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Development of ovarioles in the ovary of *Eulasia arctos anatolica* (Reitter, 1903) (Coleoptera: Glaphyridae)

Eulasia arctos anatolica (Reitter, 1903) (Coleoptera: Glaphyridae) ovaryumunda ovaryol gelişimi

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ABSTRACT

Eulasia arctos anatolica, belonging to the Coleoptera order and among the first insects to visit flowers, is associated with plant species such as *Onopordum* and *Euphorbia*, playing a significant role in floral ecology and pollination processes. Therefore, in this study, the histological and anatomical structures of the female reproductive system of *E. arctos anatolica* were examined for the first time using light microscopy and scanning electron microscopy. The female reproductive system of *E. arctos anatolica* includes a pair of ovaries, each with six telotrophic meroistic ovarioles, a pair of lateral oviducts, a common oviduct, and a genital chamber. SEM revealed that ovarioles are enclosed by a reticulated sheath from the suspensory ligament to the pedicel, with tracheal networks on the sheath surface. Each ovariole contains three to four oocytes; the choriogenic oocyte, located near the pedicel, is larger and anthracite, while the others are whitish. Ovarioles anterior ends are attached to the abdomen wall via the suspensory ligament. Histology of the germarium showed trophocytes, prefollicular cells, young oocytes, and nutritive cord. Early-stage oocytes lacked yolk granules and were surrounded by a single layer of cuboidal to short columnar follicular epithelium. In a late-stage oocyte, the ooplasm was filled with acidophilic yolk granules, and oocytes were encircled by flattened epithelial cells; chorion formation was evident. The pedicel was lined by a single layer of epithelium. This study contributes to the understanding of ovariol development in flower-visiting Coleoptera species and provides fundamental data on the reproductive biology and ecological functions of this group.

Keywords: Germarium, Nutritive cord, Ooplasma, Vitellarium, Yolk granules

ÖZ

Çiçekleri ziyaret eden ilk böceklerden olan Coleptera takımından *Eulasia arctos anatolica*, *Onopordum* ve *Euphorbia* gibi bitki türleriyle ilişkili olup, floral ekoloji ve tozlaşma süreçlerinde önemli bir role sahiptir. Bu nedenle bu çalışmada *E. arctos anatolica*'nın dişi üreme sisteminin histolojik ve anatomik yapısı ilk defa ışık mikroskobu ve taramalı elektron mikroskobu kullanarak incelenmiştir. Dişi üreme sistemi, her biri altı telotrofik meroistik ovaryolden oluşan bir çift ovaryum, bir çift lateral kanal, bir ortak kanal ve genital çemberden meydana gelmektedir. SEM analizleri, ovaryollerin asıcı bağdan pedisele kadar uzanan ağsı bir kılıf ile çevrelendiğini ve bu kılıfın yüzeyinde trakeal ağların yer aldığını göstermiştir. Her bir ovaryol üç ila dört oosit içermekte olup, pedisele yakın konumdaki koryogenik oosit daha büyük ve antrasit renkli, diğer oositler ise beyazımsı renkte görünüm sergilemektedir. Ovaryollerin ön uçları, asıcı bağ aracılığıyla abdomen duvarına tutunmaktadır. Histolojik incelemeler, germaryum bölgesinde trofositler, prefoliküler hücreler, genç oositler ve besin kordonunun bulunduğunu ortaya koymuştur. Vitellaryumda gelişimin erken evrelerdeki oositlerde besin granülleri granülleri görülmemekte olup, bu oositler kübik ya da kısa prizmatik tek katlı folikül epiteli ile çevrilidir. Gelişmenin ileri evresindeki oositin ooplazması asidofilik besin granülleriyle dolu olup, bu oositin tek katlı yassı folikül epiteliyle çevrelendiği ve koryon oluşumu görülmüştür. Pediselin duvarı tek katlı epitel ile çevrelenmiştir. Bu çalışma, çiçek ziyareti yapan Coleoptera türlerinde ovaryol gelişiminin anlaşılmasına katkı sağlayarak, bu grubun üreme biyolojisi ve ekolojik işlevleri hakkında temel veriler sunmaktadır.

