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Research Paper

The Morphology and Histology of The Female Reproductive System of *Graphosoma lineatum* (Heteroptera: Pentatomidae) based on light and scanning electron microscope studies

Biology

KEYWORDS : Ovaries, ovarioles, vitellogenesis, tropharium, vitellarium, oocyte.

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ABSTRACT

The female reproductive morphology and histology of Graphosoma lineatum (Heteroptera: Pentatomidae) was examined by light and scanning electron microscopy. We found that the female had a pair of ovaries, each with 7 meroistic telotrophic ovarioles, a pair of lateral oviducts, a common oviduct, a spermatheca, accessory glands and a genital chamber. Each ovariole is composed of four main regions: (1) the thread-like terminal filament (2) the tropharium, a chamber housing anteriorly the nurse cells and posteriorly the young oocytes, (3) a long vitellarium composed of a series of oocytes, which become progressively large towards the posterior and, and (4) the pedicel (ovariolar stalk) in which the mature eggs are lodged before passing into the lateral oviduct.

Introduction

The Heteroptera are a widely distributed and economically important group of insects. Several reports are available on different aspects of the reproductive system of these insects. Accounts on structure of female reproductive system in Heteroptera have been published (Książkiewicz, 1980; Ksiazkiewicz-Kapralska, 1985; Jawale & Ranade 1990, Ksiazkiewicz-Kapralska, 1991; Szklarzewicz, 1998; Fritz & Turner, 2002; Lemos *et al.*, 2005; Jahnke *et. al.*, 2006; Klowden, 2007; Ogorzalek, 2007; Pires *et al.*, 2007; Pluot-Sigwalt, 2008). But yet, all these completed studies are not sufficient if the Pentatomidae is taken into consideration.

The female reproductive system in insects consists of the paired ovaries, lateral oviducts, common oviduct, spermatheca and accessory glands (Kumar, 1962; Pericart, 1972; Ma & Ramaswamy, 1987; Štys *et al.*, 1998; Nation, 2002; Lemos *et al.*, 2005; Jahnke *et al.*, 2006; Klowden, 2007; Pires *et al.*, 2007). The ovaries of insects differ in the way nutrients are available for nourishment of the oocytes (Nijhout, 1998) and they can be divided in the two types: panoistic and meroistic (Davey, 1959; Chapman, 1998; Nijhout, 1998; Lemos *et al.*, 2005). Ovaries of hemipterans are composed of a variable number of telotrophic ovarioles. In each ovariole, a terminal filament is followed by a tropharium which connects to a vitellarium. The tropharium contains trophocytes and arrested oocytes. All germ cells in the ovariole are connected with a central, cell-free region of the trophic chamber, termed the trophic core: oocytes by nutritive cords, trophocytes by cytoplasmic projections. In the ovariole of Hemiptera, only one cluster of germ cells seems to exist (Büning, 1994; Szklarzewicz, 1998). The ovarioles are linked to proximally narrow to a fine tube, the pedicel, which connects to the lateral oviduct. Both lateral oviducts join in a common oviduct which opens into a genital chamber (Chapman, 1998; Nijhout, 1998; Lemos *et al.*, 2005).

The number of ovarioles present in Heteroptera is variable (Miyamoto, 1957; Ksiazkiewicz-Kapralska, 1985; Grozeva & Kuznetsova, 1992; Javahery, 1994; Lalitha *et al.*, 1997; Szklarzewicz, 1998; Vogelsang & Szklarzewicz, 2001; Lis, 2003; Jahnke *et al.*, 2006; Ogorzalek and Trochimczuk, 2009; Chiang, 2010; Szklarzewicz *et al.*, 2005; Ramírez-Cruz *et al.*, 2008).

The present study was undertaken with the aim of gaining further information regarding the structure of the parts of the female reproductive system *G. lineatum*, representative of the Pentatomidae was used in this investigation.

Materials and methods

Gross anatomy analysis

Adult females of *G. lineatum* were collected from in june and july 2008-2010, in Ankara, Turkey. Adult females were killed with ethyl acetate fumes and dissected in 70% ethyl alcohol under a steromicroscope. For the morphological analysis, the dorsal cuticle was first removed from the prothorax to the last full-sized abdominal segments. Subsequently, the epidermis and the digestive system were removed. Two insect pins were inserted laterally through the last full-sized sternite to spread apart the abdomen and to expose the base of the reproductive system attached to the genital segments. The gross morphology of the the reproductive systems of the females were examined and photographed with a Leica EZ4D stereomicroscope.

