Manuscript

Original Article

Histological organization of tephritid fruit flies (Diptera, Tephriridae) from Thailand

Pisit Poolprasert[1], Sinlapachai Senarat[2,*], Lamai Thongboon[3], Narit Thaochan[4], Ezra Mongkolchaichana[5], Woranop Sukparangsi[6], and Natthawat Charoenphon[7]

[1] Program of Biology, Faculty of Science and Technology, Pibulsongkram Rajabhat University, Phitsanulok, 65000, Thailand

[2] Department of Marine Science and Environment, Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang Campus, Sikao, Trang, 92150, Thailand

[3] Division of Biological Science, Faculty of Science, Prince of Songkla University, Songkhla 90110, Thailand

[4] Pest Management Biotechnology and Plant Physiology Laboratory, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand.

[5] Department of General Education Faculty of Science and Health Technology Navamindradhiraj University Bangkok, 10300 Thailand

[6] Department of Biology, Faculty of Science, Burapha University, Chon Buri, 20131, Thailand

[7] Department of Anatomy, Faculty of Medical Science, Naresuan University, 65000 Thailand

*** Corresponding author, Email address: Sinlapachai.s@rmutsv.ac.th**

Abstract

The structural evidence of tephritids, notably *Bactrocera albistrigata, B. dorsalis, B. umbrosa*, *Zeugodacus cucurbitae*, and *Z. tau* complex was explored using histology. These tephritids' male reproductive organs comprised two testes, ducts with deferent ducts, seminal vesicles, and tubular exocrine glands. The testicular follicle was investigated as a cyst during four stages of spermatogenesis (spermatogonium, spermatocyte, spermatid, and spermatozoa). Similarly, the female reproductive systems of these fruit flies were morphologically identical, with a pair of ovaries containing seven meroistic ovariole types. There was also a spermatheca, two lateral oviducts, a common oviduct, and a genital chamber identified. Tephritids have digestive systems (foregut, midgut, and hindgut), excretory systems (malpighian tubules), neural systems, integumentary systems (cuticle and epidermis), and adipose tissue. These data are not only significant and publicly available, but they can also be used in future studies such as histopathology, ecotoxicological assays, and phylogenic characteristics.

Keywords: Diptera, systematic histology, tephritid fruit flies, Thailand

1. Introduction

True fruit flies (Diptera, Tephritidae) have about 4,300 species categorized into 500 genera and are found all over the world. They are economically significant and are considered serious crop pests (El Harym & Belqat 2017; Rubabura Chihire, & Bisimwa, 2019). Tephritidae is divided into six subfamilies: Blepharoneurinae, Dacinae, Phytalmiinae, Tachiniscinae, Tephritinae, and Trypetinae. The Dacinae are divided into three major tribes: *Ceratitidini*, *Dacini*, and *Gastrozonini*, with the *Dacini* tribe infesting fruits the most. Pests of commercial fruits and vegetables account for around

10% of the 1,000 already identified species (Doorenweerd, Leblanc, Norrbom, Jose, & Rubinoff, 2018; Kunprom & Pramual 2016).

Histological data frequently give information on cell and tissue modifications that explain organ physiology and system response to a certain environment or therapy. It is widely employed in a variety of downstream applications, most notably aquatic ecotoxicology (Bernet et al., 1999). There are various findings on brain, ocular structure, trachea, blood vessel, and skeleton muscle (Poolprasert et al., 2020), and gonadal tissue (Boonyoung et al., 2020). The reproductive system of insects is similar to that of other invertebrates in architecture and function. The testes are composed of numerous testicular follicles or sperm tubes that produce sperms, whereas the ovaries contain numerous follicles (usually cystic or tube-like) that contain developing eggs (Gullan & Cranston, 2014).

Investigating insect histological organization is essential for comprehending how insect biology has crucial implications such as biocontrol and insect pest outbreaks. *Bactrocera albistrigata*, *B. dorsalis*, *B. umbrosa*, *Zeugodacus cucurbitae*, and *Z. tau* complex were reared and thus selected for histological study from infested different fruits to provide important baseline data on these nuisance pest populations and to be employed as comparisons with other insect groups. It could be employed in the future in terms of systematic science or agricultural management.

2. Materials and Methods

Infested fruits such as angled loofah (*Luffa acutangular* Roxb.), cempedak (*Artocarpus integer* Spreng), chili (*Capsicum annuum* L.), guava (*Psidium guajava* L.), and tropical almond (*Terminalia catappa* L.) were taken from the experimental field of the Department of Pest Management, Faculty of Natural Resources, Prince of Songkla University, Hat Yai district, Songkla province, Thailand. A smartphone was used to picture a fruit fly from the field (Figure 1A).

The larvae-infested fruits were housed in clear plastic boxes (20 centimeter x 25 centimeter x 15 centimeter) with air circulation openings on the top lid. As a pupation substrate, one centimeter of sterile (autoclaved and dried) vermiculite was placed in the bottom of a clear plastic box. Pupae were sieved and placed in a tiny clear plastic box (10 centimeter x 10 centimeter x 10 centimeter) for adult emergence after larvae pupation. Adult fruit flies were maintained in a gauze cage (30 centimeter x 30 centimeter x 30 centimeter) with cube sugar, yeast hydrolysate, and water ad libitum after emergence. Adult flies were recognized after ten days based on the morphological criteria reported by Drew and Hancock (1994), Hardy (1973) and White and Elson-Harris (1994). The species identification of collected flies were *Zeugodacus cucurbitae* (Coquillett) and *Z. tau* complex (Walker) from angled loofah; *Bactrocera umbrosa* (Fabricius) from campedak, *B. dorsalis* (Hendek) from guava and *B. albistrigata* (Meijere) from tropical almond, respectively (Figures 1B-1F). Each species was raised in its own gauze cage (30 centimeter × 30 centimeter × 30 centimeter) with cube sugar, yeast hydrolysate, and water as needed. Adult fruit flies were reared in a laboratory setting with a 12:12 h light: dark, 75-80% relative humidity (RH), and a temperature 27 ± 2 °C. In this study, 10 individuals of each species and 5 individuals of each sex of each adult fly species were employed. Adult flies were euthanized by quick cooling shock following emergence of each species, and fresh specimens were fixed with Davidson's fixative solution (~48 hr) for histological inspection (Wilson et al., 2009).

The abdominal segments of all samples were dissected and morphologically evaluated in Ringer's solution using a stereomicroscope (Leica 750; Leica Camera AG, Wetzler, Germany). The traditional histology method was then applied to all systems (Suvarna et al., 2013). Tissue was paraffin embedded, sectioned through a microtome (OSK 97LF506) at 4 m thicknesses, and stained with Harris's haematoxylin and eosin (H&E). A digital light microscope was employed after staining to analyze and photograph different histological features of these fruit files including the reproductive system, digestive system, excretory system, integumentary system, nervous system, and adipose tissue (Leica TE750-Ua, Boston Industries, Inc., USA). The details of those histological systems in both male and female fruit flies are discussed here.

3. Results

Morphology and Histology of Male Reproductive System

The male reproductive system of recognized laboratory tephritid fruit flies (*Bactrocera albistrigata*, *B. dorsalis*, *B. umbrosa*, Zeugodacus *cucurbitae*, and *Z. tau* complex) was shared under the stereomicroscope. They shared a pair of testes that linked to the deferent duct morphologically. Throughout the ejaculatory bulb, there were two pairs of seminal vesicles and two pairs of auxiliary glands (Figure 2A).

The testicular capsule protected the many follicles seen in each testis (Figures 2B-2C). It was enclosed by a peritoneal membrane and had substantial cysts within the testicular follicle, with three zones (growth, maturation, and transformation). The spermatogenic stages of all fruit flies in the follicle were usually classified into four phases based on the morphology and histological organization of chromatin: spermatogonia, spermatocyte, spermatids, and spermatozoa (Figures 2C-2D).

