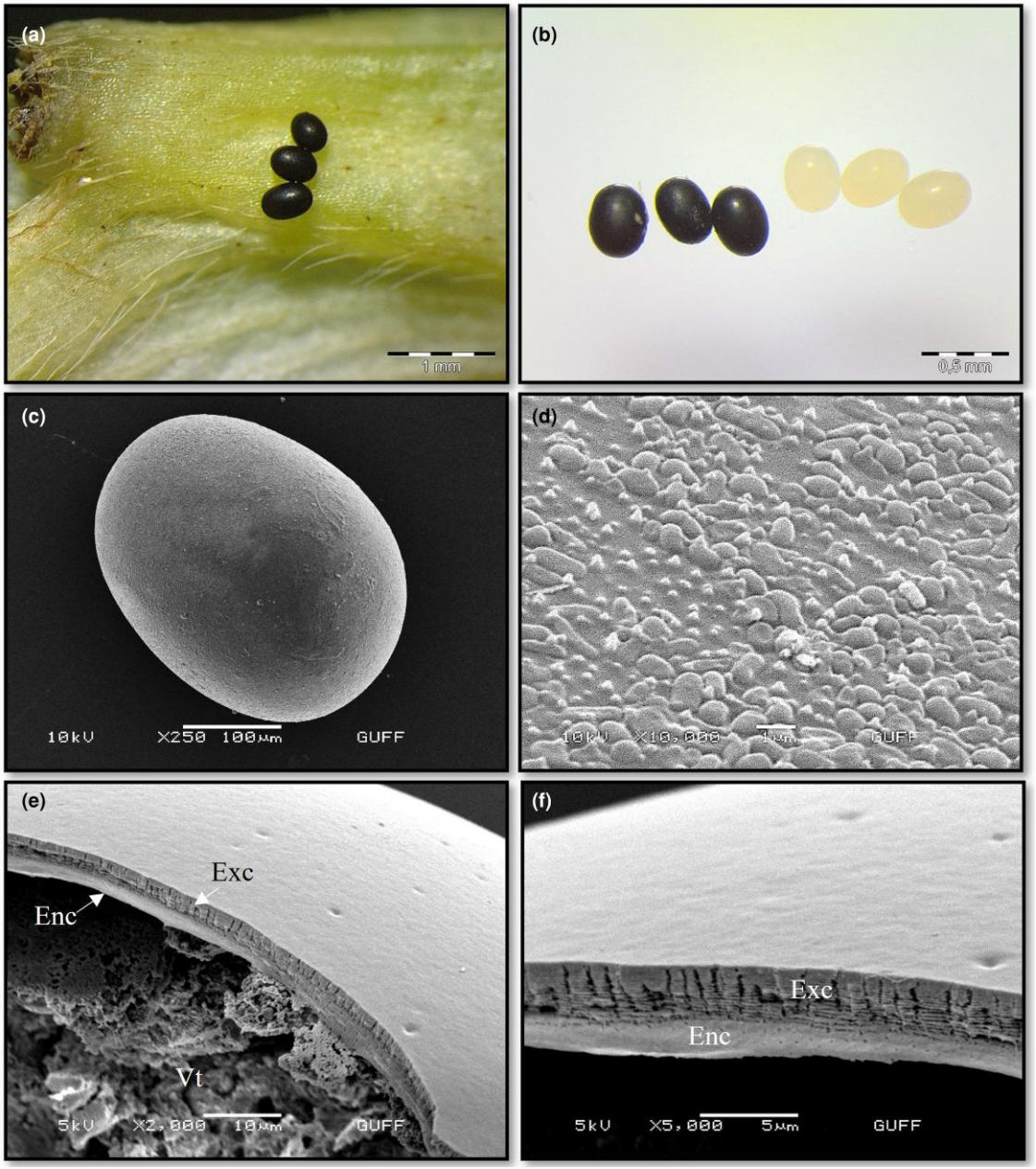



**Fig. 8.** (**a, b**) Transition of *H. postica* eggs from whitish to black coloration during embryonic development (SM). (**c**) SEM micrograph showing the general morphology of the egg. (**d**) Surface ornamentation of the egg with small tubercular and conical projections (SEM). (**e, f**) SEM micrographs of the chorion of the egg. Enc, endochorion; Exc, exochorion; Vt, vitellus.