Light microscopy

For the histological analysis, the reproductive systems of fifteen females were fixed in Bouin's for 48h. Thereafter, the tissues were washed, dehyrated in a grade series of ethanol solutions (70%,% 80,% 90, 100%) and finally embedded in paraffin. Paraffin sections were cut into 6 µm thick slides and stained with Mallory's triple stain for light microscopic examination. The sections were viewed and photographed by using a Olympus BX51 microscope.

Scanning electron microscopy

For the scanning electron microscopy, cleaned and dried specimens (Polaron CPD 7501 Critical Point Dryer) were mounted using double sided tape on SEM stubs, coated with gold in a Polaron SC 502 sputter coater, and examined with a JEOL JSM 6060 LV scanning electron microscope at accelerating voltage 10-15 kV. Photos were taken.

Results and Discussion

Morphology

The female internal reproductive system in insects generally, and in Heteroptera particularly, as in female reproductive of *G. lineatum* consists of the paired ovaries with ovarioles, lateral oviducts, a common oviduct, spermatheca and accessory glands (Figs. 1-3) (Kumar, 1962; Pericart, 1972; Nation, 2002; Lis, 2003). The ovaries lie vento-laterally in the body cavity of the abdomen on either side of the alimentary canal. The ovarioles in *G. lineatum* develop synchronously; all ovarioles mature at the same time. The same synchrony in ovarian maturity is in *Porphyrophora polonica* (Hemiptera: Margarodidae) (Szklarzewicz, 1998) and *Steingelia gorodestskia* (Hemiptera: Steingeliidae) (Koteja *et al.*, 2003). However, in *Gossyparia spuria* (Hemiptera: Eriococcidae) (Szklarzewicz, 1998) and *Dactylopius coccus* (Hemiptera: Dactylopiidae) (Ramírez Cruz *et al.*, 2008) the ovaries asynchronously. Like other Heteropters ovarioles in *G. lineatum* are egg-producing tubules that are the fundamental units of ovaries and each ovariole consists of a terminal filament, tropharium, vitellarium, pedicel (Figs. 2-5) (Ma & Ramaswamy, 1987; Davey, 1959; Chapman, 1998; Nijhout, 1998; Lis, 2003; Lemos *et al.*, 2005; Jahnke *et al.*, 2006), but a terminal filament is lacking in *Orthezia urticae* (Hemiptera : Orthezidae) (Vogelsang and Szklarzewicz, 2001) and *D. coccus* (Hemiptera:) (Ramírez Cruz *et al.*, 2008).

