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Histological characterization of the ambrosia beetle *Xylosandrus compactus* (Eichhoff, 1875) female as an important destroy pest on *Mitragyna speciosa*

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ABSTRACT: The ambrosia beetle *Xylosandrus compactus* (Eichhoff, 1875) is a serious pest of economically important plants including *Mitragyna speciosa*, but its body structure and cellular composition remain uncharacterized. In this study, we described whole-body histological characteristics of female *X. compactus*. Our observation found two district regions in the nervous system: frontal ganglion and ventral nerve cord. The frontal ganglion contained two layers: outer cortex and inner medullae, and the outer cortex was lined with three major types of cells including neurosecretory cells, neuroglia, and neurons. The integumentary system contained three different layers: epicuticle, exocuticle, and endocuticle in the head, thoracic, abdomen, and leg regions. Apparently, only skeletal muscles possessed muscle fibers in the muscular system. The main organ of the urinary system was the Malpighian tubule, the epithelium of which was lined with the simple cuboidal layer. The digestive system consisted of several distinct parts including foregut (for example esophagus, crop, and proventriculus), midgut, and hindgut (or rectum). Three different cell types were identified in the epithelial midgut. The respiratory organ was visible among the adipose tissue close to the integument system. Although this organ was not easily identified, it was covered by a simple squamous epithelium. The female reproductive system of this insect was a telotrophic meroistic ovary. The distribution of nurse cells was restricted to the anterior tropharium, connected to oocytes. All the above results provide the first histological description of *X. compactus*, which might be useful for advancing research in the related fields.

KEYWORDS: *Xylosandrus compactus*, histology, histochemistry, *Mitragyna speciosa*

INTRODUCTION

The ambrosia beetle *Xylosandrus compactus* (Eichhoff 1875) (Coleoptera: Curculionidae) is a serious pest distributed mainly in subtropical Asia including Thailand. Its body is a dark brown to black color, and the head of this insect is characterized by a convex at the front with a transverse groove above the mouthpart [1–3]. Typically, this ambrosia beetle causes considerable damage to coffee trees [1–3]. Unfortunately, *X. compactus* is also known to infect *Mitragyna speciosa* (kratom), an important plant used as herbal medicine in both Asia and Africa, as well as other ornamental trees and is being recognized as a high-risk pest for agriculture in various parts of the world [4–6]. The infection causes wilting symptoms and often leads to the death of the host partly because of phytopathogenic and symbiotic fungi of *X. compactus* [7]. Recent studies unveiled the ecological features of *X. compactus* such as distribution and morphology [8, 9], including the life cycle of *X. compactus*. The female bores an entry hole in a host plant and lays in its eggs; subsequently, the larvae inhabit in the tunnels [9]. However, a detailed histological characterization of this insect has not yet been reported. This information will be useful for developing histopathological, physiological, biochemical, and toxicological approaches to understand and control the pest, which must be used as practical tools to prevent infection. In this study, we described the systematic structure and cellular composition of female *X. compactus* using histological and histochemical methods in comparison with other insects.

MATERIALS AND METHODS

During January to March 2022, a total of 20 mature female *X. compactus* with 2.0–2.5 mm in total length were obtained by hand collecting from in-

fected *M. speciosa* nursery in Khlong Hoi Khong Research Station, Faculty of Natural Resources, Prince of Songkla University, Khlong Hoi Khong district, Songkla province, Thailand (6°51′20.0″ N/100°21′41.2″ E). Species identification was conducted according to the standardized taxonomic guideline [10]. For histological analyses, collected individuals were fixed in 10% neutral-buffered formalin for 48 h at room temperature, and the whole body was subjected to the standard histological protocol [11, 12]. They were dehydrated by the increasing concentration of gradient alcohol solutions, cleared in xylene, and embedded in paraffin. The paraffin block was sectioned at 4 µm thickness using a rotary microtome. The tissue sections were stained with Harris's hematoxylin and eosin (H&E) to assess histological structures and histochemically stained with Periodic acid-Schiff-hematoxylin (PAS-H) and Gomori methenamine silver stain (GMS) to assess chemical components and fungal organisms. Histological and histochemical slides were observed and photographed with a Nikon DS-Fi3 (Nikon, USA). The experimental protocol was approved by the Animal Care and Use Committee of Naresuan University (Protocol Review no.: NU-AEE660201).

