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RESEARCH ARTICLE

Anatomical and Histological Structures of the Female Reproductive System of the Adult Lucerne Leaf Beetle *Gonioctena fornicata* (Brüggemann, 1873) (Coleoptera: Chrysomelidae)

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ABSTRACT

Objective: The aim of this study was to determine the anatomical and histological structures of the female reproductive system of *Gonioctena fornicata* (Brüggemann, 1873) (Coleoptera: Chrysomelidae), an economically important alfalfa pest, and to contribute to studies in this field.

Materials and Methods: The anatomy and histology of the female reproductive system and egg structure of adult *G. fornicata* were described using stereo, light, and scanning electron microscopy.

Results: The female reproductive system of *G. fornicata* comprises a pair of ovaries, lateral oviducts, a spermatheca, and a common oviduct. Each ovary contains 14 telotrophic ovarioles with terminal filaments. Just below the terminal filament is the germarium. The germarium surface is smooth and has a pear-shaped appearance. There is a single oocyte in the vitellarium region, located next to the germarium. The oocyte, which completes its development, passes into the pedicel, the last part of the ovariole. The ovarioles are connected to the lateral oviduct via pedicels. Polygonal shapes are observed on the chorion surface of eggs in the lateral oviduct. A pair of lateral oviducts opens into a common oviduct. Spines extend from the intima layer of the lateral and common oviducts towards the lumen. *G. fornicata* eggs laid outside are yellowish in colour and have a cylindrical appearance. The chorion is extremely thin and has polygonal shapes on its surface.

Conclusion: The female reproductive systems of *G. fornicata* are generally similar to those of other species in the subfamily Chrysomelinae.

Keywords: Ovariol, Ovary, Trophocyte, Light microscope, Scanning electron microscope.

INTRODUCTION

The family Chrysomelidae comprises most species-rich and important families.[1,2] This family includes approximately 19 subfamilies and >2,000 genera, and the total number of species is estimated to exceed 50,000. The Palaearctic region is represented approximately 3,500 Chrysomelidae species[3-9] although recent studies indicate that the region is home to approximately 9,293 species. In Turkey, approximately 968 Chrysomelidae species have been identified, representing 11 subfamilies and 113 genera.[10] Chrysomelinae includes important pests and biological control agents and is one of the largest subfamilies in Chrysomelidae.[11,12]

One of the pest species included in the Chrysomelinae is the lucerne leaf beetle *Gonioctena fornicata* (Brüggemann, 1873), which is a pest of plants in the Fabaceae family, especially alfalfa. Both the adult and larval stages of *G. fornicata* (Chrysomelidae) are highly damaging, causing crop losses and significant damage to alfalfa. Adults and larvae of these species feed on the leaves, flowers, leaf buds, young shoots, and stem tips of alfalfa.[13-15]

In the Coleoptera female reproductive system, the ovaries are divided into two or more ovarioles that open into the oviduct.[16] There are terminal filaments, germarium, vitellarium, and pedicel in each ovariol.[17-20] Whereas the division of germ cells and detection of oocyte and follicle formation occur in the germarium, the growth of oocytes and egg formation occur in the vitellarium.[21] There are very few studies on the reproductive systems of the different species belonging to the family Chrysomelidae, which is of great importance for the reproductive system and the classification.[17,22-26] The aim of this study was to determine the female reproductive system and egg structure of *G. fornicata* (Chrysomelidae) anatomically and histo-

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logically and to contribute to the development of more effective control methods against this pest.

MATERIALS AND METHODS

Lucerne Leaf Beetle Samples

The 20 adult female specimens of *G. fornicata* (Chrysomelidae) used in this study were collected in May 2022 from an agricultural field in Elazığ, Türkiye.

Stereo Microscopy

The female reproductive organs of insects anaesthetised with ethyl acetate smoke and dissected in 0.1 M sodium phosphate buffer at pH 7.2, examined, and photographed under an Olympus SZX7 stereomicroscope.