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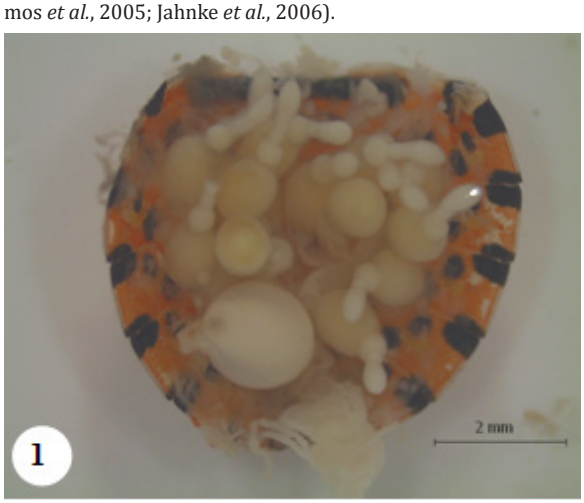
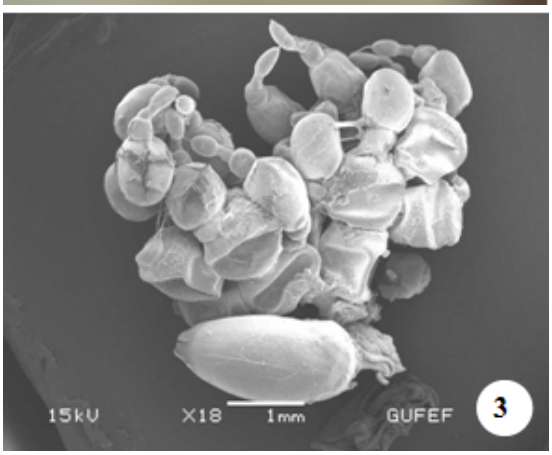
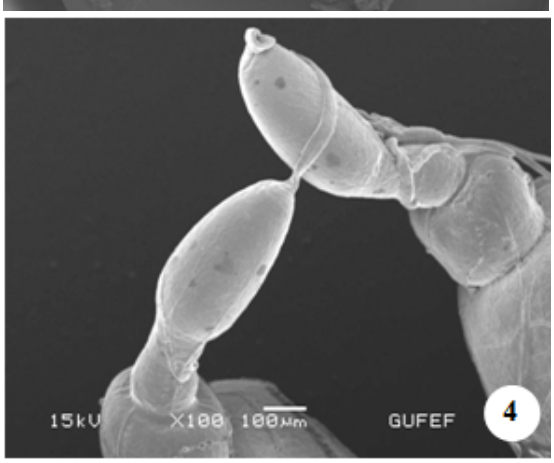
The number of ovarioles in ovaries present in Hemiptera is extremely variable, for example, there are 8-12 in *Parapiesma quadratum* (Hemiptera: Piesmidae) (Grozeva and Kuznetsova, 1992), 10 in *Abedus ovatus* (Hemiptera: Belastomatidae) (Lalitha *et al.*, 1997), 14 in *Cosmolopius nigroannulatus* (Hemiptera: Reduviidae), *Leptoglossus occidentalis* (Heteroptera: Coreidae) and *Coreus marginatus* (Hemiptera: Coreidae) (Jahnke *et al.*, 2006; Chiang, 2010; Ogorzalek, 2007), 14 in *Legnotus limbosus*, *Ochetostethus tarsalis* and *Tritomegas bicolor* (Hemiptera: Cydnidae) (Lis, 2003), 30 in *Orthezia urticae* (Hemiptera: Orthezidae) (Vogelsang & Szklarzewicz, 2001), 48 in *Elasmucha grisea* (Heteroptera: Acanthosomatidae) (Ogorzalek and Trochimczuk, 2009), 100 in *Palaecoccus fuscipennis* (Hemiptera: Monophlebidae) (Szklarzewicz *et al.*, 2005), between 100 and 200 in *Dysmicoccus newsteadi* (Hemiptera: Pseudococcidae), *Kermes quercus* (Hemiptera: Kermesidae), *Eriococcus buxi* (Hemiptera: Eriococcidae), *G. spuria*, *Cryptococcus fagisuga* (Hemiptera: Cryptococcidae), *Pseudochermes fraxini* (Hemiptera: Cryptococcidae), approximately 300 in *P. polonica* (Szklarzewicz, 1998), 400 in *D. coccus* (Ramírez-Cruz *et al.*, 2008).

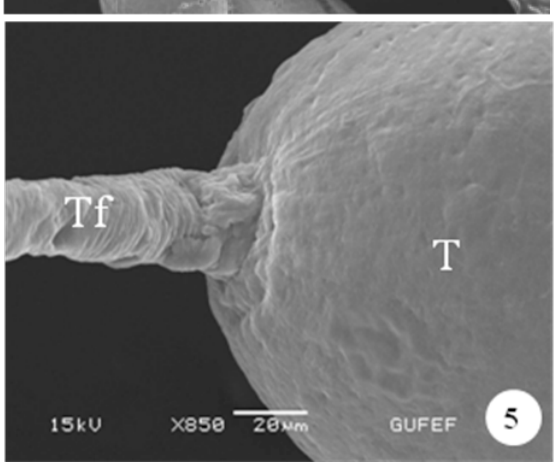
The ovarioles resemble long filaments and they involve by a tracheal network (Figs. 2-4). Each ovary of the *G. lineatum* have ovarioles join through the terminal filaments (Fig. 5) and forming a compact bunch shape structure. The other region of ovarioles in *G. lineatum* is constituted by vitellarium. In the vitellarium there was always from three to four oocyte in vitellogenesis, contrasting with *O. urticae*, *D. coccus* and *E. grisea* in which always only one oocyte in vitellogenesis (Vogelsang & Szklarzewicz, 2001; Ramírez-Cruz *et al.*, 2008). The vitellarium is characterized by oocytes in three different development stages: Previtellogenesis, vitellogenesis and choriogenesis. Oocyte surface in the previtellogenesis stage is not development (Fig. 6). Oocyte surface in the vitellogenesis stages, variable shaped polygons are clearly seen by a scanning electron microscope (Figs 6-8). Oocyte surface in the choriogenesis stages, extend the series of chorionic spines (Figs 9-12). Candan & Suludere (1999) stated that chorionic spins in surface of eggs of *G.lineatum* extend from the egg in an upright position and are connected with each other by somewhat flattaned ridges arranged to form an irregular polygonal pattern.