Anahtar Kelimeler: Germaryum, Besin kordonu, Ooplazma, Vitellaryum, Besin granülleri

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Introduction

Glaphyridae (Coleoptera) includes six genera and approximately 220 species worldwide. The Turkish fauna includes 89 recorded taxa (species and subspecies) of Glaphyridae, accounting for roughly 40% of the diversity known for the entire family (Nikodým & Bezděk, 2016). Members of the Glaphyridae family are generally known to be flower-associated beetles (floricolous), feeding on floral resources, which suggests a potential role in pollination processes and flower-ecology interactions within ecosystems. The subspecies *Eulasia arctos anatolica* has been reported to feed on certain plant species within the families Brassicaceae and Euphorbiaceae, including *Sinapis, Medicago, Vicia, Onopordum, and Euphorbia.* Its classification as a flower-visiting beetle and its documented association with specific plant taxa (e.g., *Onopordum*, *Euphorbia*) indicate a potential ecological role (Rozner & Rozner, 2009).

To date, the reproductive system of this species, as well as that of the genus and the entire family, has not been thoroughly investigated. Determining the anatomical and histological characteristics of the female reproductive organs would provide valuable insights into the systematic position of the species and contribute to interspecific comparisons within the Glaphyridae family. Therefore, a detailed anatomical and histological investigation of the female reproductive system of *E. arctos anatolica* is of significant scientific importance, both for a more comprehensive understanding of the species' biological features and to fill this gap in the literature.

In the order Coleoptera, the female reproductive system shows some variation among species but generally follows a similar structural pattern. Its organization provides key morphological characteristics relevant to reproductive biology and systematic classification. In general, the female reproductive system in Coleoptera consists of a pair of ovaries, oviducts, and a genital chamber. Each ovary contains multiple ovarioles, which are usually of the meroistic telotrophic type and play a critical role in oocyte development and nourishment (Büning, 1994). The lateral oviducts unite to form a common oviduct that functions in the transport of mature eggs (Beutel & Leschen, 2005; Gillott, 2005).

Despite the ecological and systematic studies of Glaphyridae, detailed investigations into the female reproductive morphology of species within this family remain lacking. Likewise, studies examining the female reproductive system across other coleopteran taxa are limited in scope. In this context, the present study aims to provide a morphological characterization of the ovariol development in *E. arctos anatolica*. The results are expected to advance our understanding of reproductive anatomy within Glaphyridae and related beetle lineages, while comparative analyses with other taxa may offer valuable insights for systematic studies.

Methods

Insects and Stereomicroscopic (SM) Examination

Eulasia arctos anatolica 4♀♀ were collected in April 2024 from grassland habitats in Kızılcahamam, Ankara, Türkiye (40.427900° N, 32.668888° E; altitude 1147 m). The beetles were first anesthetized with ethyl acetate vapors and subsequently dissected in 0.1 M sodium phosphate buffer (pH 7.2) under laboratory conditions. Morphological examinations and photographic documentation of the general appearance were carried out using an Olympus SZX7 stereomicroscope (SM).

Preparation of Samples for Light Microscopy

For histological examinations, specimens from the female reproductive system were fixed in 10 % formaldehyde for 24 hours. Following fixation, the organs were washed under running tap water for 24 hours to remove excess fixative. The organs were then dehydrated through a graded ethanol series (50%, 70%, 80%, 90%, and 99%) and subsequently cleared in xylene, first at room temperature and then at 65 °C in an incubator, each step lasting 15 minutes. Afterward, the samples were gradually infiltrated with paraffin and finally embedded in pure paraffin blocks. Sections of 6-7 µm thickness were obtained from the paraffin blocks and stained with Hematoxylin-Eosin stain for light microscopic observation. The prepared slides were examined under an Olympus BX51 light microscope (Özyurt Koçakoğlu et al., 2025a).

Preparation of Samples for Scanning Electron Microscopy (SEM)

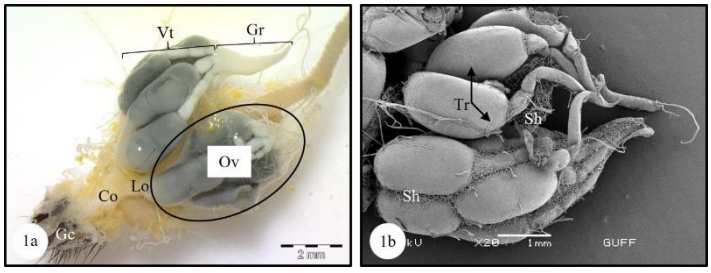
The organs dissected from the abdomens of adult female beetles were fixed in 2.5% glutaraldehyde. Then they were dehydrated through a graded ethanol series (80%, 90%, 96%, and 99%), followed by treatment with hexamethyldisilazane (HMDS) and air-dried. The dried specimens were mounted on SEM stubs using double-sided adhesive tape and coated with a thin layer of gold under vacuum using a Polaron SC502 sputter coater. The organs were examined using a JEOL JSM 6060 LV SEM at the Faculty of Science, Gazi University (Özyurt Koçakoğlu et al., 2025b).