The spermatogonium was found at the follicle's apex. It was the biggest cell, measuring 10-12 m in diameter. This stage's nucleus was big and included eosiophilic cytoplasm (Data not shown). Mitotic division transforms the spermatogonium into spermatocytes. It had an oval-spherical form (Figure 2C). The resultant nucleus of primary spermatocytes originally compacted the chromatin at the growing zone. The spermatocyte then formed, going through the second meiotic division. This process is also known as "spermiogenesis." At the maturation zone, the spermatid's chromatin in the nucleus was severely compressed, but its eosinophilic cytoplasm was rare (Figure 2D). Spermatozoa were the final stage of the spermatogenesis process. It could be discovered in a transition zone. Spermatozoa with expanded heads and tails were discovered in abundance (or flagellum). Spermatozoa from the testis were released into the deferent duct.

The lumen of the seminal vesicle includes a significant number of free spermatozoa (Figure 3A). A single layer columnar epithelium lined the auxiliary gland wall, which was externally bordered by a thin muscle layer (Figure 3B).

Morphology and Histology of Female Reproductive System

All tephritid fruit fly female reproductive systems are morphologically formed of a paired ovary with ovarioles, lateral oviducts, a common oviduct, and spermatheca (Figure 4A). Each ovariole displayed three distinct regions: terminal filament, germarium (trophic chamber), and vitellarium. Oocyte differentiation stages were also discovered in the ovary (Figure 4B).

Typically, the germarium was split into three stages: oogonium, previtellogenic (Pv), and vitellogenic (Vg). However, in all samples, only the Pv phrase was detected in the ovary. It had an oval form. They were distinguished by a single layer of elongated cuboidal follicle cells and a central nucleus with homogeneous basophilic cytoplasm (Figures 4C-4D). There was a huge nurse cell with a well-developed nucleus, as specified by the term "polytrophic meroistic type" (Figure 4C).

The common oviduct stood out, linking the spermatheca with a slender duct (Figure 4A). The oviduct was commonly lined with simple columnar epithelium and was rarely surrounded by a muscle layer (Figure 4E).

Histology of Digestive System

The digestive tracts of the five fruit fly species examined were histologically similar, with three fundamental sections: foregut, midgut, and hindgut (Figure 5A). The foregut was a short tract that included the esophagus and the month (Data not shown). The esophageal valve was situated between the foregut and the midgut (Figure 5B). A model of this valve was exhibited, including epithelial and muscular protrusion from the foregut into the midgut. This valve contained two cell layers: inner and outer (Figure 5C). The outer cell layer was lined by epithelial cells with a high density of simple columnar cells and a predominant oval basophilic nucleus, whereas the inner cell layer was lined by a simple cuboidal epithelium (Figure 5C). The midgut was the alimentary canal's longest segment (Figure 5A). It was lined with several cell kinds (epithelial and basal cells). The epithelial cell had a big columnar cell with a microvilli-covered surface (or brush border) surrounded by acidophilic cytoplasm. A tiny basal cell was found in the epithelium's basal area (Figure 5D). It was spherical and had a prominent basophilic nucleus. The digestive tract's final portion was the hindgut (Figure 5A). A projecting epithelial layer, surrounded by a thick layer of muscle tissue, was observed (Figure 5F).

Histology of Excretory System

The malpighian tubules (MTs) were the most abundant organs in the excretory system (Figure 6A). The MTS were situated between the midgut and the hindgut. A single layer of pavement cells lined the epithelium, which was covered by a massive nucleus projecting into the tubule lumen (Figure 6B).

Histology of Nervous System

The brain and ventral nerve cord comprised the central nervous system (CNS) of the tephritids (subesophageal ganglion and thoracico-abdominal ganglion). The brain was a bilobed structure positioned dorsally in the head, with a cortex part housing the neural cell and a medullar region carrying the neural fiber (Figures 6C-6D). A perineurium protective sheath with a connective tissue layer was created (Figure 6D). Histological slides revealed that the ganglia of the ventral nerve cord were similarly organized. The ganglion contains neurons, neurosecretory cells, and supporting cells, whereas the medullary area contains neural fibers (Figure 6E).

Histology of Adipose Tissue

Histological tests revealed that tephritids' adipose tissue was widely dispersed throughout the body (Figure 7A). It was mostly composed of oenocytes and trophocytes (Figures 7B-7C). Oenocytes possessed a distinct perinuclear basophilic cytoplasm, and peripheral chromatin has been identified in the nucleus (Figure 7B). Eosinophilic granules and vacuoles kept the round-trophocytes mostly contained (Figure 7C).

Histology of Integument

The integument was shielded by two layers (cuticle and epidermis) (Figure 7B). A light microscopic examination revealed two layers: endocuticle (thin layer) and exocuticle (thick layer) (Figure 7D).

Histology of Respiratory System

The respiratory structure of tephritids may be found all over the body (Figure 7E). This structure was observed among the adipose tissue (Figures 7E-7F) and was an elongated tube (Figure 7F).

4. Discussion

The histological organization of tephirid fruit flies namely *Bactrocera albistrigata*, *B. dorsalis*, *B. umbrosa*, *Z. cucurbitae* and *Zeugodacus tau* complex gathered from Thailand was explored in this time. They all had a pair of testes implanted in a mass of fat bodies above the intestine of flies, followed by ducts comparable to deferent ducts, seminal vesicles, and tubular exocrine glands, as proposed by Shehata et al (2011) in *B. zonata*. Histologically, the testicular follicle appeared as a cyst with four distinct spermatogenesis phases (spermatogonium, spermatocyte, spermatid, and spermatozoa). These histological traits were nearly similar found to other insects such as predatory stinkbugs, *Podisus nigrispinus* (Lemos, Ramalho, Serrao, & Zanuncio, 2005), hairy shieldbugs, *Dolycoris baccarum* (Özyurt, Candan, & Suludere, 2012), shieldbugs, *Graphosoma lineatum* (Hemiptera: Pentatomidae) (Özyurt, Candan, Suludere, & Amutkan, 2013), tortoise beetles, *Aspidimorpha sanctaecrucis* (Coleoptera: Chrysomelidae) (Boonyoung et al., 2020) and giant water bugs, *Belostoma* spp. (Hemiptera, Belostomatidae) (Munhoz Serrão, Dias, Lino-Neto, de Melo, &

Araújo, 2020). Similarly, the internal structural features of the female reproductive system of these common fruit flies were equivalent, with a pair of ovaries constituted of seven different ovariole types. A spermatheca, a lateral oviduct, and a genital chamber were also found as Chou et al. (2012) hypothesized in *B. dosalis*. In the same manner, these features were relative similarly to that of insects for example *Aedes aegypti* (Diptera: Culicidae), *Perillus bioculatus* (Hemiptera: Pentatomidae) and *Trypophloeus klimeschi* (Coleoptera: Curculionidae) (Adams, 2000, 2001; Gao, Wang, & Chen, 2021; Zhang, Goh, Ng, Chen, & Cai, 2023). It had a well-developed nucleus, as designed "polytrophic meroistic type with the nurse cells contained with the follicle," as previously described in other insect orders (Hymenoptera and higher Diptera) (Dong, Ye, Guo, Yu, & Hu, 2010; Okada, Miyazaki, Miyakawa, Ishikawa, Tsuji, & Miura, 2010). Nurse cells are necessary for oocyte development because they produce RNA and proteins that are transported to the follicular epithelium of the oocyte (De Loof, Geysen, Cardoen, & Verachtert, 1990). The oviduct was mostly covered by simple columnar epithelium and was rarely bordered by muscle. *Acromyrmex balzani*, *A. landolti* and *A. landolti balzani* (Hymeoptera: Formicidae) have these features (Cardoso, Fortes, Cristiano, Zanuncio, & Serro, 2008; Ortiz & Camargo-Mathias, 2007). Conversely, some insect ovaries, designated as panoistic ovaries, occurring in Orthoptera and Dictyoptera, lack nurse cells (Gullan, & Cranston, 2014; Çakıcı, 2016).