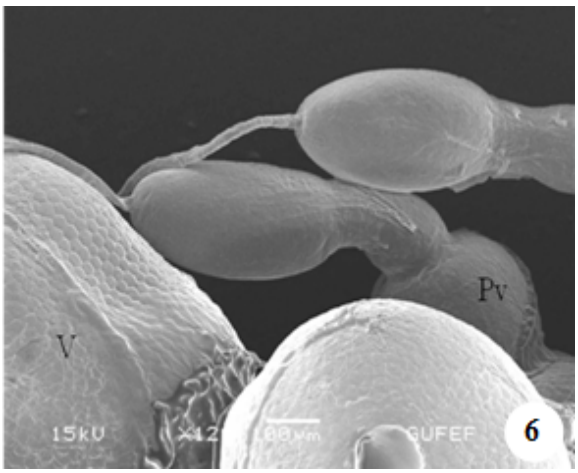
Each ovariole opens through a pedicel in the calix of the lateral oviduct. It joins in the middle line of the abdominal cavity, forming the common oviduct, which is very short relative to the total size of the reproductive system. The common oviduct opens ventrally the genital chamber. The oocytes leave the terminal portion of the ovariole towards the lateral oviduct and are fertilized when going through the genital chamber. This structures are similar *Podisus nigrispinus* (Heteroptera: Pentatomidae) and *Cosmoclopius nigroannulatus* (Hemiptera: Reduvidae) (Lemos *et al.*, 2005; Jahnke *et al.*, 2006).



