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Systemic Histology of the Marine Water-Strider *Halobates hayanus* (Heteroptera, Gerridae)

(Histologi Sistemik Water-Strider Marin *Halobates hayanus* (Heteroptera, Gerridae))

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ABSTRACT

The structure and cell types of the organs of *Halobates hayanus* from Libong Island, Thailand, were investigated using a histological method. The integumentary system of this species consists of three layers: epicuticle, exocuticle, and endocuticle. Throughout the body, the muscular system exclusively included skeletal muscle. In the excretory system, we found four fully formed Malpighian tubules, each one lined with a straightforward cuboidal epithelium harbouring secretory granules. The digestive system comprised a foregut, midgut, and hindgut. The midgut had three distinct cell types: basal cells, epithelium, and absorptive cells. The nervous system comprised two regions: A ventral nerve cord and a frontal ganglion, which is connected to the eye structure. The frontal ganglion was composed of two lobes each containing an outer cortex and inner medulla. The outer cortex presented neurosecretory cells, neuroglia cells, and neurons. The neurosecretory cells were large and contained secretory granules in their cytoplasm. This histological study also showed the reproductive system of this gerrid species, including the reproductive tract, and the accessory organ.

Keywords: Histological structure; Libong Island; oceanic *Halobates* species; systemic organs

ABSTRAK

Struktur dan jenis sel organ *Halobates hayanus* dari Pulau Libong, Thailand, dikaji menggunakan kaedah histologi. Sistem integumen spesies ini terdiri daripada tiga lapisan: epikutikel, eksokutikel dan endokutikel. Pada seluruh badan, sistem otot secara eksklusif merangkumi otot rangka. Dalam sistem perkumuhan, kami menjumpai empat tubul Malpighian yang telah terbentuk sepenuhnya, setiap satu dilapisi dengan epitelium kuboid lurus yang menyimpan butiran rembesan. Sistem pencernaan terdiri daripada usus depan, usus tengah dan usus belakang. Usus tengah mempunyai tiga jenis sel yang berbeza: sel basal, epitelium dan sel penyerap. Sistem saraf terdiri daripada dua kawasan: kord saraf ventral dan ganglion frontal, yang bersambung dengan struktur mata. Ganglion frontal terdiri daripada dua lobus setiap satu mengandungi korteks luar dan medula dalam. Korteks luar membentangkan sel neurorembesan, sel neuroglia dan neuron. Sel neurorembesan adalah besar dan mengandungi butiran rembesan dalam sitoplasmanya. Kajian histologi ini juga mendedahkan sistem pembiakan spesies gerrid ini, termasuk saluran pembiakan dan organ aksesori.

Kata kunci: Organ sistemik; Pulau Libong; spesies *Halobates* lautan; struktur histologi

INTRODUCTION

More than forty species of water striders belong to the genus *Halobates* (Hemiptera, Gerridae), sometimes known as sea skaters. *Halobates* spp. have been the subject of studies examining the occurrence and abundance of certain species and their adaptation to an epineustonic lifestyle along coasts and in protected marine regions (Cheng & Mishra 2022; Mahadik, Agustí & Duarte 2019). Originally from the Gulf of Aden, *H. hayanus* White, 1883 is now known to inhabit waters around Singapore, Malaysia, Indonesia, Thailand (Phuket), China, Vietnam, Australia, and a number of Pacific Island groups. The range of this species covers more than 70% of the planet's surface (Andersen & Cheng 2004; Andersen & Foster 1992; Mahadik, Agustí & Duarte 2019; Mahadik et al. 2020). It has been hypothesized that *H. hayanus* was restricted to a known geographic region from where it migrated from island to island by adhering to ocean currents (Cheng 1985). The original description of the oceanic *H. hayanus* by White (1883) was based on morphological features. Later studies of taxonomy, phylogeny, ecology, and zoogeography showed the biological characteristics of the species (Andersen & Cheng 2004; Andersen & Weir 1994; Cheng 1974; Mahadik, Agustí & Duarte 2019).