RESULTS AND DISCUSSION

X. compactus individuals were collected from infected *M. speciosa* seedling (Fig. 1A). Females were found in the stem bore (Fig. 1B) likely digging the hole (Fig. 1C). The size of mature females was about 0.87 mm within the entrance hold (Fig. 1D–F). We also found prominent eggs and larvae (Fig. 1G,H) in the infected *M. speciosa*. At the light microscope level, we could clearly observe the whole body of *X. compactus* from several views. Integrated views were used to identify multiple systems according to the localization, organ properties, and staining patterns. Each system is described in Figs. 2–7.

Nervous system

The central nervous system of insects typically contains 2 distinct parts: the brain (or frontal ganglion) and ventral nerve cord [13–15], which was also in the case of *X. compactus* (Fig. 2A–D). The frontal ganglion was centrally located in the head (Fig. 2A–F), surrounded by a thin layer of connective tissue called the "cranial capsule" or "neuronal capsule" (Fig. 2D). Two components (protocerebrum and deutocerebrum) were found in the anterior horn of the frontal ganglion, whereas the ventral horn contained the tritocerebrum (Figs. 2F and 2G). These structures are similar to those of *Locusta migratoria* (migratory locust) [16] and other insects [14, 17, 18].

In addition to the frontal ganglion, the 2 ganglia [sub-esophageal ganglion (Fig. 2B) and abdominal ganglion (Fig. 2B)] were remarkable in the ventral nerve cord as reported for the general insect nerves [19]. The structures of each ganglion were similar to each other, divided into 2 layers: the inner medullae and the outer cortex (Fig. 2H–J). The nerve fibers and the neuroglia were embedded in the inner medullae (Fig. 3E), whereas the clusters of neuronal cells were generally present in the outer cortex (Fig. 2J).

The neuronal cells could be classified into 3 types according to the sizes and histological characteristics. The neurosecretory cells (Nc) had an oval shape and the largest diameter of about 6 µm. The nucleus of

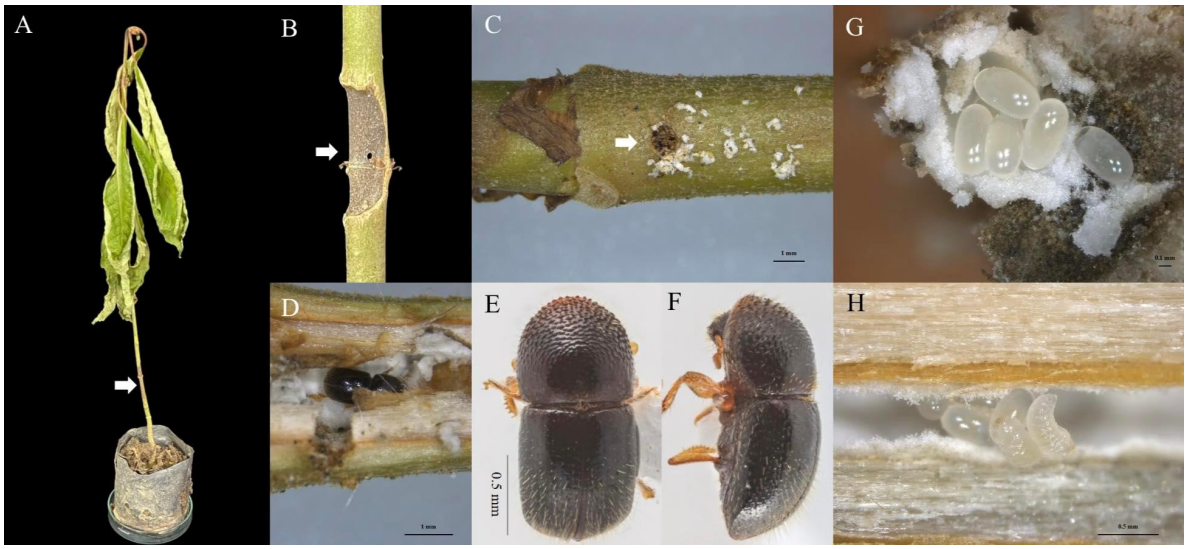

Fig. 1 The external morphology of *X. compactus* and injury in the *M. speciosa* seedling. The seedling dried after the insect attack and nursery (A). An entrance hole built by beetle females (white arrow) (B and C). Adult females (D–F), eggs (G), and larvae (H) observed inside the gallery.