Light Microscopy

For histological examination, 10 female reproductive systems were dissected and fixed in 10% neutral formalin for 24 h. After fixation, the specimens were rinsed with tap water and dehydrated in an ethanol gradient from 50% to 100%. They were then clarified in two batches of xylene for 15 min each and gradually switched from xylene to paraffin. The tissues were then definitively embedded in liquid paraffin at 65°C and solidified at room temperature. 5-6 µm thick sections were obtained from these paraffin blocks using a Microm HM 310 microtome. Finally, sections were stained with Haematoxylin and eosin (H&E) and Mallory's trichrome (M) and visualised and photographed using an Olympus BX51 LM microscope.

Scanning Electron Microscopy (SEM)

For SEM examination, initially, 10 samples were fixed in 2.5% glutaraldehyde and then rinsed with sodium phosphate buffer (pH 7.2). The samples were then dehydrated using a series of ethanol solutions ranging from 50% to 100%. Subsequently, the samples were air-dried using hexamethyldisilazane. Finally, the specimens, which were affixed to SEM stubs with double-sided tape, received a thin gold coating using a Polaron SC 502 sputter coater before examination. Images were captured using a JEOL JEM 6060 LV SEM at 5 kV.

RESULTS

The female reproductive system of *G. fornicata* comprises one pair of ovaries, one pair of lateral oviducts, and one common oviduct. Each ovary consists of four parts; terminal filament, germarium, vitellarium, and pedicel. The ovary consists of 14 telotrophic ovarioles with terminal filaments (Figure 1). The ovariole ends with a terminal filament (Figures 2a and 2b). The germarium is located in the second half of the ovariole. When histological sections are examined, it is seen that the trophocyte proximal to the germarium had large spherical and basophilic nuclei (Figures 2b, 2c and 2d). Germarium has a pear-shaped appearance and a smooth surface (Figure 2e). The germarium length is 291 µm and width is 144 µm, and the width of the terminal filament is 12.9 µm. At the same time, trophocyte were found in the broken samples (Figure 2f).

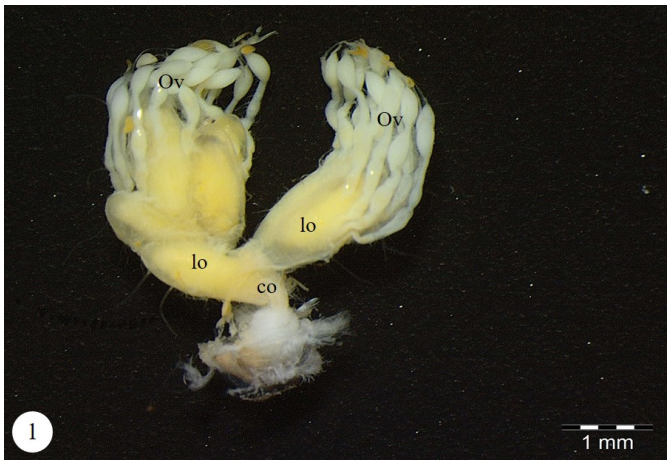

Figure 1. General view of *Gonioctena fornicata* female reproductive system under steromicroscope. co: common oviduct, lo: lateral oviduct, ov: ovariole.