Only recently has the study of Poolprasert et al. (2022) on the comprehensive structure of marine water-strider, *Asclepios annandalei* (Hemiptera, Gerridae), the closely related species, been investigated. The integumentary system of this species has three layers: epicuticle, exocuticle, and endocuticle. The muscular system is made up of only skeletal muscle. The urinary system has well-developed Malpighian tubules, which are lined with simple cuboidal epithelial cells. The digestive system is composed of three distinct parts: foregut, midgut, and hindgut. The respiratory system is made up of a respiratory organ, which is lined with simple squamous epithelial cells. The nervous system consists of two regions: the frontal ganglion and the ventral nerve cord. Each ganglion has two layers: the outer cortex and the inner medullae. The outer cortex contains three types of cells: neurosecretory cells, neuroglial cells, and neurons. The inner medullae layer contains neuroglia and neurons. The female reproductive system of this species is composed of the ovarian structure, the reproductive tract, and the accessory organ.

The internal anatomy and physiology of *H. hayanus* White, 1883, however, have not been covered in great detail. The publication of more detailed morphological and histological information may lead to a better understanding of the physiological and evolutionary adaptations that enabled *Halobates* spp. to proliferate across oceans. Using histological techniques, we examined the systemic structural organization of *H. hayanus* to elucidate characteristic features of this genus.

MATERIALS AND METHODS

Twenty mature of *H. hayanus* were obtained from the waters around Libong Island, Thailand. The total lengths of the samples ranged from 0.6-0.8 cm. The treatment of the animals was strictly in accordance with the recommendations of the Animal Care and Use Committee of the Faculty of Science and Fisheries Technology, Trang Campus, Rajamangala University of Technology Srivijaya (Protocol Review No. IAC 13-03-64). The entire body of each specimen was fixed in Davidson's fixative for about 48 h and stored in 70% ethanol. According to routine histological methods (Presnell & Schreibman 1997; Suvarna, Layton & Bancroft 2013), the samples were dehydrated through ethanol series, cleared in xylene and embedded in Paraplast® (Merck, Darmstadt, Germany). The paraffin block was sectioned longitudinally and sagittally with a rotary microtome at a thickness of 4 µm. Sections were stained with Harris's hematoxylin and eosin (H&E). The 3DHISTECH Panoramic Viewer (3DHISTECH, Hungary) coupled with a digital scanner was used to view and photograph samples.

RESULTS

We categorized the organizational structure of *H. hayanus* into unique systems based on localization, organ features, tissue/cell composition, and staining patterns by examining whole-mount slides under a light microscope, as illustrated in Figures 1-7. Each system is described in specific sections of the text.

INTEGUMENTARY AND MUSCULAR SYSTEMS

The integumentary system was evident throughout the body (Figure 1(A)). All selected regions of the integument, including head (Figure 1(B)), upper abdomen (Figure 1(C)) and middle abdomen Figure 1(D)), were similarly structured with three primary layers of epicuticle, exocuticle, and endocuticle (Representative Figure 1(C)). The epicuticle with the sensilla, a sensory organ of arthropods had a substantial, has a brown outer surface (Figure 1(C)). Exceptionally, this structure was absent from the integuments of the head and abdomen, possibly due to an artefact created during the histological processing (Figures 1(C)-1(D)). The exocuticle was the middle layer of the integument and thicker than the other two layers. The exocuticle was eosinophilic and had a pink tint (Figure 1(D)). Along the lateral side of the body, the oenocytes contained a small vacuole (Figure 1(E)), whereas trophocytes had a central and oval nucleus with a larger vacuole (Figure 1(F)). Skeletal muscle was mostly observed in longitudinal sections, notably in the abdomen (Figure 1(G)), and was mainly composed of eosinophilic muscular bundles located close to the trachea (Figure 1(G)) containing several muscle fibers (or muscle cells) (data not shown).