The digestive tract of tephritid fruit flies was investigated histologically and revealed a basic tube-like structure divided into three major sections, namely the stomodeum (foregut), mesenteron or ventriculus (midgut), and proctodeum (hindgut). In the transition between the foregut and the midgut, an esophageal valve set showed the extension of the epithelium and muscle from the foregut into the midgut. The longest area was the midgut, which was lined with various cell types such as epithelial and basal cells. The epithelial cell was characterized by a large columnar cell with a microvilli-covered surface (or brush border) and acidophilic cytoplasm. Meanwhile, the hindgut was the digestive tract's final portion. A projecting epithelial layer surrounded by a thick layer of muscular tissue was observed. These feathers relatively differed from recent observation of Somala et al. (2020) who speculated that mucosal foregut of arboreal bicoloured ant, *Tetraponera rufonigra* (Hymenoptera: Formicidae) was lined by a simple squamous epithelium. The mucosal layer of its midgut was comprised two sub-layers: epithelium and muscular sub-layers. Lastly, the hindgut two layers; epithelium and musculari were clearly observed. Additionally, the malpighian tubules (MTs), the main osmoregulatory and excretory organs of insects, are thin fingerlike extensions connected to the intestinal tract between the midgut and the posterior gut or hindgut (Gullan & Cranston, 2014). Histologically, the MTs were mainly lined with simple cuboidal epithelium. Similar structures have been discovered in stick insect, *Pylaemenes mitratus* (Phasmida: Basillidae) (Harris, Azman, & Othman, 2019), firefly, *Pteroptyx tener* (Coleoptera: Lampyridae) (Othman, Nur Hudawiyah, Roslim, Nur Khairunnisa, & Sulaiman, 2018) and arboreal bicoloured ant, *Tetraponera rufonigra* (Hymenoptera: Formicidae) (Somala et al. 2020).

In general, the insect CNS is separated into three parts: central, visceral, and peripheral sensory nervous systems (Gullan & Cranston, 2014). The CNS of these tephritids was observed to include the brain and ventral nerve cord (subesophageal ganglion and thoracico-abdominal ganglion), with the ventral ganglion formed by the combination of suboesophageal, thoracic, and abdominal neuromeres, as seen in dipterans (Boleli & Paulino-Simes, 1999; Fritz, 2002). Histologically, the ganglion was ovoid structure and had a large nerve fiber extending anteriorly to the thoracico-abdominal ganglion. In this regard, the ganglia in the ventral nerve cord were similarly structured. The cortical region having neurons, neurosecretory cell and supporting cell classified in the ganglion, whereas the neuronal fibers was seen in the medullary region, as similar visualized in some insects like arboreal bicoloured ant *T. rufonigra* (Somala et al, 2020) and striped blister beetle, *Epicauta waterhousei* (Langkawong et al., 2013). Interestingly, the fused thoracic + abdominal ganglia (thoracico-abdominal ganglion) of these tephritid fruit flies could be explored. In terms of insect evolution, in this case, it was indicated that these flies are considered as advanced insect groups (Diptera), as similar exhibited in the research of Fritz (2002) (see the arrangements of the ventral ganglia in various insects).

The histological properties of these tephritidae were abundantly displayed throughout the body in terms of adipose tissue. The oenocytes and trophocytes (adipocytes) are important storage organ cells in this finding, and the term adipocyte is sometimes used in the literature to refer to the principal fat body cell. The fat body in insects is the principal storage location for lipid, glycogen, and protein, and is analogous to the adipose tissues and liver in vertebrates. In addition, it is also the site of haematopoiesis and secretion of many immune compositions, antibacterial compounds as well as blood clotting proteins (Azeez, Meintjes, & Chamunorwa, 2014; Vilmos & Kurucz, 1998). Recently, in one case of honeybee, oenocytes and trophocytes of *Apis mellifera* workers presented aging phenotypes. On the other hand, those of *A. mellifera* queens exhibited pro-longevity phenotypes (Lu, Weng, Tan, & Hsu, 2021).

An insect's exoskeleton, also known as the integument, is the outer layer of tissue that covers the surface of the insect. In this observation, the integument was obviously protected by two distinct layers (cuticle and epidermis). Epicuticle and cuticulin envelop, procuticle (exocuticle, mesocuticle, and endocuticle), epidemis, and basement membrane were the three epithelial layers seen in insects (basal lamina). Nonetheless, two layers of a thin endocuticle and a broader layer of exocuticle were visible at a light microscopic level, in contrast to previous observations (Gullan & Cranston, 2014). A transmission electron microscope (TEM) is required to analyze certain integument features.

The respiratory system of these insects could be seen all over the bogy. The trachea was identified histologically amidst the fat tissue. The tracheal system and spiracles spread throughout the insect body and operate as the respiratory system. The respiratory system is in charge of both delivering enough oxygen (O_2) to all cells in the body and eliminating carbon dioxide (CO_2) as a consequence of cellular respiration (Gullan & Cranston, 2014). These findings were conformed to several previous observations, for example, arboreal bicoloured ant *Tetraponera rufonigra* Somala et al, 2020, coffee berry borer, *Hypothenemus hampei* (Alba-Tercedor, Alba-Alejandre, & Vega, 2019), milkweed bug, *Oncopeltus fasciatu* (Hanna & Popadić, 2020) and mosquitoes, *Anopheles sinensis* and *Aedes togoi* (Ha, Yeom, Ryu, & Lee, 2017) etc.

4. Conclusions

All tephritid fruit flies shared a structurally similar systemic arrangement. The findings of this work add to our understanding of the structural systems of tephritid fruit flies that are linked to their histochemistry, physiology, and hormone control. This could, in particularly, be employed as a comparative control with histopathology to quantify apoptosis caused by the application of insecticides or medicinal pla nt extracts in commercial agricultural systems.

Acknowledgments

The authors are thankful to the Microtechnique Laboratory, Division of Biological Science, Faculty of Science, Prince of Songkla University for their supports and laboratory. We also thank Mr. Stuart Grandger, UK for critically reading the manuscript.

References

Adams, T. S. (2000). Effect of diet and mating status on ovarian development in a predaceous stink bug *Perillus bioculatus* (Hemiptera: Pentatomidae). *Annals of the Entomological Society of America*, *93*, 529–535. doi: 10.1603/0013-8746(2000)093[0529:EODAMS]2.0.CO;2

Adams, T. S. (2001). Morphology of the internal reproductive system of the male and female two spotted stink bug *Perillus bioculatus* (F.) (Heteroptera: Pentatomidae) and the transfer of products during mating. *Invertebrate Reproduction and Development*, *39*, 45–53. doi: 10.1080/07924259.2001.9652466

Alba-Tercedor, J., Alba-Alejandre, I., & Vega, F. E. (2019). Revealing the respiratory system of the coffee berry borer (*Hypothenemus hampei*; Coleoptera: Curculionidae: Scolytinae) using micro-computed tomography. *Scientific Reports*, *9*, 1–17. doi: 10.1038/s41598-019-54157-3

Azeez, O. I., Meintjes, R., & Chamunorwa, J. P. (2014). Fat body, fat pad and adipose tissues in invertebrates and vertebrates: the nexus. *Lipids in health and disease, 13* (71), 1–13. doi: 10.1186/1476-511X-13-71

Bernet, D., Schmidt, H., Meier, W., Burkhardt-holm, P., & Wahli, T. (1999). Histopathology in fish: proposal for a protocol to assess aquatic pollution. *Journal of Fish Diseases, 22*, 25–34. doi: 10.1046/j.1365-2761.1999.00134.x

Boleli, I. C., & Paulino-Simões, Z. L. (1999). Mapping of serotonin-immunoreactive neurons of *Anastrepha obliqua* Macquart larvae. *Revista Brasileira de Zoologia, 16*(4), 1099–1107. doi: /10.1590/S0101-81751999000400019

Boonyoung, P., Senarat, S., Kettratad, J., Jiraungkoorskul, W., Thaochan, N., Sing, K-W., Pengsakul, T., & Poolprasert, P. (2020). Mature gonadal histology and gametogenesis of the tortoise beetle *Aspidimorpha sanctaecrucis* (Fabricius, 1792) (Coleoptera: Cassidinae: Chrysomelidae): Histological observation. *Songklanakarin Journal of Science and Technology, 42*(4), 873–878. doi: 10.14456/sjst-psu.2020.112

Çakıcı, Ö. (2016). A Histological and Histochemical Investigation on Oocyte Development in Gryllus bimaculatus (Orthoptera: Gryllidae). *KSÜ Doğa Bilimleri Dergisi*, 19 (3), 256–260.