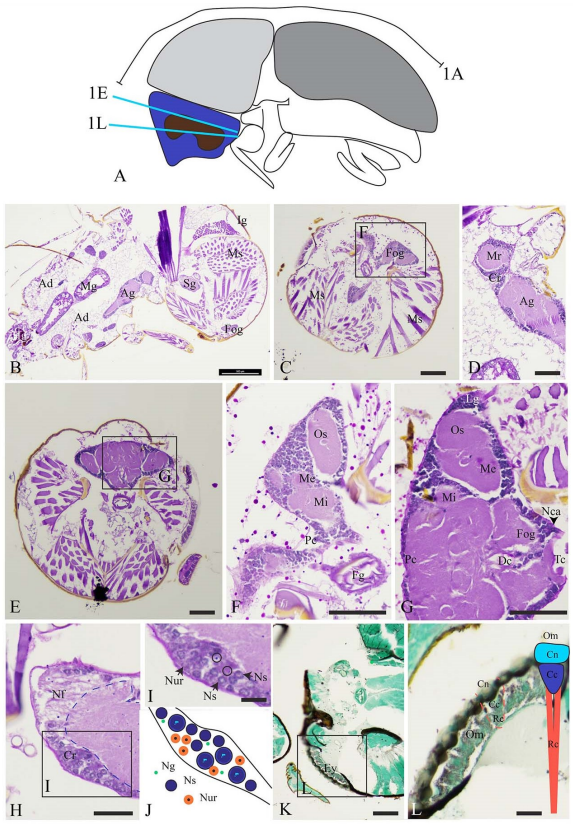

Fig. 2 Light microscopic observation and schematic diagram showing the nervous system of *X. compactus*. (A) Schematic diagram of the whole body in the longitudinal view. (B) Longitudinal section showing the frontal ganglion (Fog), sub-esophageal ganglion (Sg), and abdominal ganglion (Ag). (C) The frontal ganglion (Fog) in the head. (D) Abdominal ganglion having 2 regions including cortex region (Cr) and medullar region (Mr). (E) The large area of frontal ganglion in cross section. (F and G) Different areas of frontal ganglion. (H–J) Neurosecretory cell (Ns), neuron (Nur), and neuroglia (cycle) in the cortex region (Cr). (K and L) The regular ommatidium (Om) in the eye. Abbreviations: Ad = adipose cell; Cc = crystalline cone; Cn = cornea; DC = deutocerebrum; Fog = frontal ganglion; Ig = integument; Me = medulla externa; Mg = mid-gut; Mi = medulla interna; Mg = midgut; Ms = muscle; Nca = neuronal capsule; Nf = neuronal fiber; Os = optic stalk; Pc = protocerebrum; Rc = retinula cell; and Tc = tritocerebrum. Scale bar: (B) = 500 µm; (C–E) = 100 µm; (F–H) and (K) = 50 µm; (I) = 10 µm; and (L) = 10 µm. Staining methods: (B–I) = PAS-H and (K) = GMS.

Nc had one or 2 central nucleoli and was surrounded by eosinophilic nucleoplasm (Figs. 2I and 2J) as similarly observed in *Blaberus craniifer* (death's head cockroach), *Periplaneta americana* (American cockroach) [20, 21], and *Drosophila melanogaster* (common fruit fly) [22]. This cell is a part of the neuroendocrine system of insects and produces the neurosecretory hormone to support the growth and reproductive activity [23]. The middle-sized cells, the neuronal cells, had a diameter of about 3 µm, a spherical shape, and a small-oval nucleus (Fig. 2I). The smallest cells were neuroglia with a diameter of 2 µm, which normally scattered among other types of cells (Figs. 2I and 2J). The oval nucleus of this cell was surrounded by an eosinophilic cytoplasm, similar to the structure reported in *P. americana* [23].