EXCRETORY SYSTEM

Four Malpighian tubules were prevalent in a variety of locations between the midgut and the hindgut. The homogenous eosinophilic matrix included the tubular lumen (Figures 2(A)-2(B)). The tubules comprised two layers, an outer layer of peritoneal membrane and an epithelial layer (Figure 2(C)). lined with simple cuboidal epithelium (Figure 2(C)). H&E staining revealed that the cytoplasm of these cells was extremely basophilic and that their euchromatic nuclei were centrally positioned.

NERVOUS SYSTEM

The brain (or frontal ganglion), ventral nerve cord and ventral ganglia were identified. The central nervous system (CNS) was in the middle of the head at the frontal ganglion (brain) (Figure 2(A)). The longitudinal section of the brain (Figure 2(D)) showed frontal ganglia implanted inside a cranial capsule protected by a thin layer of connective tissue (or neuronal capsule). The CNS comprised anterior and ventral horns (Figure 2(D)). The anterior horn presented a protocerebrum and deutocerebrum, whereas the ventral horn presented the ventral tritocerebrum. Paired lobes, also known as corpora pedunculata or mushroom bodies, attached the ocular structure to the lateral protocerebrum. The optic nerve divided this region (or optic stalk). Under high magnification, the optic nerve showed three parts: the medulla interna, the medulla externa, and the inmina ganglionaris (Figure 2(D)). Two ganglia - the sub-esophageal ganglion (Figure 2(E)) and the abdominal ganglion (data not shown)- were identified in the ventral nerve cord. Each ganglion presented an inner medulla and outer cortex, identically arranged forming two layers in each ganglion, (Figure 4(E)). The inner medullae were composed of nerve fibres and neuroglia (Figure 3(E)).

Extensive multilayers of neuronal cells in the outer cortex were classified into three categories based on size and histological characteristics. The largest of the three cell types was the oval-shaped neurosecretory cell (Nc), which had a diameter of roughly 5-6 µm. The oval nucleus of the Nc exhibited one or two central nucleoli and the cell was surrounded by eosinophilic nucleoplasm (Figure 2(F)). The neuronal cell was oval-shaped with a major axis of 3-4 µm. The smallest cells were the neuroglia, with a diameter of around 2 µm. This cell was often situated among other cell types (Figure 4(G)). Eosinophilic cytoplasm surrounded its spherical nucleus. Three categories based on size and histological characteristics. The largest of the three cell types was the oval-shaped neurosecretory cell (Nc), which had a diameter of roughly 5-6 µm.

A pair of compound eyes were evident in sagittal sections (Figure 3(A)). Ommatidia formed a consistent pattern of densely packed facets (Figure 3(B)). The outer and inner zones of each ommatidium were separated by an extended form. A clear bi-convex cornea comprised the outer zone of the eye (Figure 3(A)). Underneath the structure of the cornea, the inner zone was visible. All around the ommatidium, several cell types were present, including crystalline cone and photoreceptor cells (commonly known as retinular cells). The ommatidia were separated by rhabdomeres (Figure 3(B)). Additionally, an elongated pigment cell containing various brown pigments was situated within the ommatidium (Figure 3(C)).

DIGESTIVE SYSTEM

Based on location and histological structure, the foregut, midgut, and hindgut of the digestive tract were separated into three sections in the longitudinal view (Figures 4(A)-4(B)). From the mouth, which was mainly composed of cuticle and epidermal layer (Figure 4(C)), the foregut was identified as having a very thin, simple squamous epithelium without microvilli; however, the muscular layer was vary sparse (Figure 4(D)). The most significant organ in the digestive system was the midgut. It was circular in form (Figure 4(E)). The mucosal layer of the midgut showed as a longitudinal fold (Figure 4(E)). Several cell types were identified in the midgut, including a non-ciliated low simple cuboidal epithelium, labelled 'enterocyte' (Figure 4(F)). Each epithelium was distinguished by empty vesicles that had a foamy appearance (Figure 4(F)). Other layers shared the same structure as the foregut. Simple cuboidal epithelium bordered the mucosal hindgut (Figure 4(G)). A proliferation of epithelium was observed in the hindgut (Figure 4(H)). No rectal papilla could be found in the epithelial layer of the hindgut.