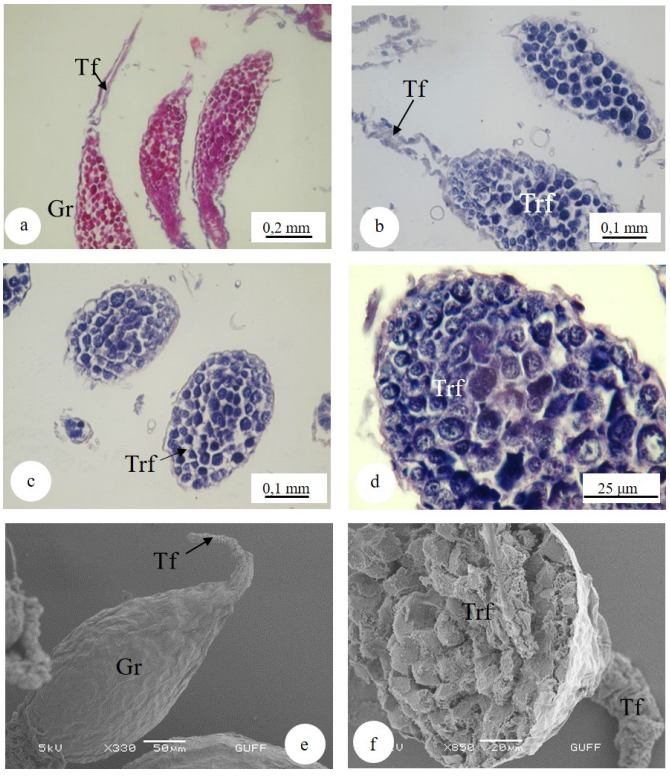

Figure 2. (**a**) Terminal filament extending from the germarium under light microscope: Haematoxylin and eosin (H&E) and Mallory's trichrome (M) staining. (**c,d**). Germarium under light microscope: H&E staining (**e**). Germarium and terminal filament general view under scanning electron microscope. (**f**). Nutrient granules in germarium under scanning electron microscope. Gr: Germarium, Tf: Terminal filament, Trf: Trophocyte.

The germarium contains trophocyte (large nurse cells), young oocytes, and prefollicular cells (Figure 3a). There is a single oocyte in the vitellarium region, located next to the germarium.

The previtellogenic oocyte is surrounded by several layers of follicular epithelium. No nutrients were stored in the ooplasm (Figure 3b). The vitellogenic oocyte is surrounded by a single-layered cylindrical follicle epithelium. Nutrients began to be stored in the ooplasm (Figures 3c and 3d). When examined under a scanning electron microscope, nutrient granules were observed in the ooplasm of the choriogenic oocyte, and the chorion layer was formed (Figure 3e). At this stage, polygonal shapes are distinguished on the chorion surface (Figure 3f).

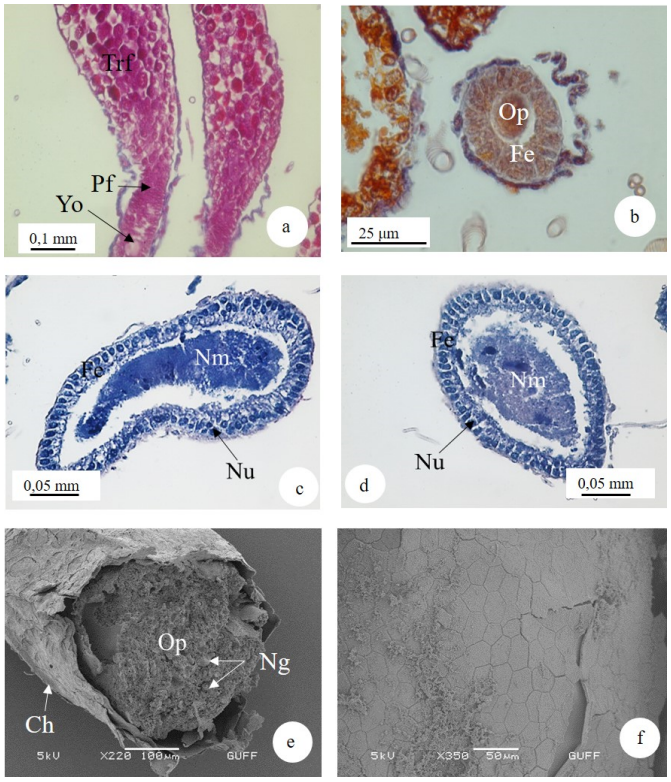

Figure 3. (a) General appearance of trophocytes, prefollicular cells, and young oocytes in the germarium under light microscope: Mallory's trichrome (M) staining. (**b**). Section of previtellogenic oocytes under light microscope: M staining, (**c,d**). Vitellogenesis under light microscope: Haematoxylin and eosin staining. (**e**). Choriogenic oocyte under scanning electron microscope. (**f**). The surface of choriogenic oocyte, under scanning electron microscope. Ch: Chorion, Fe: Follicular epithelium, Nm: Nutrient material, Nu: Nucleus, Ng: Nutrient granules, Op: Ooplasma, Pf: Prefollicular cells, Trf: Trophocyte, Yo: Young oocytes.