The paired compound eyes were observed in *X. compactus* with regularly structured ommatidia (Figs. 2K and 2L), a common cellular arrangement of insect compound eyes [14, 24]. Each ommatidial structure had an elongated shape and was classified into the outer and the inner zones. The outer zone was a transparent bi-convex cornea of the eye (Fig. 2L), whereas the inner zone was located below the cornea structure. It contained crystalline cones and the photoreceptor cells (or retinular cells). Clusters of each ommatidium were separated by rhabdomeres. The pigment cells were found among the ommatidium with an elongated shape and several brown pigments (Fig. 2L).

Integumentary and muscular systems

The integumentary system is the outermost layer of the body (Fig. 3A), which protects insects from desiccation, toxic external molecules, and predators [18, 25, 26]. The integument system was similarly structured in different regions (head, thoracic and abdomen regions, and legs) with 3 layers: epicuticle, the exocuticle, and the endocuticle from outside to inside (Fig. 3B–D, PAS method) as commonly found in insects [18, 25, 27]. The integument system had a thickness of 8.9 ± 0.8 µm. This is thicker than those of other insects [(0.5 µm in *Frankliniella occidentalis* (western flower thrips) [28], 0.23 µm in *Diloboderus abderus* (bull bug), and 0.36 µm in *Golofa claviger* (giant rhinoceros beetle) [29], which may confer extra protection to *X. compactus*. Although the cuticle thickness of insect is related to the size, location, and molting process [18], biological control of *X. compactus* may target the effective penetration of the integument system.

The brown-colored epicuticle is known to be permeable, but it was a thick layer. The sensilla (or setae) was specifically found in this region (Fig. 3C). The layer below the epicuticle was an exocuticle (Fig. 3E) with a characteristic pink color. The endocuticle was the innermost layer and was much thicker than other layers. It was weakly stained by the H&E method. The epidermal layer showed a monolayered cubic organization (Fig. 3E). It consisted of specialized epidermal cells and gland cells (Fig. 3E–G). These cells likely secrete wax and proteins via the integument pores as reported by Arnosti et al [30].

The skeletal muscle was observed in both circular and longitudinal striated muscle regions along the

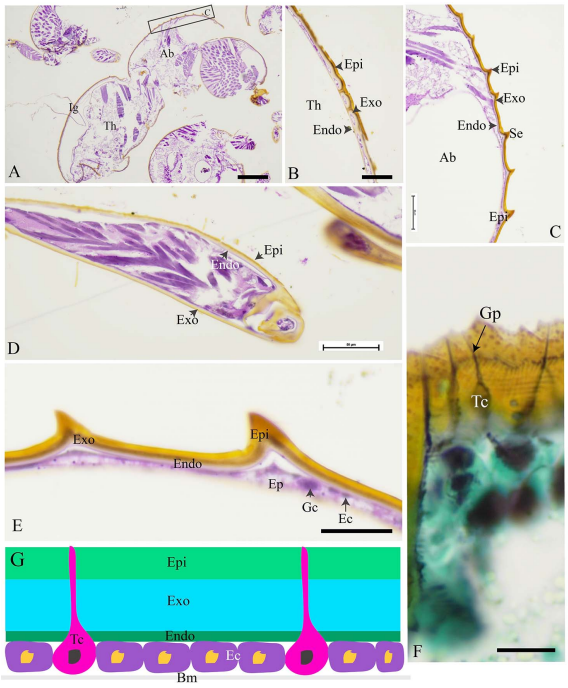

Fig. 3 Light photomicrographs (A–F) and a schematic diagram (G) of the *X. compactus* integument system. (A) The overall structure of integument system (Ig) in the longitudinal view along the body. Different areas of integument including thorax (B), abdomen (C), and leg (D). (E–G) Three distinct types of layers: epicuticle (Epi), exocuticle (Exo), and endocuticle (Endo) observed throughout the epidermis (Ep). Several cell types including gland cell (Gc), epidermal cell (Ec), and tricogen cell (Tc) were identified. Abbreviations: Bm = basement membrane and Gp = granular pit. Scale bar: (A) = 100 µm; (B–E) = 50 µm; and (F) = 20 µm. Staining methods: (A–E) = PAS-H and (I) = GMS.