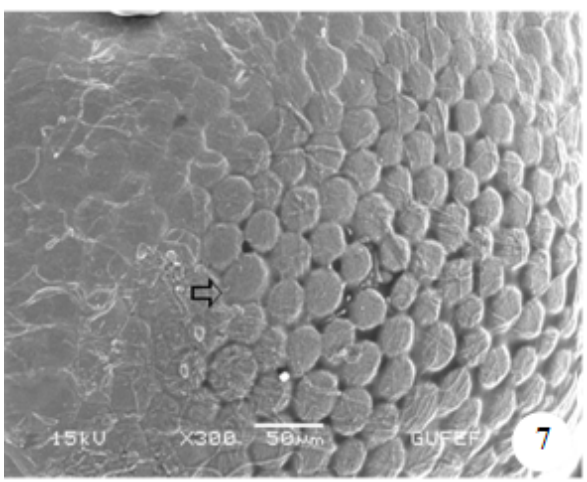


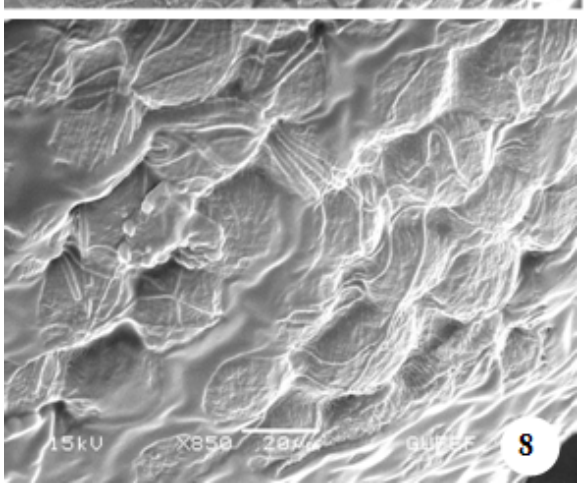


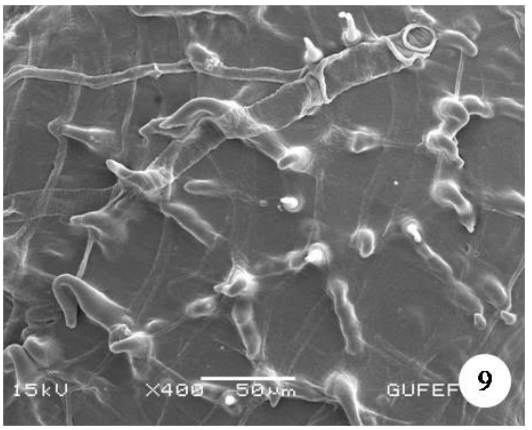


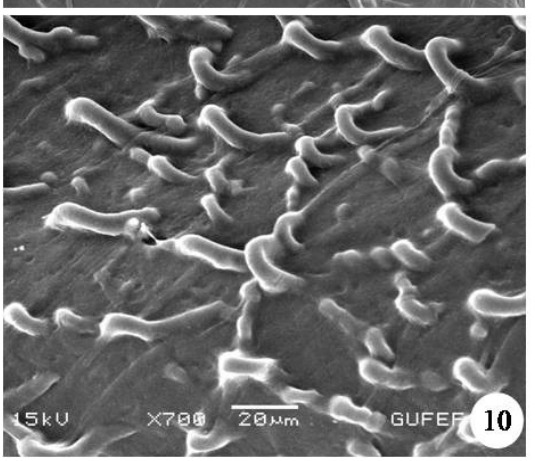


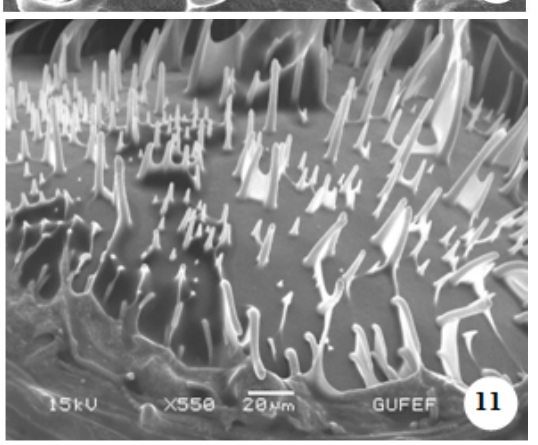
Figs. 1-3. General view (Light and SEM photos) of female reproductive system of *G. lineatum* in abdomen cavity. **Fig. 4.** Terminal filaments extending ovariole of *G. lineatum*. **Fig. 5.** Tropharium (T) and terminal filament (Tf). **Fig. 6.** Previtellogenic ovarian follicle (Pv), vitellogenic ovarian follicle (V).

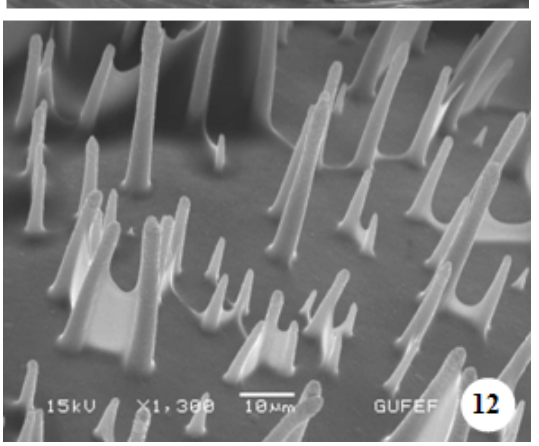












Figs 7-8. Polygonal shape in oocyte surface in the vitellogenesis stages (♋) **Figs 9-10.** Chorionic spines below the sheath in the choriogenesis stages. **Figs. 11-12.** Chorionic spines in oocyte surface in the choriogenesis stages.