Cardoso, D. C., Fortes, J. C., Cristiano, M. P., Zanuncio, J. C., & Serrão, J. E. (2008). Spermathecae and associated glands of the ants *Solenopsis saevissima* and *Acromyrmex subterraneus subterraneus* (Hymenoptera: Myrmicinae). *Sociobiology, 52*, 377–385.

Chou, M. Y., Mau, R. F., Jang, E. B., Vargas, R. I., & Piñero, J. C. (2012). Morphological features of the ovaries during oogenesis of the Oriental fruit fly, Bactrocera dorsalis, in relation to the physiological state. *Journal of insect science, 12,* 1–12. https://doi.org/10.1673/031.012.14401

De Loof, A., Geysen, J., Cardoen, J., & Verachtert, B. (1990). Comparative developmental physiology and molecular cytology of the polytrophic ovarian follicles of the blowfly Sarcophaga bullata and the fruitfly *Drosophila melanogaster*. *Comparative Biochemistry & Physiology*, *96*(2), 309–321. doi: 10.1016/0300-9629(90)90698-r.

Dong, S. Z., Ye, G. Y., Guo, J. Y., Yu, X. P., & Hu, C. (2010). Oogenesis and programmed cell death of nurse cells in the endoparasitoid, *Pteromalus puparum*. *Microscopy Research and Technique, 73*(7), 673–680. doi: 10.1002/jemt.20808.

Doorenweerd, C., Leblanc, L., Norrbom, A. L., Jose, M. S., & Rubinoff, D. (2018). A global checklist of the 932 fruit fly species in the tribe Dacini (Diptera, Tephritidae). *ZooKeys*, *730*, 19–56. doi: 10.3897/zookeys.730.21786

Drew, R. A. I., & Hancock, D. L. (1994). The *Bactrocera dorsalis* complex of fruit flies (Diptera: Tephritidae: Dacinae) in Asia. *Bulletin of Entomological Research Supplement Series*, *2*, 1–68. doi:10.1017/S1367426900000278

El Harym Y., & Belqat, B. 2017. First checklist of the fruit flies of Morocco, including new records (Diptera, Tephritidae). *ZooKeys*, *702*, 137–171. doi.org/10.3897/zookeys.702.13368

Fritz, A. H. (2002). A Single, Abdominal Ganglion in *Anastrepha suspensa* (Diptera: Tephritidae) and its innervation of the female sperm storage organs. *Annals of the Entomological Society of America*, 95(1), 103–108.

Gao, J., Wang, J., & Chen, H. (2021). Ovary Structure and Oogenesis of *Trypophloeus klimeschi* (Coleoptera: Curculionidae: Scolytinae). *Insects, 12*(12):1099. https://doi.org/10.3390/insects12121099

Gullan, P. J., & Cranston, P. S. (2014). *The insects: An outline of entomology*. 5th Edition, Wiley Blackwell.

Ha, Y. R., Yeom, E., Ryu, J., & Lee S. J. 2017. Three-dimensional structures of the tracheal systems of *Anopheles sinensis* and *Aedes togoi* pupae. *Scientific Reports*, *7*, 1–8. doi.org/10.1038/srep44490

Hanna, L., & Popadić, A. (2020). A hemipteran insect reveals new genetic mechanisms and evolutionary insights into tracheal system development. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(8), 4252–4261. doi.org/10.1073/pnas.1908975117

Hardy D. E. (1973). *The Fruit flies (Tephritidae - Diptera) of Thailand and bordering countries*. Pacific insects monographs 31, Bishop Museum, Honolulu.

Harris, M. N., Azman, S., & Othman, N. W. (2019). Gross anatomy and histology of alimentary system of stick insect, *Pylaemenes mitratus* (Phasmid: Basillidae). *Serangga*, *24*(1), 151–158.

Kunprom, C., & Pramual, P. (2016). DNA barcode variability and host plant usage of fruit flies (Diptera: Tephritidae) in Thailand. *Genome*, *59*(10), 792–804. doi.org/10.1139/gen-2015-0110

Lemos, W. P., Ramalho, F. S., Serrao, J. E., & Zanuncio, J. C. (2005). Morphology of female reproductive tract of the predator *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) fed on different diets. *Brazilian Archives of Biology and Technology*, *48*(1), 129–138. doi.org/10.1590/S1516-89132005000100017

Langkawong, K., Senarat, S., Chantarasawat, N., Boonyoung, P. & Poolprasert, P. (2013). Identification of some organ by histology and histochemistry of female blister beetles, *Epicauta waterhousei* (Haag-Rutenberg, 1880): The abdominal part. *Journal of* Science *and Technology*, *Ubon Ratchathani University*, *15*, 1–10.

Lu, C. Y., Weng, Y. T., Tan, B., & Hsu, C. Y. (2021). The trophocytes and oenocytes of worker and queen honeybees (*Apis mellifera*) exhibit distinct age-associated transcriptome profiles. *GeroScience*, *43*, 1863–1875. doi.org/10.1007/s11357-021-00360-y

Munhoz, I. L. A., Serrão, J. E., Dias, G., Lino-Neto, J., de Melo, A. L., & Araújo, V. A. (2020). Anatomy and histology of the male reproductive tract in giant water bugs of the genus *Belostoma* Latreille, 1807 (Heteroptera, Belostomatidae). *International Journal of Tropical Insect Science*, *41*, 303–311. doi.org/10.1007/s42690-020-00207-7

Okada, Y., Miyazaki, S., Miyakawa, H., Ishikawa, A., Tsuji, K., & Miura, T. (2010). Ovarian development and insulin-signaling pathways during reproductive differentiation in the queenless ponerine and *Diacamma* sp. *Journal of Insect Physiology*, *56*, 288–295.

Ortiz, G., & Camargo-Mathias, M. I. (2007). Spermatheca of four species of ants of the tribe Attini (Hymenoptera: Myrmicinae). Morphological specialization. *American Journal of Biological Sciences*, *2*(1), 5–12.

Othman, N. W., Nur Hudawiyah, A., Roslim, R., Nur Khairunnisa, S., & Sulaiman, N. (2018). Mouthpart and digestive tract morphology of the synchronized firefly, *Pteroptyx tener* (Coleoptera: Lampyridae). *Serangga*, *23*(2), 170–182.

Özyurt, N., Candan, S., & Suludere, Z. (2012). The morphology and histology of the male reproductive system in *Dolycoris baccarum* Linnaeus 1758 (Heteroptera: Pentatomidae)-Light and scanning electron micoscope studies. *Micron*, *44*, 101–106.

Özyurt, N., Candan, S., Suludere, Z., & Amutkan, D. (2013). Morphology and histology of the male reproductive system in *Graphosoma lineatum* (Heteroptera: Pentatomidae) Based on optical and scanning electron microscopy. *Journal of Entomology and Zoology Studies*, *1*, 40–46.