Results

The female reproductive system of *E. arctos anatolica* consists of a pair of ovaries, each containing six telotrophic meroistic-type ovarioles, a pair of lateral oviducts, a common oviduct, and a genital chamber, all extending along both sides of the alimentary canal (Figures 1a, b). Scanning electron microscopy (SEM) images revealed that the ovarioles are surrounded by a reticulated sheath extending from the suspensory ligament to the distal end of the pedicel, with tracheal networks occasionally observed on the surface of this sheath (Figures 2a, 3a, c, 4d). Each ovariole contains 3-4 oocytes, with the oocyte closest to the pedicel being distinctly larger and anthracite in color, while the remaining portions of the ovariol appear whitish (Figure 1a). In *E. arctos anatolica*, each ovariole consists of a germarium, vitellarium, and pedicel. All ovarioles converge at their anterior ends and are attached to the abdomen by a suspensory ligament (Figures 1b, 2a).

Figure 1.

a, b: The general morphology of the female reproductive system in E. arctos anatolica (SM, SEM). Co-common oviduct, Gc-genital chamber, Gr-germarium, Lo-lateral oviduct, Ov-ovary, Sh-sheath, Tr-trachea, Vt-vitellarium.



Histological sections of the germarium reveal the presence of trophocytes, prefollicular cells, young oocytes, and nutritive cords (Figure 2b). The nuclei of the trophocytes are large and round (Figures 2c, d). The nuclei of the prefollicular cells are smaller and oval-shaped compared to those of the trophocytes (Figures 2b, c). Figure 3a shows the surface of oocytes at different developmental stages in the vitellarium region, located immediately posterior to the germarium, together with associated tracheal networks. During the early stages of oocyte development in the vitellarium, no accumulation of yolk granules is observed in the ooplasm, which exhibits a basophilic appearance. The ooplasm is surrounded by a single layer of epithelial cells ranging from cuboidal to short columnar, with oval nuclei containing patches of heterochromatin (Figures 3b, d). Interfollicular cells are present between adjacent oocytes (Figure 3d). SEM micrograph also show that the oocytes are enclosed by a network-like sheath associated with tracheal nets (Figure 3c).

Figure 2.

a: SEM image of the suspensory ligament extending from the tip of the ovarioles; b: Longitudinal section of the germarium showing trophocytes, prefollicular cells, nutritive cord, and young oocyte (LM, H&E); c, d: LM (H&E), and SEM images of trophocytes in the germarium. Gr-germarium, Nc-nutritive cord, Prf-prefollicular cells, Sh-sheath, Sl-suspensory ligament, Trf-trophocytes, Yo-young oocyte.