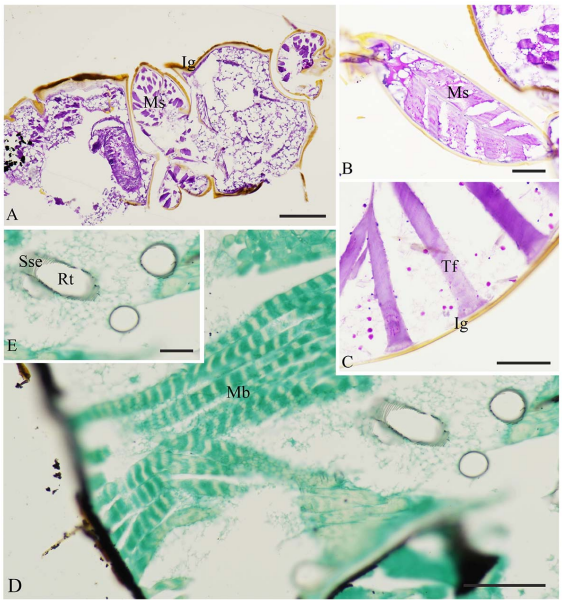

Fig. 4 Light photomicrographs of the muscular system of *X. compactus*. (A) Distribution of muscular system (Ms) along the body. (B and C) Muscular system (Ms) in leg and head. (D) The striated muscle containing the muscular bundle (Mb). (E) The respiratory tract (Rt) lining the simple squamous epithelium (Sse). Abbreviations: Ig = integument and Tf = tonofibrillae. Scale bar: (A) = 200 µm; (E) = 100 µm; (B and C) = 50 µm; and (F) = 10 µm. Staining methods: (A–C) = PAS-H and (E and F) = GMS.

lateral side of the body (Figs. 4A and 4B). This muscle formed muscle bundles together with the eosinophilic muscle (Figs. 4C and 4D, PAS method). Similar structures have been reported in other insects [31, 32] such as *Epicauta waterhousei* (striped Blister Beetle) [33] and *Oligotoma saundersii* (webspinners) [34].

Respiratory system

Spiracles and trachea are the major structure in the respiratory system of insects in the literature [18, 27, 35, 36]. A network of small respiratory organs was prominently distributed in the adipose tissue between the thorax and abdomen (Figs. 4E and 4F), but it was difficult to identify spiracles and trachea in *X. compactus*. The respiratory organs had a highly specialized oval-spherical shape, and each tubule was lined with simple squamous epithelium on the thin layer of connective tissue (Fig. 4F). Since the primary function of the respiratory system is the uptake of oxygen from the air and elimination of carbon dioxide [18, 27], the predominant network structure of this organ in *X. compactus* might support the life in wood.

Digestive system

The complex digestive system accounted for the main body part in *X. compactus* [37] and could be divided into 3 parts including foregut, midgut, and hindgut according to the location and histological characteristics (Fig. 4). The first part was the slender foregut, but it was hard to identify this part even at higher magnifications. The foregut consisted of esophagus, crop, and proventriculus parts. The esophagus wall had 3 layers including the mucosal fold lining with thin simple squamous epithelium, muscularis having the thin muscular tissue, and serosa (Figs. 5A and 5B). The crop continued from the esophagus, and their structures were similar. The crop wall showed the prominent mucosal folds and contained materials of the plant origin (Fig. 5C). Proventriculus is the last part of the foregut, and its wall had prominent sclerotized spines and deep folds on the muscularis (Fig. 5D–F). These well-developed structures of digestive organs suggest the concentration on the digestive activity as well as the production of digestive enzymes