Poolprasert, P., Chitchamnong, A., Boonyoung, P., Senarat, S., Dokchan, P., Mongkolchaichana, E., Likittrakulwong, W., & Berry, G. (2020). COI nucleotide sequence confirms the species of common posy butterfly, Drupadia ravindra Horsfield, 1829 and histological feature reveals its microanatomical structure of nervous system and sense organs. *Khon Kaen Agriculture Journal*, *48*(1), 755–764.

Rubabura, K. J. A., Chihire, B. P., & Bisimwa, B. E. (2019). Diversity and abundance of fruit flies (family: Tephritidae) in the Albertine rift zone, Democratic Republic of the Congo, and preliminary prospects for biological control. *Advances in plants & Agriculture Research*, *9*(1), 41–48.

Shehata, N. F., Younes, M. W. F., & Mahmoud, Y. A. (2011). Anatomical and Histological Studies on the Peach Fruit Fly, *Bactrocera zonata* (Saund.) Female Reproductive System. *Journal of Applied Sciences Research*, *7*(7): 1212–1217.

Somala, N., Senarat, S., Para, C., Jiraungkoorskul, W., Kaneko, G., Poonpet, T., & Poolprasert, P. (2020). Systemic organization of *Tetraponera* *rufonigra* (Jerdon, 1851) (Hymenoptera: Formicidae): histological observation. *Serangga*, *25*(1), 53–67.

Suvarna, K. S., Layton, C., & Bancroft, J. D. (2013). *Bancroft's Theory and Practice of Histological Techniques*. 7th ed. Canada, Elsevier.

Vilmos, P., & Kurucz, E. (1998). Insect immunity: evolutionary roots of the mammalian innate immune system. *Immunology letters*, *62*(2), 59–66. doi.org/10.1016/S0165-2478(98)00023-6

White, I. M. & Elson-Harris, M. M. (1994). *Fruit flies of economic significance: their identification and bionomics*, CAB International, Wallingford, U.K.

Wilson, J. M., Bunte, R. M., & Carty, A. J. (2009). Evaluation of rapid cooling and tricaine methanesulfonate (MS222) as methods of euthanasia in zebrafish (*Danio rerio*). *American Association for Laboratory Animal Science*, *48*, 785–789.

Zhang, H., Goh, F. G., Ng, L .C., Chen, C. H. & Cai, Y. (2023) *Aedes aegypti* exhibits a distinctive mode of late ovarian development. *BMC Biology*, *21* (11): 1–18.

Figure legends

Figure 1. Field study shows (A) the tephritid fruit flies on the green leave. (B-F) Representative figures of the tephritid fruit flies present such as *Bactrocera albistrigata* (B), *Bactrocera umbrosa* (C), *Zeugodacus curcubitae* (D), *Batrocera dorsalis* (E), and *Zeugodacus tau* complex (F).

Figure 2. Morphology and light microscope of male reproductive system of the tephritid fruit flies. (A) A Representative morphology shows the reproductive system of *Zeugodacus curcubitae* was observed. (B-C) Histological structure of the testis (Ts) containing the testicular follicle was identified from *Bactrocera albistrigata*

and *Zeugodacus tau* complex. (D) Each follicle was separated into three zones [growth zone (Gz), maturation zone (Mz) and transitional zone (Tz). Several stages of male germ cell were classified including spermatocytes (Sc), spermatids (St) and spermatozoa (Sz). Abbreviations: At = adipose tissue, Ea = ejaculatory apodeme, Dd = deferent duct, He = head of spermatozoa, Lt = long tubular, Mt = mulpigian tubule, Ta = tail of sperm, Tc = testicular capsule, Tt = testes, Sag = short accessory gland.

Figure 3. Representative light microscope of (A) seminal vesicle and (B) contains the accessory gland of *Bactrocera umbrosa*. Abbreviations: Ep = epithelium, Ms = muscular layer, Sz = spermatozoa.

Figure 4. Morphology and Light microscope of female reproductive system of the tephritid fruit flies. (A) Representative morphology exhibits the reproductive system of *Zeugodacus curcubitae*. (B-D) Reproductive histology from *Bactrocera albistrigata* displays the ovary and previtellogenic stage (Pv). E: The oviduct is also seen. Abbreviations: Bc = basophilic cytoplasm, Ep = epithelium, Co = common oviduct, Fc = follicle cell, Hg = hindgut, Nc = nurse cell, Ov = ovaries, Rp = rectal pad, Spe = spermatheca.

Figure 5. Light microscope showing the digestive system of the tephritid fruit flies. (A) The digestive tract of *Zeugodacus curcubitae* is divided into two parts [midgut (Mg) and hindgut (Hg)]. (B) The esophageal valve (Ev) and the midgut (Mg) are longitudinally sectioned. (C) High magnification appears the esophageal valve, which is lined by the inner cell layer (Icl) and the outer cell layer (Ocl). (D-E) The feature of midgut (Mg) with lining of epithelium (Ep) and rare muscular layer. (F) The characterization of hindgut having the epithelium (Ep) and the

obvious muscular layer is noted. Abbreviations: Bc = basal cell, Mcs = microvilli-covered surface.

Figure 6. Light microscope showing the excretory system and the nervous system of the tephritid fruit flies. (A-B) Representative figures the Malpighian tubule (Mt) between *Bactrocera albistrigata* (A) and *Bactrocera umbrosa* (B) are found. (C-D) The histological description of brain is divided into cortex region (Cr) and medullar region (Mr). E: These regions also similarly showed in the ganglion. Abbreviations: Nec = neural cell group, Neu = neuron, Nf = neuronal fiber, Nsc = neurosecretory cell, Pn = perineurium, Pv = pavement cell, Sc = supporting cell.

Figure 7. Light microscope shows the adipose tissue (A-C), integument [Ig] (B, D) and respiratory system (E-F) of the tephritid fruit flies. A: The composition of adipose tissue (At) is in the abdominal body. B-C: High magnification reveals the oenocytes (Oc) and trophocyte (Tp). B,D: High magnification demonstrates that the cuticle (Cu) and epidermis (Epi) of *Zeugodacus curcubitae* (B) and *Zeugodacus tau* complex (D) are located in integument layer. Endocuticle (Ed) and exocuticle (Ex) are also seen. E-F: The respiratory structure (Rs) from *Zeugodacus curcubitae* (E) and *Zeugodacus tau* complex (F) is identified. Abbreviations: Nuc = nucleus.