**Table 1.** Number of Ovarioles per Ovary in Some Coleoptera Species.

| Species | Family | Number of ovarioles per ovary | References |
|---|---|---|---|
| *H. postica* | Curculionidae | 2 | This study |
| *Curculio caryae* | Curculionidae | 2 | Bissell (1937) |
| *Hyperodes bonariensis* | Curculionidae | 2 | Goldson and Emberson (1981) |
| *Smicronyx fulvus* | Curculionidae | 2 | Korman and Oseto (1989) |
| *Sitophilus oryzae* | Curculionidae | 2 | Perez-Mendoza et al. (2004) |
| *Hypothenemus hampei* | Curculionidae | 2 | Rubio et al. (2008) |
| *Trypophloeus klimeschi* | Curculionidae | 2 | Gao et al. (2021) |
| *Rhynchophorus ferrugineus* | Curculionidae | 2 | El Naggar et al. (2010) |
| *Listronotus bonariensis* | Curculionidae | 2 | Barker (1989) |
| *Blepiarda undulata* | Curculionidae | 4 | Calder (1990) |
| *Leptopius colossus* | Curculionidae | 6 | Calder (1990) |
| *Leptopius duponti* | Curculionidae | 6 | Calder (1990) |

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*H. postica* eggs are black and elliptical in appearance. The surface of *H. postica* eggs has small buds and conical protrusions. In *R. ferrugineus* (Curculionidae), freshly laid eggs are soft, whitish and gloomy in color. A few hours later, they begin to harden and become white, calm, and transparent at the bilateral two external edges. The eggs are ovo-cylindrical shaped and egg shell surface is uniform, smooth or densely set with small, raised projections (Curculionidae) (Al-Dosary et al., 2010). In *S. granarius* (Curculionidae), mature ovary eggs are cylindrical; the egg surface displays a greatly wrinkled coat (Gaino & Fava, 1995). Overall, these observations indicate that *H. postica* eggs exhibit a distinct morphological pattern characterized by dark coloration, elliptical shape, and a uniquely sculptured chorion surface, differentiating them from other Curculionidae species while still reflecting family-level structural diversity. When examining egg sizes across different Curculionidae species, it is clear that there are morphological differences among species. *H. postica* eggs are about 0.2 mm wide and 0.3 mm long. The eggs of *S. granarius* measure 0.6 mm in length and 0.27 mm in width (Gaino & Fava, 1995). *Rhynchophorus schach* eggs are 2.4 mm long and 0.9 mm wide (Bong et al., 2008). *R. ferrugineus* was found to be 1.09 mm long and 0.43 mm wide (Al-Dosary et al., 2010). These comparisons indicate that *H. postica* eggs are considerably smaller than those of other Curculionidae species and highlight a wide interspecific variation in egg size within the family.

## Conclusion

The microscopic examination of the female reproductive system and eggs of *H. postica* is of great importance for understanding the reproductive biology of the species. Additionally, this information provides detailed data on the pest's reproductive capacity and egg development. In biological control studies, knowledge of the egg structure and protective layers helps assess the potential of parasitoids to penetrate or infect the eggs. The microscopic features of the female reproductive system also contribute to the development of reproductive-inhibiting biotechnical methods. In summary, such microscopic investigations provide a scientific basis for the targeted, effective, and environmentally friendly planning of biological control strategies.

## Availability of Data and Materials Statement

All data in the current study can be obtained from the corresponding author upon reasonable request.

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## Author Contributions Statement

Concept—N.Ö.K., H.A., S.C.; Design—N.Ö.K., H.A., S.C.; Data Collection and/or Processing—N.Ö.K., H.A., S.C.; Analysis and/or Interpretation—N.Ö.K., H.A., S.C.; Literature Search—N.Ö.K., H.A., S.C.; Writing Manuscript—N.Ö.K., H.A., S.C.; Resources—N.Ö.K., H.A.; Critical Review—N.Ö.K., S.C.; Supervision—S.C.

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## Conflict of Interest

The authors declare that they have disclosed no conflict of interest in this work.

## Ethics Standards

Since insects are used, ethics committee approval is not required.

## Additional Information

This article was initially submitted by the authors in English and was first published here.

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