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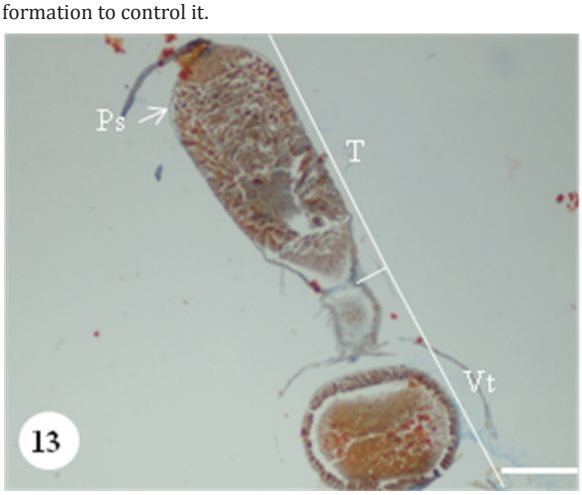
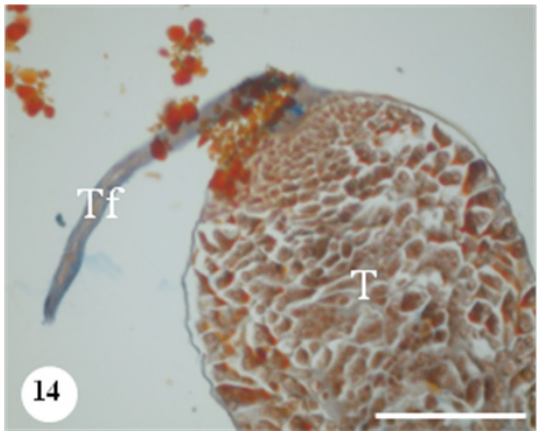
Histology

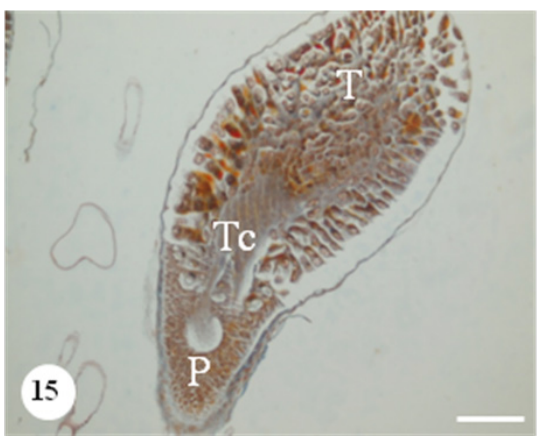
The ovary of *G. lineatum* as in other Heteropterans has the telotrophic-meroistic type and each ovary is covered by the peritoneal sheath (Fig 13) (Ma & Ramaswamy, 1987; Jawale & Ranade, 1990; Lemos *et al.*, 2005). Each ovariole divide in terminal filament, a tropharium (trophic chamber), a vitellarium, a pedicel (Fig. 13). The terminal filament is built with spindle-shaped cells, orient perpendicularly to the long ovariole axis (Fig. 14). The apical part of each ovariole is formed by a tropharium region with germinal cells and nurse cells. The central part of the tropharium was occupied by the trophic core, made up of acellular structure. The nurse cells were connected to the trophic core by trophic processes. The trophic core and the trophic processes had a fibrillar structure that ran lengthwise through them. At the base of the tropharium some prefollicular cells of approximately elongated form were distinguished (Fig. 15). The other region of ovarioles in *G. lineatum* is constituted by vitellarium. This region is characterized by oocytes in three different development stages: previtellogenesis, vitellogenesis and choriogenesis (Fig. 16). In the previtellogenic stage, oocytes descend into the apical of vitellarium and increase in size, while maintaning contact with the trophic core by means nutritive cords. This oocyte is surrounded by prefollicle epithelium (Fig. 16). At the vitellogenesis and choriogenesis, the oocytes is located at the basal of vitellarium. During vitellogenesis, the oocyte is surrounded by a single layer follicular epithelium, in ooplasm accumulate substances-yolk granules and lipid droplets (Figs. 16-18) and is attached to the trophic core by the trophic cord (Fig. 17). In this stage, ovarioles are characterized by the development of the intercellular spaces in the follicular epithelium. Follicular cells originate as a result of proliferation of prefollicular cells. At the beginning of oocyte growth (previtellogenesis and early vitellogenesis) follicular cells are cylindirical, during advanced vitellogenesis they transform into cubes (Figs 17-19). Vitellogenesis ends with the formation of vitelline membrane on the oocyte surface. During choriogenesis, an egg shell consisting of an external layer (exochorion) and an internal one (endochorion) is formed on the surface of the vitelline membrane (Fig. 20). There isn't nutritional cord at oocyte in this stage.