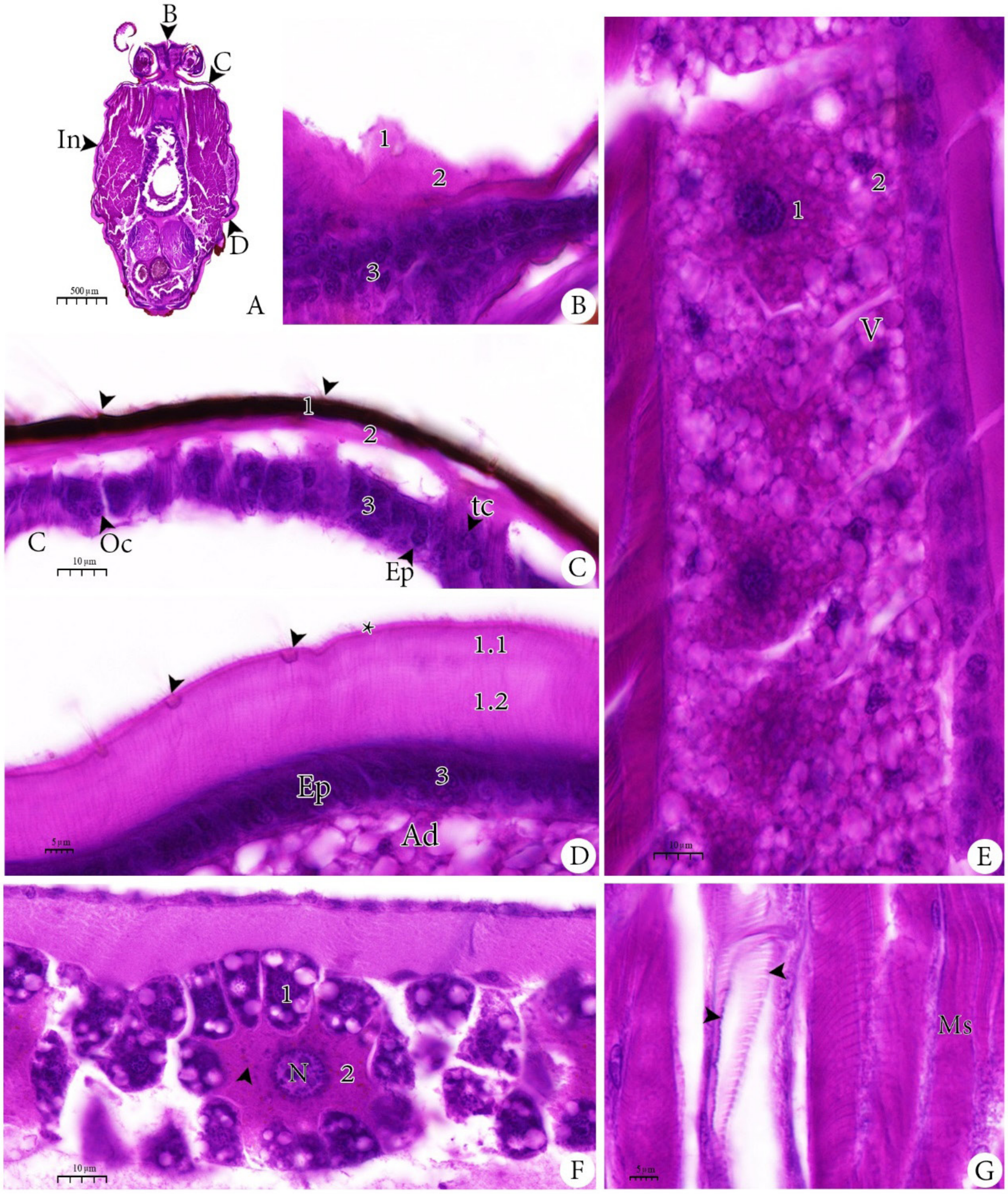

FIGURE 1**.** The photomicrograph shows the integument system (In), the adipose tissue (Ad) and the muscular system (M) in *Halobates hayanus*. The lettered arrows indicate the areas of the integument which appear in photomicrographs at higher magnification. B: Integument in the head region shows the epicuticle (1), endocuticle (2) and epidermal layer (3). C: In the upper abdomen, the epicuticle (arrows) covers the dark-colored exocuticle (1) on top of the endocuticle covering the epidermis (3). In the epidermis, several cell types are indicated by arrows, including an oenocyte (Oc), a trichogen (tc) and an epidermal cell (Ep). D: The integument in the middle body, shows the epicuticle (asterisk), containing several sensilla (arrow heads) in the apical area. The exocuticle is composed of two sub-layers: the upper exocuticle (1.1) and the lower exocuticle (1.2). The epidermal layer (Ep) comprises epidermal cells (3). E: The oenocyte (1) shows a small vacuole (V) in the cytoplasm (2). F: The trophocyte has a larger vacuole (1) in the cytoplasm (2). Small granules (arrow head) were found in the cytoplasm of this cell. G: Longitudinal muscle (Ms) packed muscular cell (Mc) is visible parallel to the trachea (arrows). Staining method: Harris's hematoxylin and eosin (H&E). Abbreviations: Mg = midgut, N = nucleus