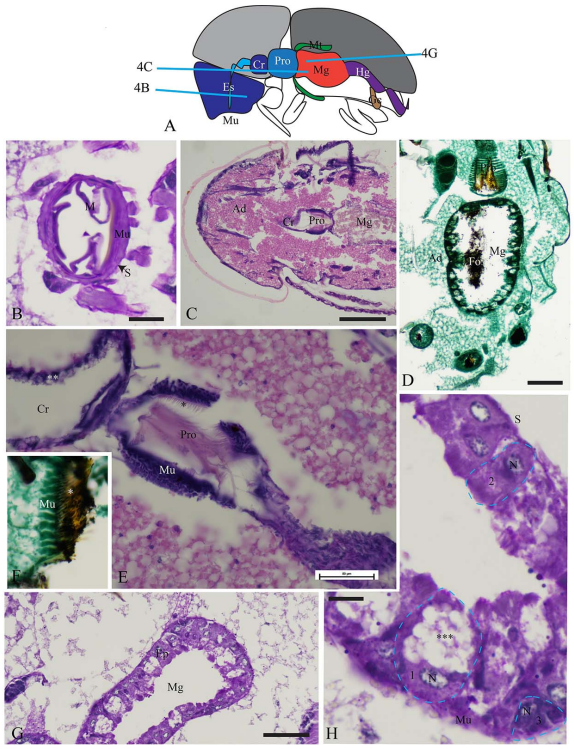

Fig. 5 Light photomicrographs and a schematic diagram of the digestive system of *X. compactus*. (A) The digestive system composed of mount (Mu), esophagus (Es), crop (Cr), proventriculus (Pro), midgut (Mg), and hindgut (Hg). (B) Esophagus. (C–F) The crop (Cr) and proventiculus (Pro). (G and H) The midgut structure showing 3 cell types including type 1 cell (1), type 2 cell (2), and type 3 cell (3). Abbreviations: Ad = adipose cell; Ep = epithelial layer; Fo = food; M = mucosa; Mu = muscularis; N = nucleus; S = serosa; * = the prominent sclerotized spines; ** = epithelium; and *** = foamy appearance. Scale bar: (C) = 200 µm and (B-D) and (G) = 50 µm. Staining methods: (C) and (E) = H&E; (B), (G), and (H) = PAS-H; and (D) and (F) = GMS.

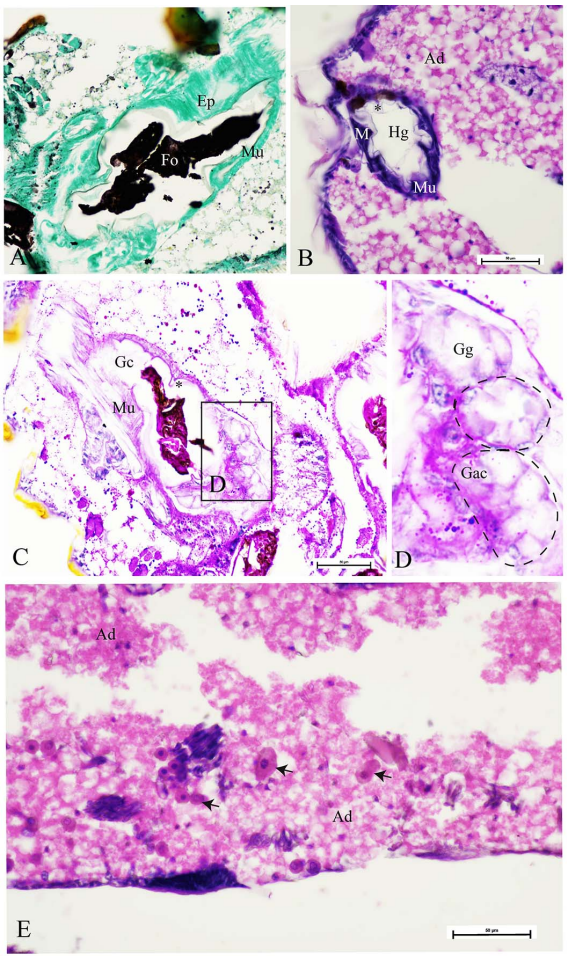

Fig. 6 Light photomicrographs of the digestive system and circulatory system of *X. compactus*. (A and B) The prominent structure of hindgut showing the longitudinal fold in mucosa (M). (C and D) The gastric caeca (Gc) containing the gastric gland (Gg). (E) The distribution of hemocytes (arrows) was observed among the adipose tissue (Ad). Abbreviations: Ad = adipose tissue; Ep = epithelial layer; Fo = food; and Gac = gastric cell. Scale bar: (B), (C), and (E) = 50 µm. Staining methods: (A) = GMS; (B) and (E) = H&E; and (C) and (D) = PAS-H.