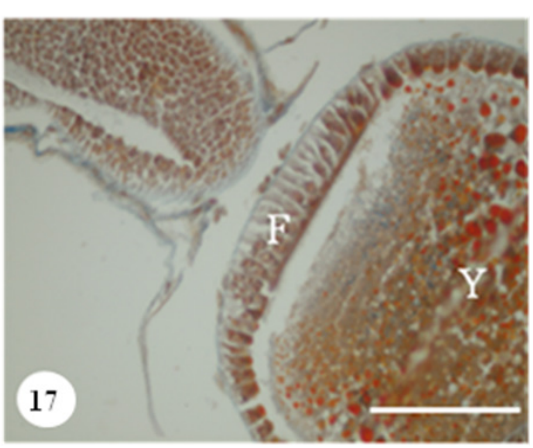
Our studies have also shown that the histology of the ovariole of *G.* lineatum is similar to that of another representative of Heteroptera (Szklarzewicz, 1998; Pires *et al.*, 2007; Ramìrez-Cruz *et al.*, 2008; Chiang, 2010).

This study aimed to describe the morphology and histology of the female reproductive system of *G. lineatum*, to contribute for a better understanding of its biology, to provide additional information to control it.









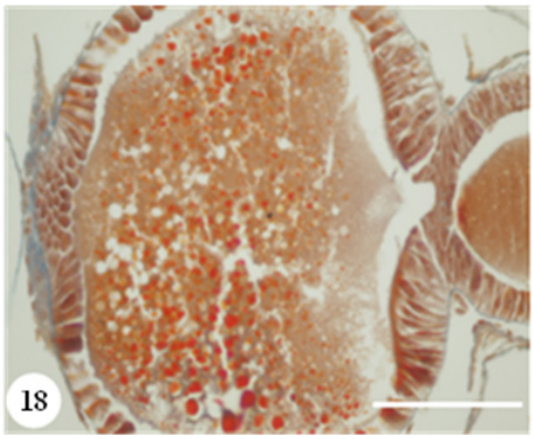
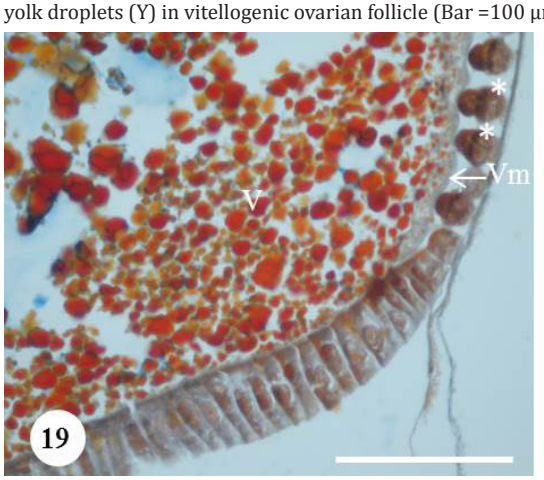


Fig. 13. Longitudinal section of ovariol is covered by the peritoneal sheath (Ps) of *G. lineatum*, Tropharium (T), vitellarium (Vt) (Bar =200 µm) **Fig.14.** Terminal filament (Tf), Tropharium (G). **Fig 15.** Tropharium (T), trophic chamber (Tc), prefollicular cells (P). **Fig. 16.** Previtellogenic ovarian follicle (Pv), vitellogenic ovarian follicle (V). **Figs. 17-18.** Follicular epithelium (F), yolk droplets (Y) in vitellogenic ovarian follicle (Bar =100 µm).



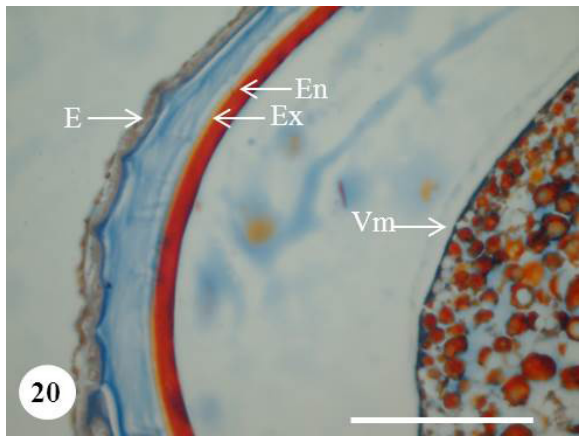


Fig. 19. Vitellus (V), vitellin membrane and intercellular spaces (*) in the follicular epithelium of vitellogenic ovarian follicle. **Fig. 20.** Epithelium (E) Exochorion (Ex), endochorion (En), vitellin membrane (Vm) in choriogenic ovarian follicle (Bar =500 µm).

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