Figure

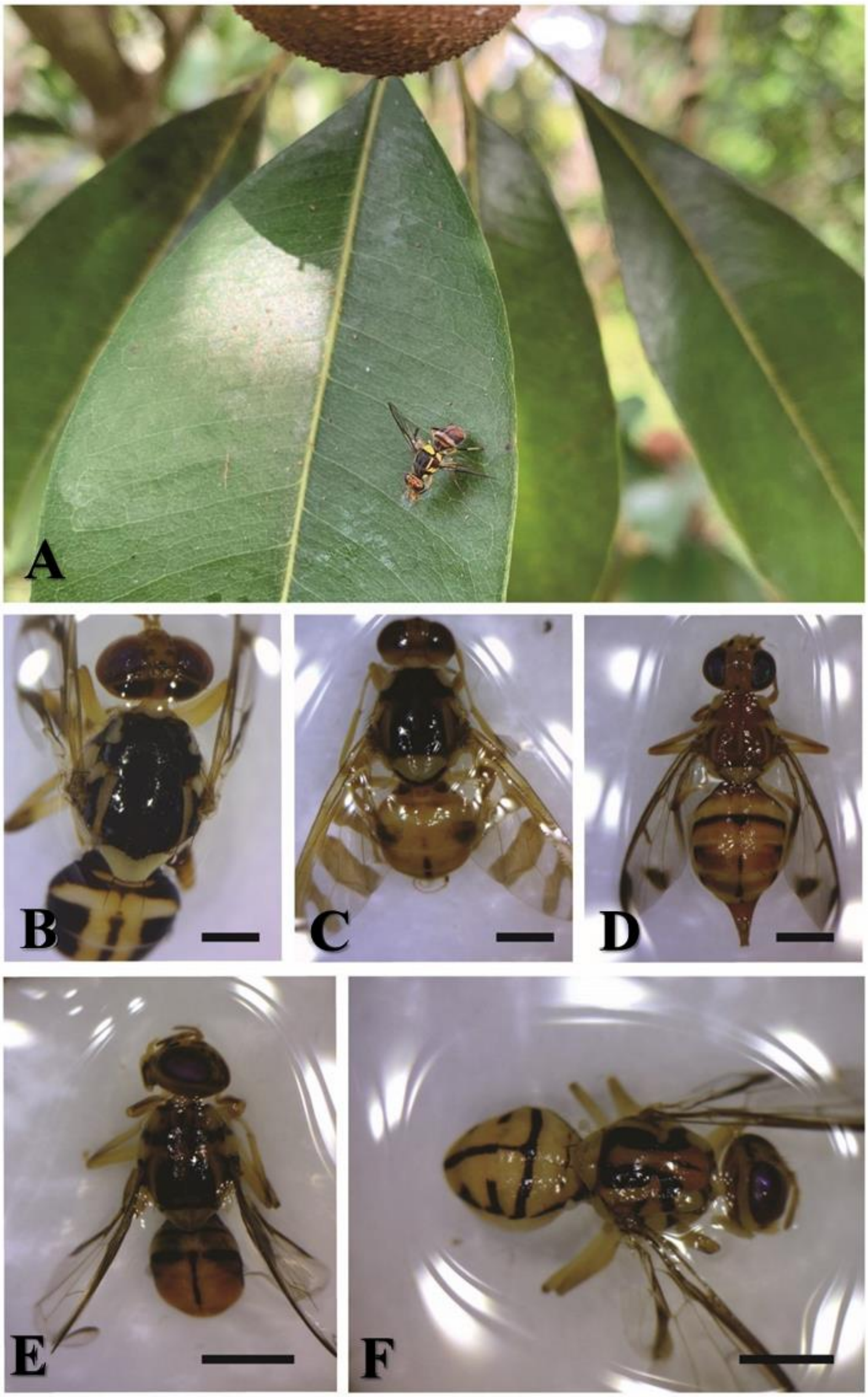

Figure 1.

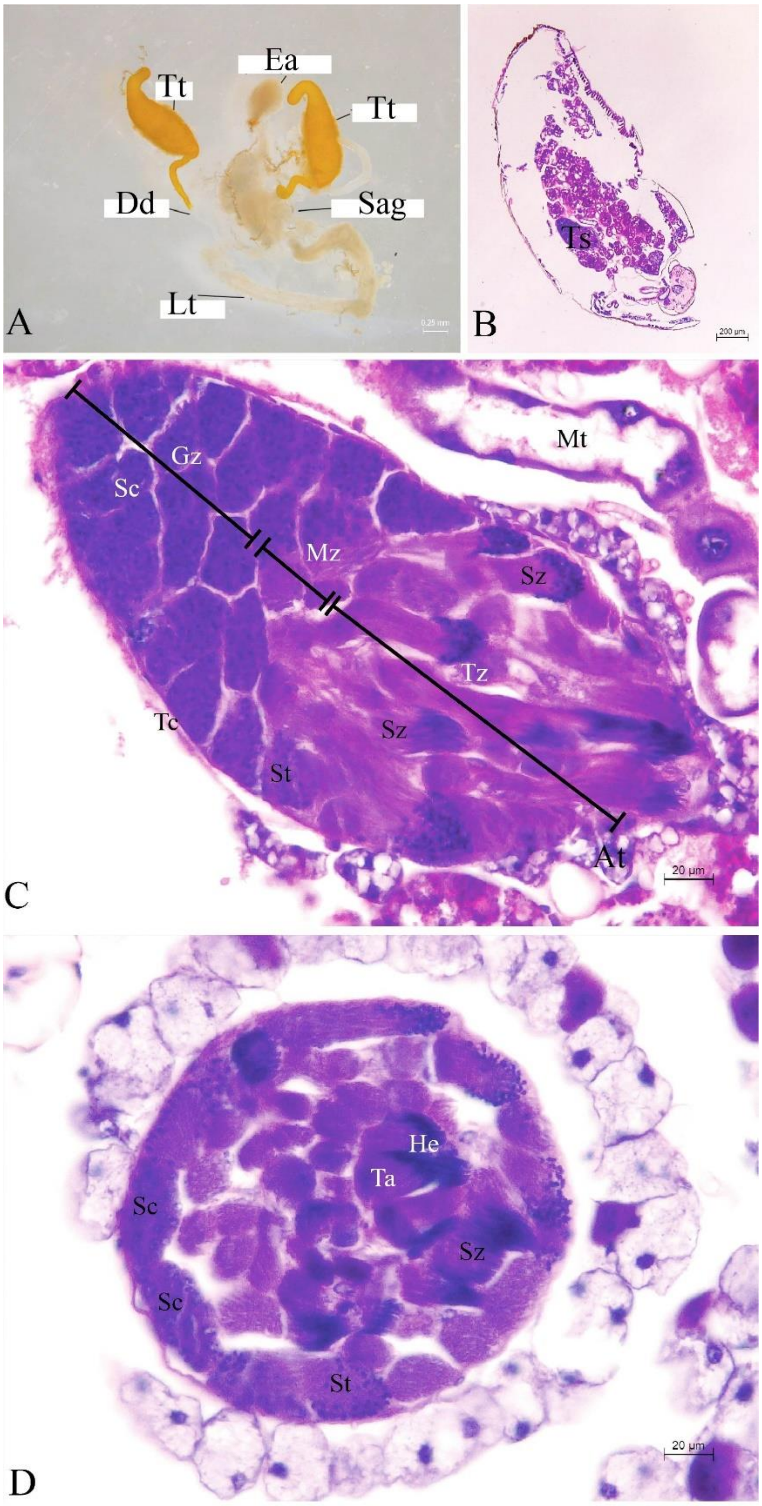
Tt
Ea
Tt
Dd
Sag
Lt
A
Ts
B
Gz
Sc
Mt
Mz
Sz
Tz
Tc
Sz
St
At
C
He
Ta
Sc
Sz
Sc
St
D

Figure 2.

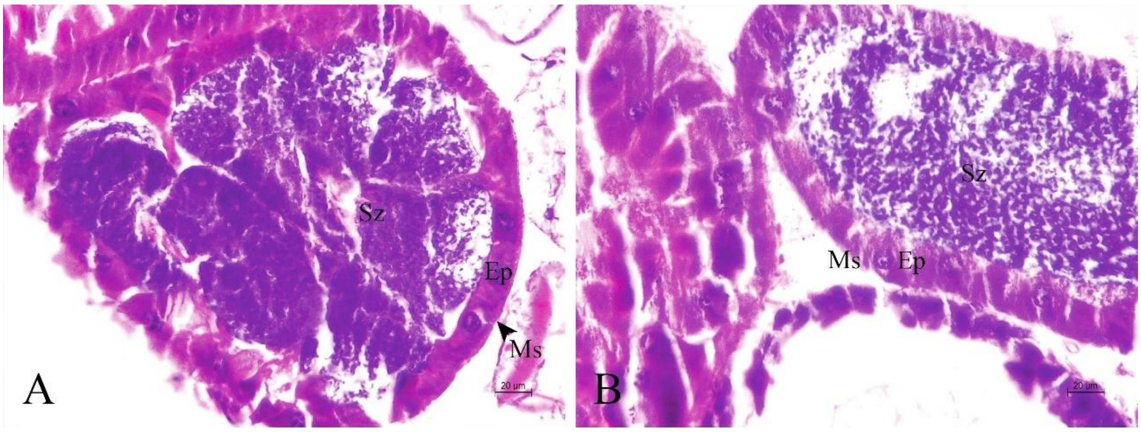

Figure 3.