(for example the carbohydrate and protein digestion) [14, 15, 18], which may help *X. compactus* to infect to *M. speciose*.

The midgut accounted for a large part of the digestive system (Figs. 5A and 5C). The midgut was a circular organ (Fig. 5D). The mucosal layer of the midgut showed fewer folds compared with those of the foregut and was composed of different cell types (Fig. 4G), which possibly is a feature of phytophagous insect [38]. There were 3 cell types in the midgut (types 1, 2, and 3), which is the first observation from *X. compactus*. The type 1 cells had an oval shape and an eccentric nucleus (Fig. 5H). The prominent and unstained vacuoles (or foamy appearance) were identified in the cytoplasm. It is possible that the midgut epithelium plays a key role in the production of digestive enzymes, absorption, and secretion [39]. The type 2 cells were the columnar cell with a central nucleus, and the type 3 cells were the smallest cell with a triangular shape located on the basal lamina (Fig. 5H). We assume that the type 3 cell is the stem cell that will develop to other cell types.

The mucosal layer of hindgut contained marked longitudinal folds (Figs. 6A and 6B) and was lined with the simple cuboidal epithelium (Fig. 6B). In contrast to the midgut, only one cell type was identified in the epithelium of hindgut (Fig. 6B), implying their different functions. It is noted that the gastric caeca were observed in the hindgut of *X. compactus* as a noticeably visible gastric gland Figs. 6C and 6D).

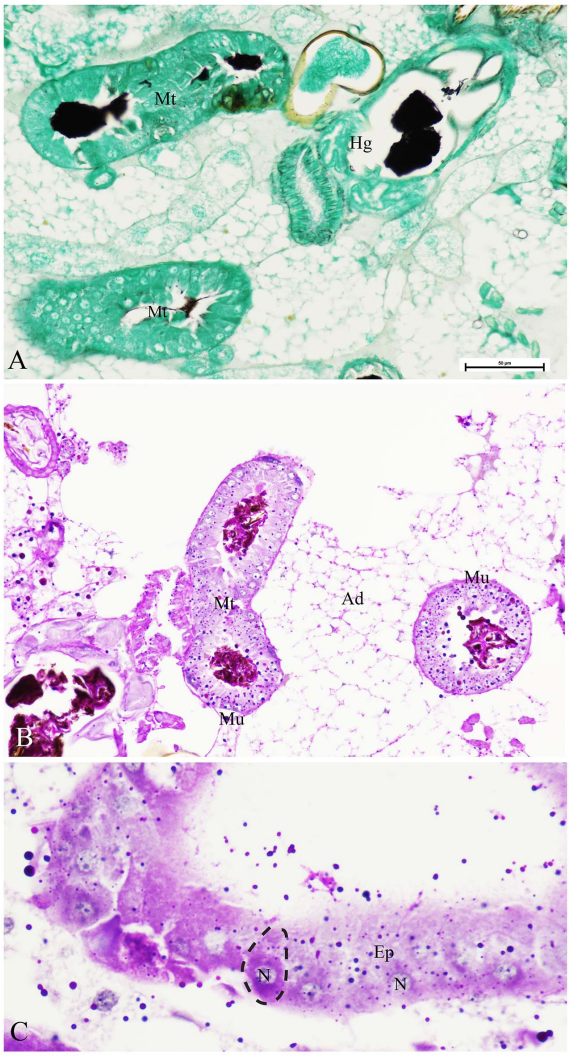

Fig. 7 Light photomicrographs of the urinary system of *X. compactus*. (A and B) Malpighian tubule (Mt) in close to the hindgut (Hg) and among the adipose tissue (Ad). (C) The tubule lining with high simple cuboidal epithelium (Ep). Abbreviations: Mu = muscularis and N = nucleus. Scale bar: (A) = 50 µm. Staining methods: (A) = GMS and (B and C) = PAS-H.