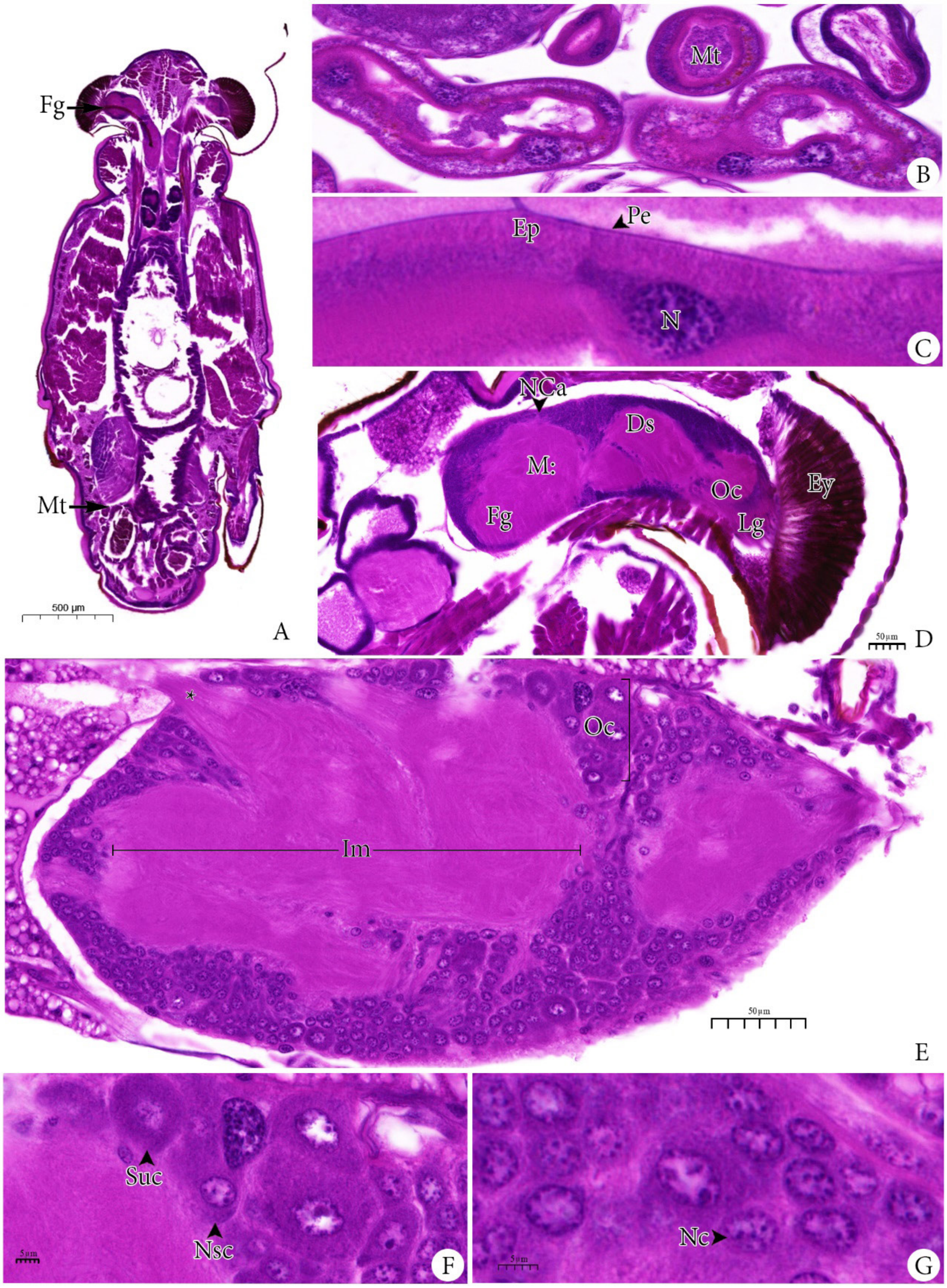

FIGURE 2. Photomicrographs of the urinary system (A-C) and the nervous system (E-F) in *Halobates hayanus*. A-C: Various Malpighian tubules (Mt) can be seen close to the midgut. Higher magnification shows this tubule lined with a simple cuboidal epithelium and peritoneal membrane (Pe). The nervous system show the forebrain (Fg, D) and ganglion (E-G). Abbreviations: DC = deutocerebrum, Ey = eye, Ic = inner optic chiasma, Im = inner medulla, Me = medulla externa, Mi = medulla interna, Nsc = neurosecretory cell, Nca = neuronal capsule, Ne = neuron, Ng = neuroglia, OC = outer cortex, Oc = outer optic chiasma. Staining method: Harris's hematoxylin and eosin (H&E)