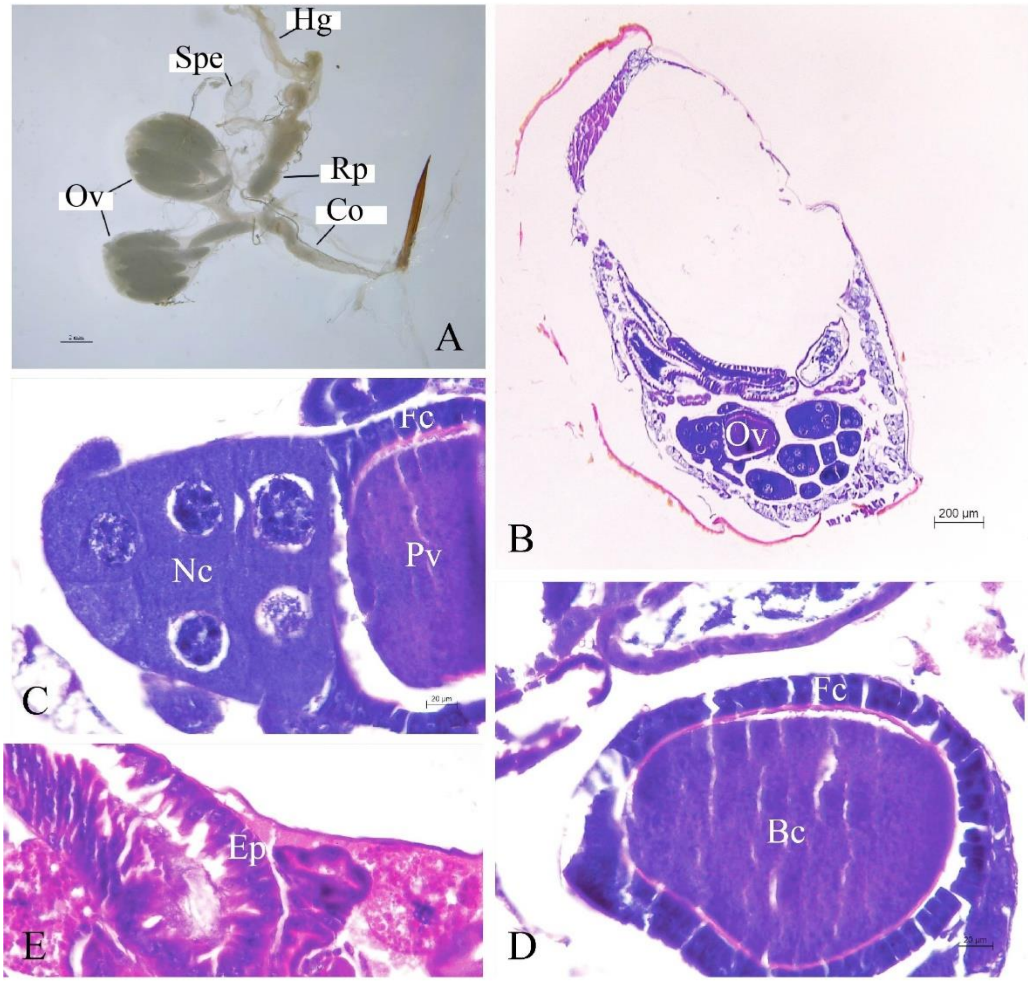

Figure 4.

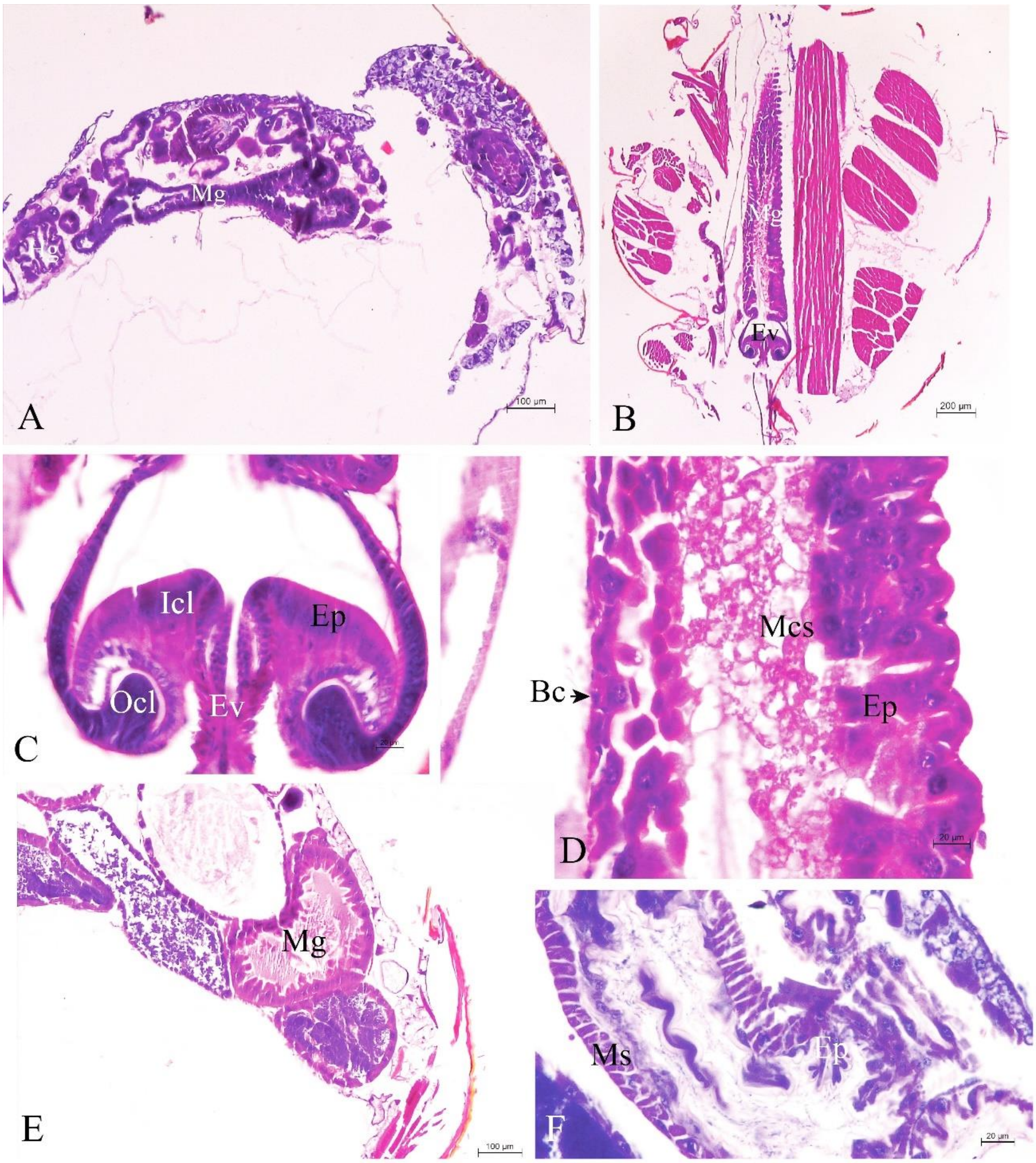
Hg
Mg
100 µm
A
Mg
Ev
200 µm
B
Icl
Ep
Ocl
Ev
C
Bc
Mcs
Ep
20 µm
D
Mg
100 µm
E
Ms
Ep
20 µm
F

Figure 5.