The choriogenic oocyte passes into the pedicel, which is the last part of the ovariole. The ovarioles are connected to the lateral oviduct by pedicels (Figure 4a). The last part of the ovarioles is the pedicel, which is surrounded by a single-layer epithelium (Figures 4a and 4b). The pedicels connect to the lateral oviduct, which has a wider lumen (Figure 4a). Histological sections are examined, it is seen that it is surrounded by a single-layer epithelial layer and forms folds towards the lumen (Figures 4c and 4d). The oval nuclei are located towards the middle of the epithelium. When histological sections taken from the lateral oviduct were examined, abundant spine-like structures were observed on the intima (Figures 4c and 4d).

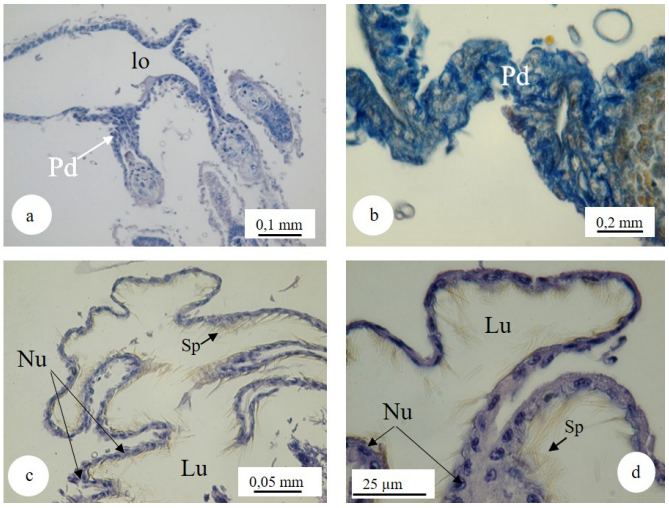

Figure 4. (a) Attachment of the pedicels to the lateral oviduct under light microscope: Haematoxylin and eosin (H&E) staining. (**b**) Longitudinal section of the pedicel under light microscope: Mallory's trichrome staining, (**c,d**). Spines located in the lateral oviduct under light microscope: H&E staining. Sp: Spins, lo: lateral oviduct, Lu: Lumen, Nu: Nucleus, Pd: Pedicel.

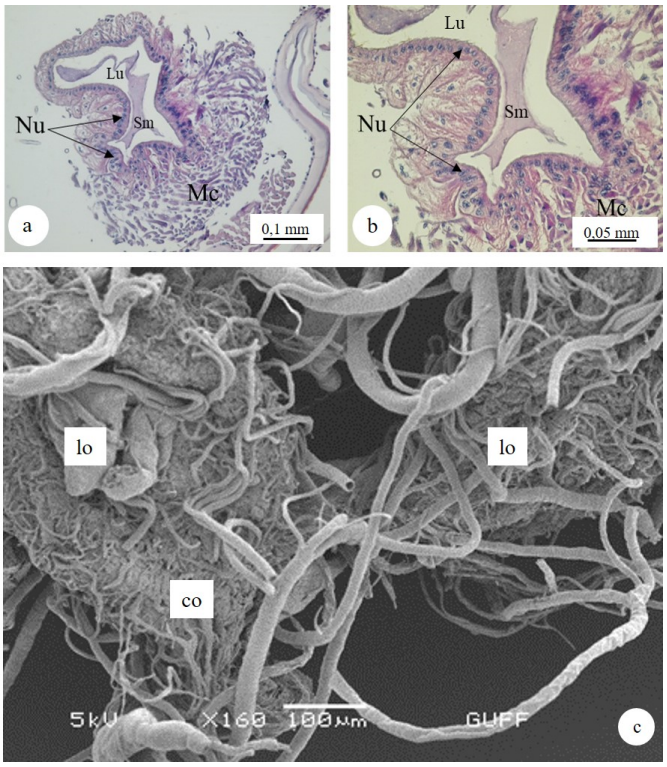

Figure 5. (a,b) Common oviduct histological section under light microscope: Haematoxylin and eosin staining. (**c**). Connection of the lateral oviduct to the common oviduct under scanning electron microscope. co: common oviduct, lo: lateral oviduct, Lu: Lumen, Nu: Nucleus, Sm: Secretory materials, Mc: Muscles.