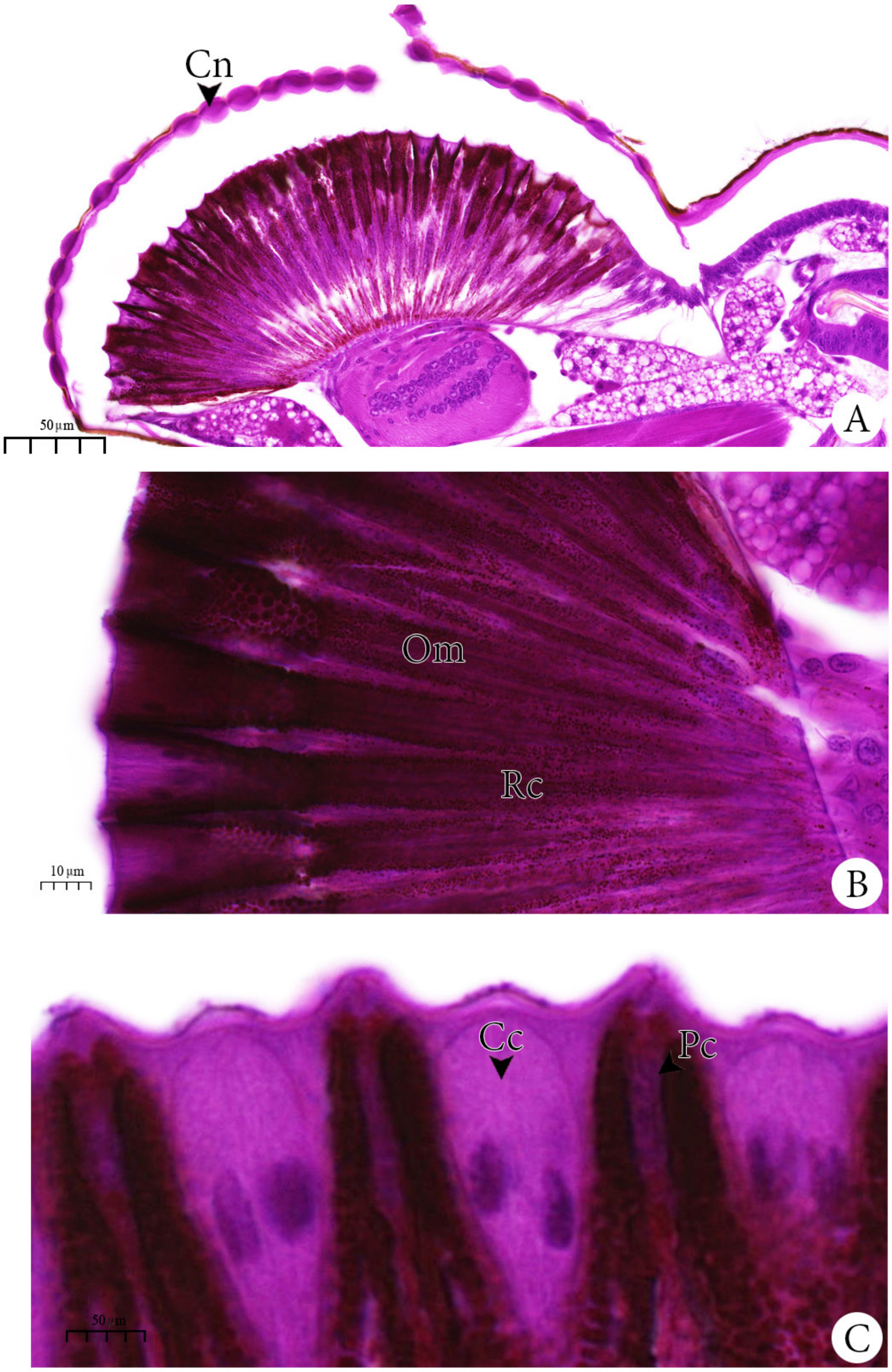

FIGURE 3. Photomicrographs of the eye structure show the regular ommatidium (Om) in *Halobates hayanus*. Staining method: Harris's hematoxylin and eosin (H&E). Abbreviations: Cc = crystalline cone, Cn = cornea, Rc = Retinular cell