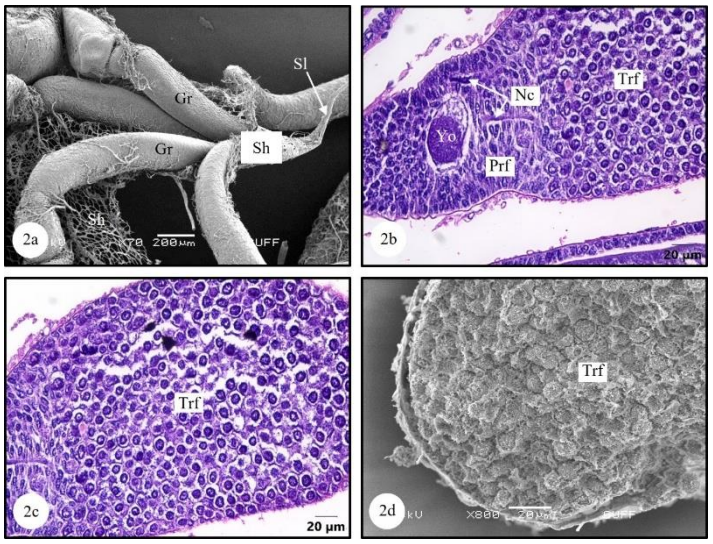
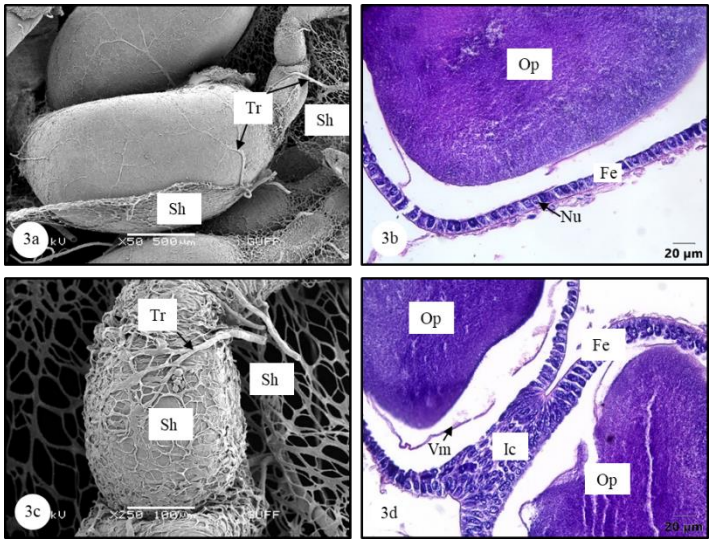


Figure 3.

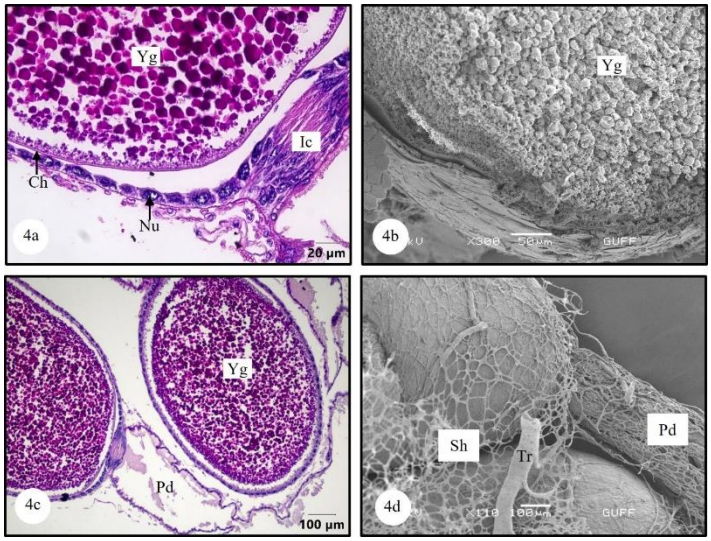
a: SEM image of oocytes at different developmental stages in the vitellarium, located posterior to the germarium; b: Early-stage oocyte showing ooplasm and follicular epithelium (LM, H&E); c: Tracheal network on the sheath surrounding an early-stage oocyte (SEM); d: Interfollicular cells between oocytes (LM, H&E). Fe-follicular epithelium, Ic-intercellular cells, Nu-nucleus, Op-ooplasma, Sh-sheath, Tr-trachea, Vm-vitellin membrane.



In the later stages of oocyte maturation (choriogenic oocyte), the ooplasma becomes filled with acidophilic yolk granules, and the oocytes are surrounded by a single layer of flattened epithelial cells possessing nuclei dense in heterochromatin (Figures 4a–c). Beneath this epithelial layer, the formation of the chorion becomes evident (Figure 4a). The terminal portion of the ovariole, the pedicel, is lined with a single layer of epithelial cells, and the continuation of the reticulated sheath is also observed on its surface (Figures 4c, d).

Figure 4.

a, b: Accumulation of yolk granules in the ooplasm of a late-stage oocyte (LM, H&E, SEM); c: Histological section of the pedicel, the terminal portion of the ovariole (LM, H&E); d: SEM image of the sheath surrounding the pedicel. Ch-chorion, Ic-intercellular cells, Nu-nucleus, Pd-pedicel, Sh-sheath, Tr-trachea, Yg-yolk granules.