The lateral oviduct open into the common oviduct, and in the histological sections of the common oviduct, a single-layered epithelium with a round nucleus forming wide folds towards the lumen is observed. Beneath the epithelium, spines extend from the intima layer towards the lumen. Secretory material is remarkable in the lumen. There is a dense amount of muscle

around the common oviduct (Figures 5a and 5b). Numerous trachea networks can be seen on the lateral and common oviduct surfaces (Figure 5c).

G. fornicata (Chrysomelinae) eggs lying outside are yellowish in colour and have a cylindrical appearance (Figure 6a). Eggs are 1.35 mm long and 0.5 mm wide. The egg has a micropyle region (Figure 6c) and polygonal shapes on its surface (Figures 6a-6e). In the SEM image (Figure 6f), the opening made by the larva as it hatches from the egg can be distinguished. Aeropyle openings are evident on the egg side (Figures 6g and 6h). The egg chorion is very thin (~500 nm) (Figures 6ı and 6j).

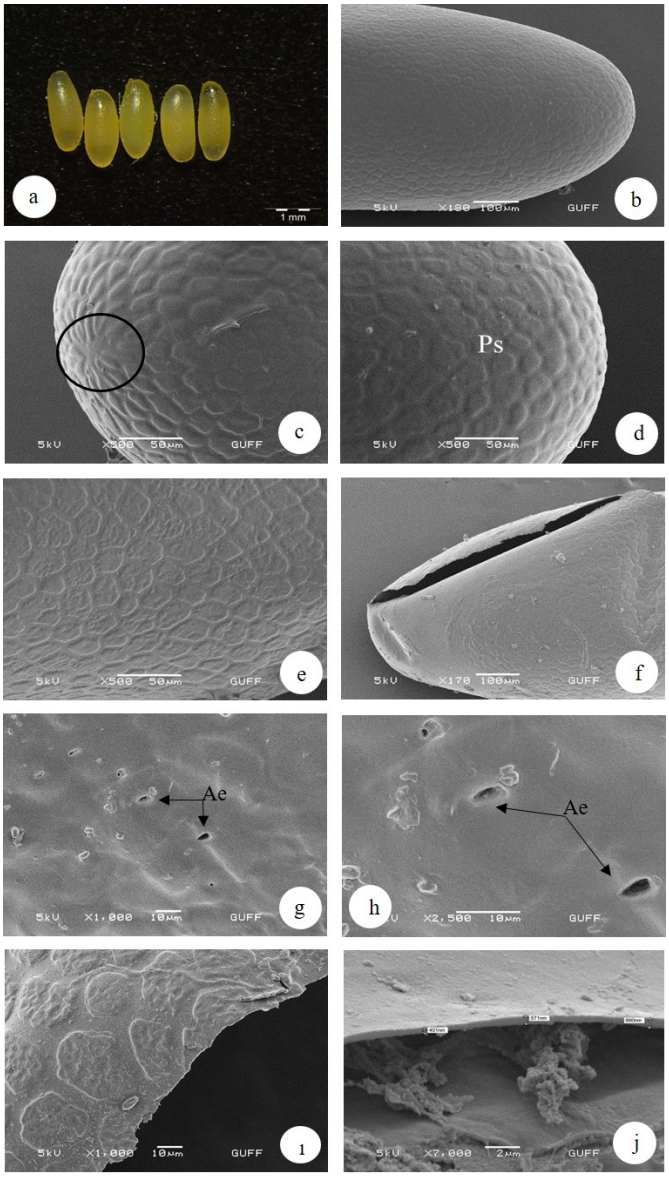

Figure 6. (**a**) *Gonioctena fornicata* eggs in the general view under stereomicroscope. (**b-e**). Micropyle opening and polygonal patterns on the egg surface under scanning electron microscope. (**f**). Opening on the surface of the egg. (**g,h**). Aeropyles on the egg surface under scanning electron microscope. (**i,j**). Chorionic thickness of the egg under scanning electron microscope. Ae: Aeropyle opening, Ps: Polygonal shapes.