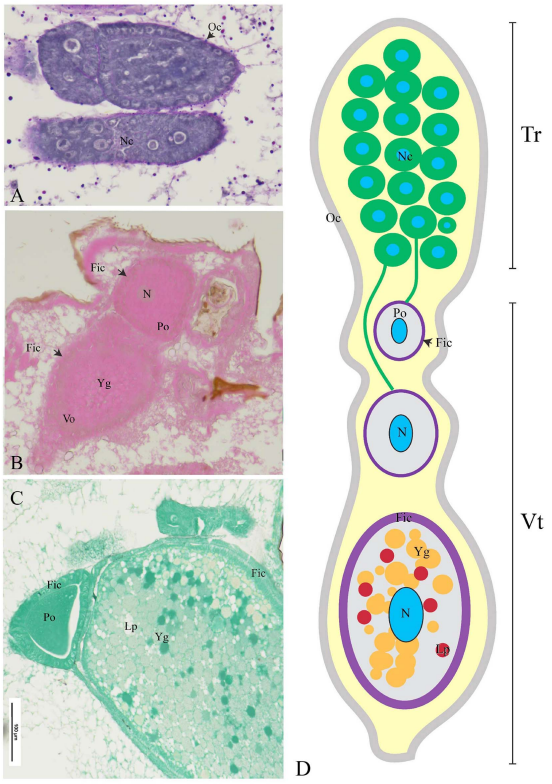

Fig. 8 Light photomicrographs (A–C) and a schematic diagram (D) of the female reproductive system of *X. compactus* containing nurse cell (Nc) in the terminal tropharium (Tr) and oocytes in differentiating stages including previtellogenic oocyte (Po) and vitellogenic oocyte (Vo). Abbreviations: Fic = follicular cell; Lp = lipid droplet; Oc = ovarian capsule; N = nucleus; Vt = vitellarium; and Yg = yolk granules. Scale bar: (C) = 100 µm. Staining methods: (A) = PAS-H; (B) = H&E; and (C) = GMS.

Circulatory system

X. compactus had an open circulatory system like other insects. Hemocytes were found in the hemolymph and in several other organs and tissues (Fig. 6E). Hemocytes had a round shape, a central nucleus, and acidophilic cytoplasm. As previously reported, insect hemocytes are analogous to vertebrate macrophages, which play a key role in the insect immune system [14].

Excretory system

The Malpighian tubule, the main organ of the excretory system, was located between midgut and hindgut in *X. compactus* (Fig. 7A). There were 2 layers in the Malpighian tubule: the epithelial layer and muscular layer (Fig. 7B). The single epithelial layer was covered with simple high cuboidal cells (Fig. 7C). Each epithelial cell had a centrally located euchromatic nuclei and was surrounded with eosinophilic homogeneous cytoplasm (H&E method, Fig. 7C) as similarly found in other scarab beetles [40].

Female reproductive system

Histologically, the female reproductive system of *X. compactus* consisted of 2 parts: 2 ovaries and an oviduct. Each ovary showed 2 subregions including tropharium and vitellarium, considered a telotrophic meroistic ovary (Fig. 8).

It is noted that several nurse cells were found in the terminal tropharium (Fig. 8A), whereas oocytes in several differentiating stages were present in the vitellarium (Fig. 8B–D). In this study, we classified

the oocytes into 3 successive stages [previtellogenic oocyte (Po), vitellogenic oocyte (Vo), and mature oocyte (Ms)] (Figs. 8B and 8C). The Po contained a centrally oval nucleus surrounded by the strongly basophilic ooplasm (H&E method). Oocytes in this stage were surrounded by a layer of simple follicle cells (Fig. 8B). The Vo were larger and had abundant small spherical yolk granules and lipid droplets (Fig. 8C). The follicular cells became low cuboidal epithelium cells (Fig. 8C).

CONCLUSION

Our study identified the essential systems of female *X. compactus*. To our knowledge, the thick integument system and the 3 cell types in the midgut are new information added to the literature. It will provide crucial information for further in-depth studies of this beetle species and other species in the genus *Xylosandrus*.

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