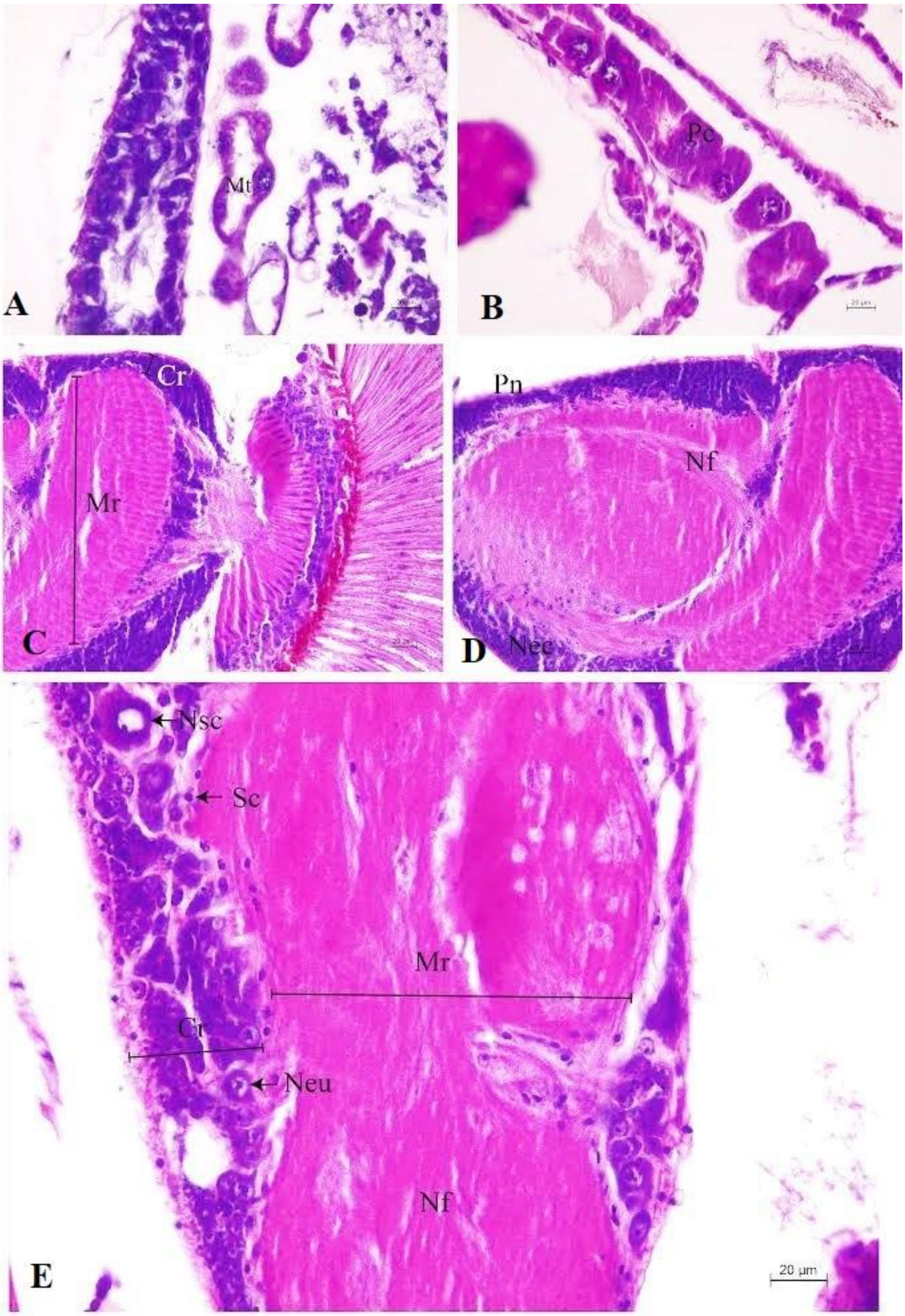

Figure 6.

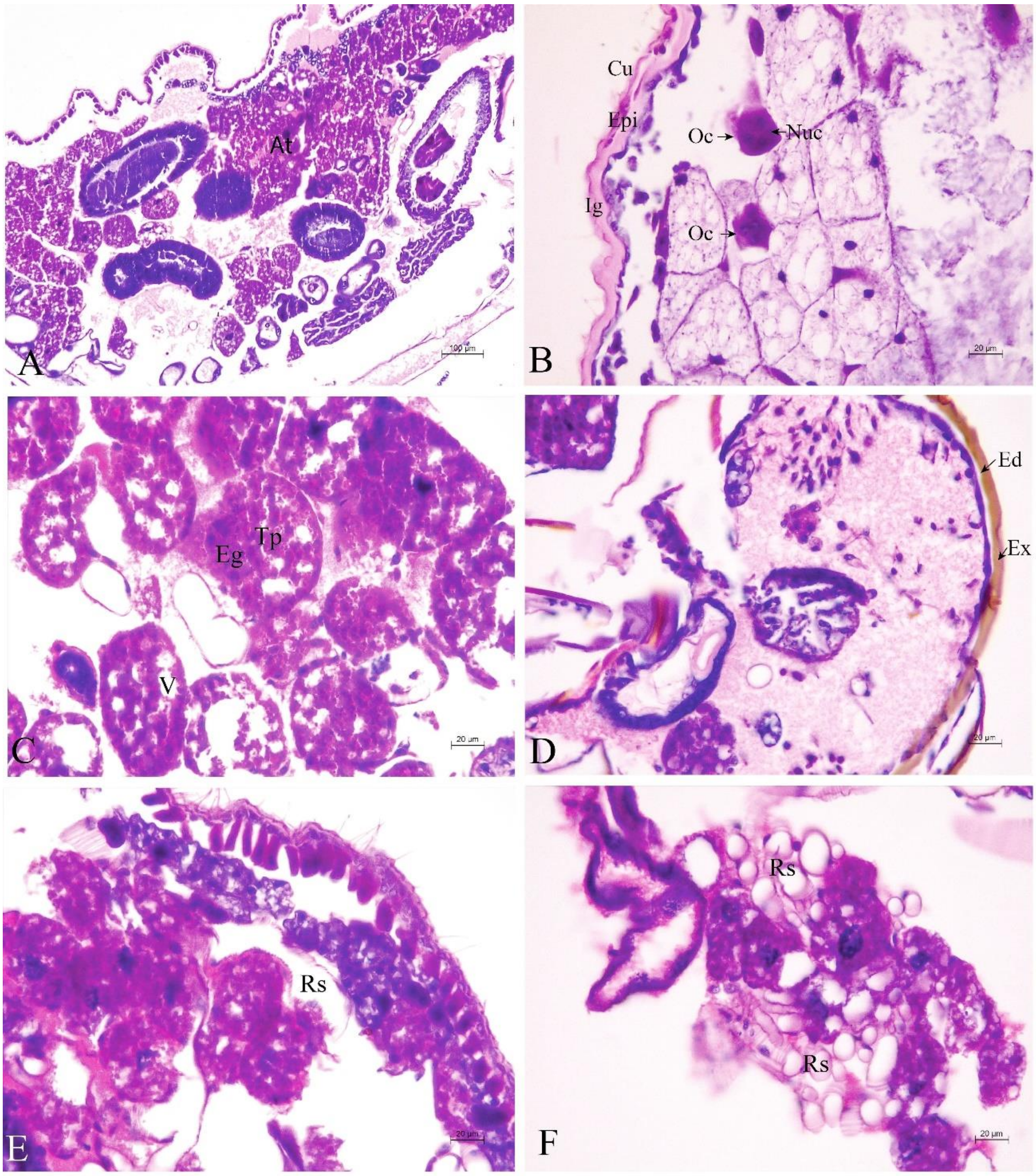
A
At
100 µm
B
Cu
Epi
Ig
Oc
Nuc
Oc
20 µm
C
Eg
Tp
V
20 µm
D
Ed
Ex
20 µm
E
Rs
20 µm
F
Rs
Rs
20 µm

Figure 7.