DISCUSSION

The female reproductive systems of *G. fornicata* are generally similar to those of other Chrysomelinae species such as *Zygogramma exclamationis* (Fabricius, 1798), *Chrysomela populi* (Fabricius, 1798), *Chrysolina herbacea*, (Duftschmid 1825), and *Phaedon brassicae* (Baly, 1874).[23, 27-29]

Insect eggs develop in highly protected structures called ovarioles.[30] The ovariole is important both systematically and in terms of life history.[31,32] The number of ovarioles affects fertility because each ovary produces its own egg.[33-35] Ovariole numbers play a crucial role in reproductive fitness.[36] Taxonomists find them valuable because of the fluctuation in the ovariole counts of insects over evolutionary time, ranging from increases to decreases and stabilisation. Extreme ovariole numbers often mirror extreme egg production levels, providing insights into evolutionary adaptations.[37] When the number of ovarioles of the studied leaf beetles is analysed, it was observed that the number varied from species to species. For example, the number of ovarioles in *G. fornicata* (Chrysomelinae) consists of 14 telotrophic ovarioles. However, each ovary of *C. herbacea* (Chrysomelinae) contains 18 telotrophic ovarioles.[28] In *Stolas conspersa* (Cassidinae), each ovary contains 28 ovarioles.[17] However, in *Longitarsus nigripennis* (Galerucinae), each ovary comprises 5–7 telotrophic ovarioles.[24] Each ovary of *Callosobruchus maculatus* (Fabricius 1775), (Bruchinae) comprising 6 ovarioles.[26] The ovariole of *G. fornicata* (Chrysomelinae) consists of four parts: terminal filaments, germarium, vitellarium, and pedicel. These structures were similarly reported in *Aspidimorpha sanctaecrucis* (Fabricius, 1792) (Cassidinae), *C. populi* (Chrysomelinae), *C. herbacea* (Chrysomelinae), and *P. brassicae* (Chrysomelinae).[22,27-29]

In *G. fornicata* (Chrysomelinae), spines were observed in groups on the intima of the lateral and common oviducts. In *Chrysomela scripta* (Chrysomelinae), the lateral oviduct intima has spines.[38] In *C. populi* (Chrysomelinae), spines were observed on the intima of the common oviduct.[27]

The pedicel of *G. fornicata* exhibits a singular-layered structure with inward folds directed towards the lumen. Conversely, in *A. sanctaecrucis* (Cassidinae), the ovarian duct is enveloped by a straight forward columnar epithelium.[22]

The surface patterns and shapes of insect eggs are essential. Egg characteristics generally support higher taxonomic groups.[38-40] Additionally, egg size, colour and surface structure vary among genus and are systematically important. *G. fornicata* eggs are yellowish and cylindrical. The eggs of *Sennius bondari* (Pic,1929) (Bruchinae) and *Sennius nappi* Ribeiro-Costa & Reynaud 1998 (Bruchinae) are oval.[39] Similarly, *C. populi* (Chrysomelinae) eggs are light yellow and long.[27] *G. fornicata* (Chrysomelinae) eggs are polygonal, similar to *C. herbacea* (Chrysomelinae) eggs.[28] These structures

were similarly reported in Callosobruchus spp. (Bruchinae) and *Zabrotes subfasciatus* (Boheman, 1833), (Bruchinae) are smooth.[40] Egg size varies in different genera of the Chrysomelidae. *G. fornicata* (Chrysomelinae) egg size is 1.35 mm in length and 0.5 mm in width. *C. populi* (Chrysomelinae) is approximately 1.6 mm in length and 0.6 mm width.[27] *C. herbacea* (Chrysomelinae) eggs are 1.60 mm long and 0.75 mm in width.[28] The eggs of *S. nappi* (Bruchinae) were avoid, 0.74 mm in length and 0.39 mm wide, and were covered by an ornamented membranous composed of small triangles.[39]

CONCLUSION

The morphological structures of the female reproductive organ and egg structure of insects are relatively complex and vary from species to species. Examining these structures is crucial for the fight against these pests. Therefore, in this study, we focused on describing the female reproductive organ and egg structure of *G. fornicata* and presented its similarities and differences with other species. This study intends to contribute to female reproductive biology studies in Coleoptera and other insect order species, which may be useful for future taxonomy studies of insect histoanatomy.

Ethics Committee Approval: Ethics committee approval is not required for the study.

Peer Review: Externally peer-reviewed.

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