Discussion

The female reproductive system of *E. arctos anatolica* generally exhibits similarities to the overall morphological characteristics of the order Coleoptera. In this species, the presence of paired ovaries, lateral oviducts, a common oviduct, and a genital chamber reflects the typical organization of the female genital system reported in many insect species (Carrillo-Ruiz et al., 2008; Ferreira & McKinlay, 2001; Martínez et al., 2001; Stringer, 1988). Variations in the female reproductive system arise from features such as the type of ovarioles in each ovary, the number of ovarioles, the presence or absence of suspensory ligaments, and the number of oocytes in the vitellarium, all of which are of systematic significance.

In the two major suborders of Coleoptera, Polyphaga and Adephaga, most Polyphaga species possess telotrophic meroistic ovarioles, whereas Adephaga species typically exhibit polytrophic meroistic ovarioles. This distinction highlights the evolutionary diversity of reproductive structures between the suborders and provides valuable characters for systematic and phylogenetic analyses (Büning, 2006). In *E. arctos anatolica*, telotrophic-meroistic ovarioles were observed.

The Glaphyridae belongs to the superfamily Scarabaeoidea and is phytophagous. the family exhibits particularly close phylogenetic relationships with the family Scarabaeidae (Mckenna et al., 2015). Differences in the number of ovarioles among insect species are generally regarded as a reflection of the diversity of reproductive strategies (Chapman, 2013; Heming, 2018; Klowden, 2013). In female *E. arctos anatolica*, each ovary consists of six ovarioles. This number may vary among species. For example, each ovary of *Cephalocyclus hogei* (Aphodiidae) contains five ovarioles (Martínez et al., 2001), while species such as *Costelytra zealandica* (Scarabaeidae), *Prodontria lewisii* (Scarabaeidae), and *Hoplia subcostata* (Melolonthidae) possess six ovarioles per ovary (Carrillo-Ruiz et al., 2008; Stringer, 1988; Ferreira & McKinlay 2001). In contrast, ovariole numbers in the genus *Ataenius* (Scarabaeidae) vary considerably: three in *A. cribrithorax*, eight in *A. apicalis*, nine in *A. sculptor*, and eleven in *A. paraperforatus* (Martínez et al., 2001). Additionally, each ovary of *Aphodius opisthius* (Scarabaeidae) contains nine ovarioles (Martínez et al., 2001). In *Chrysomela populi* (Chrysomelidae), each ovary has 14 ovarioles (Özyurt Koçakoğlu et al., 2021), whereas *Stolas conspersa* (Chrysomelidae) and *Isotomus speciosus* (Cerambycidae) each have 28 ovarioles per ovary (Özyurt Koçakoğlu & Candan, 2025; Simões, 2012). In *Mylabris cernyi* (Meloidae), each ovary contains 50 ovarioles (Özyurt Koçakoğlu & Candan, 2025). These differences indicate that the number of ovarioles is directly related to the egg production capacity of a species. Therefore, the presence of six ovarioles in *E. arctos anatolica* suggests a moderate reproductive potential. Variation in ovariole number may be influenced by factors such as environmental conditions, life cycle duration, availability and continuity of food resources, and the ecological niche occupied by the species.

The structural components of ovarioles also exhibit variation and are of systematic importance. Each ovariole typically comprises a terminal filament, germarium, vitellarium, and pedicel (Carrillo-Ruiz et al., 2008; Malekpour et al., 2010; Mohamed et al., 2015; Özyurt Koçakoğlu et al., 2021, 2022; Stringer, 1988). In *E. arctos anatolica,* each ovariole consists of a germarium, vitellarium, and pedicel. All ovarioles converge at their anterior ends and are attached to the abdomen via a suspensory ligament. In contrast, the ovarioles of *Cephalodesmius armiger* (Scarabaeidae) consist only of a germarium and vitellarium (López-Guerrero, 1995; Monteith & Storey, 1981). In this species, terminal filaments are absent, and the germarium is connected to abdominal tissues through tracheal connections (López-Guerrero, 1995; Monteith & Storey, 1981).

As *Chrysolina herbacea* (Chrysomelidae), each ovariole of *E. arctos anatolica* contains three to four oocytes (Özyurt Koçakoğlu et al., 2022). In *Liophloeus lentus* (Curculionidae), the vitellarium contains 40-50 oocytes arranged linearly in consecutive stages of oogenesis (Świątek & Górkiewicz, 2006). The number of oocytes may represent an important character reflecting reproductive strategies and systematic differences among species.

Conclusion and Recommendations

In summary, the aim of this article is to identify features that may be useful in future studies on the morphology of the female reproductive system of the order Coleoptera, particularly the order Glaphyridae.

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