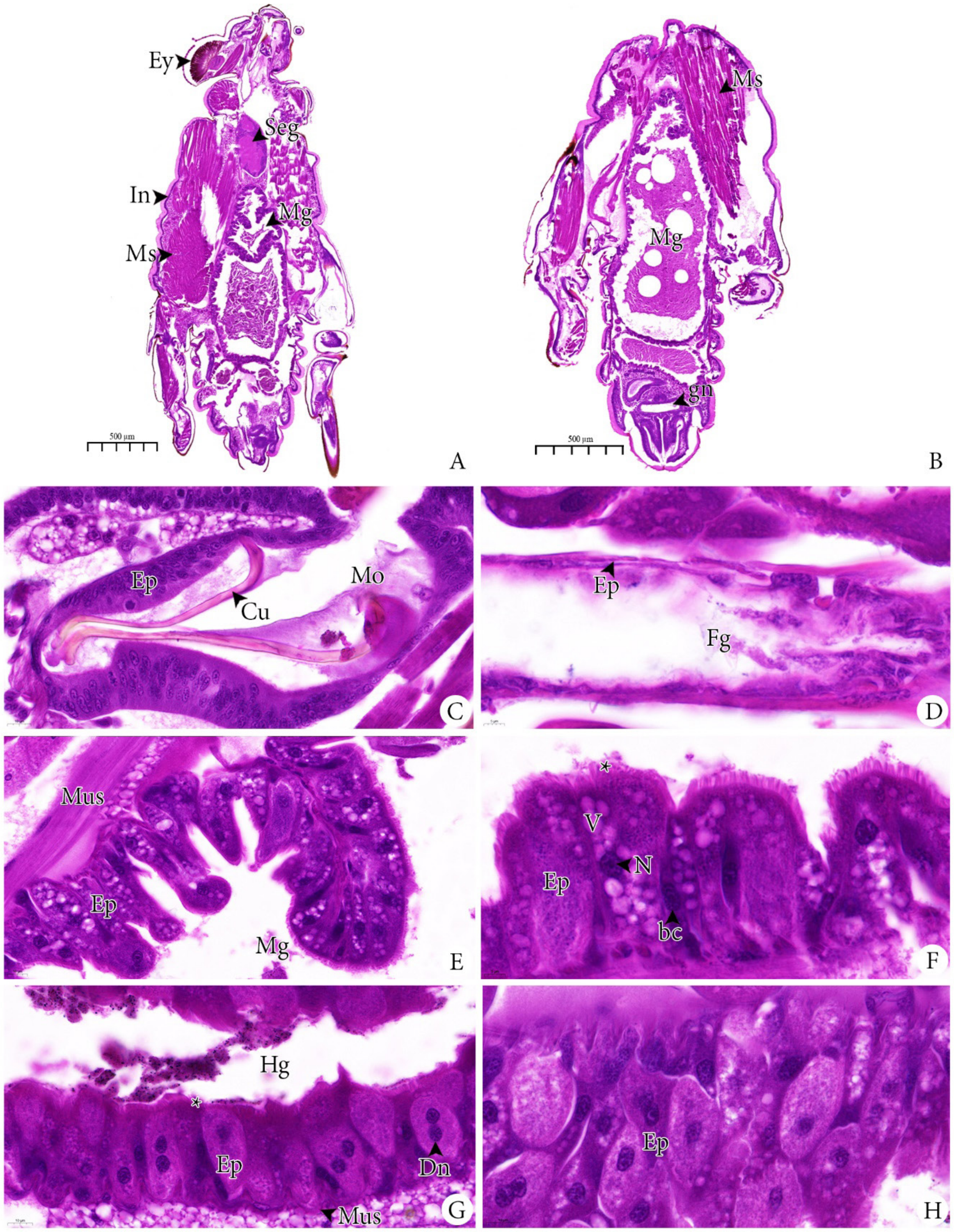

FIGURE 4. Photomicrographs of the digestive system of *Halobates hayanus* show three regions including foregut (Fg), midgut (Mg) and hindgut (Hg) (A-B). C: The mouth structure (Mo) in the foregut is lined with cuticle (Cu) and epithelial (Ep) layers. D: The esophagus was continuous and present in the foregut (Fg). It was covered with a thin layer of epithelium (Ep). E-F: The mucosa (Mu) of the midgut formed into a longitudinal fold (Lf), surrounded by the muscularis (Mus). High magnification shows three types of epithelial cell, including epithelium (Ep), absorptive cell with a foamy appearance or vacuoles (V) and basal cell (bc). G-H: The midgut was lined with a simple columnar epithelium (Ep) having microvilli (asterisk) at the apical surface. Some cells are shown during cell division (Dn). Staining method: Harris's hematoxylin and eosin (H&E)

REPRODUCTIVE SYSTEM

The male reproductive system comprised a pair of testes (Figures 5(A)-5(B)) and accessory organs. The testes were connected to vas deferentia, which are connected to seminal vesicles (Figure 5(C)). This vesicle was lined with a single layer of epithelium and a clear lumen where the maturing sperm bundles were visible (Figure 5(C)). We also identified an accessory organ that was covered with tall cuboidal epithelium (Figure 5(D)). The middle nucleus of each cell was observed and surrounded by the basophilic cytoplasm (Figure 5(D)). The testis was surrounded by a thin layer of testicular capsule (Figure 6(A)). Each follicle had germ cells at different stages of development. A large number of spermatogonium

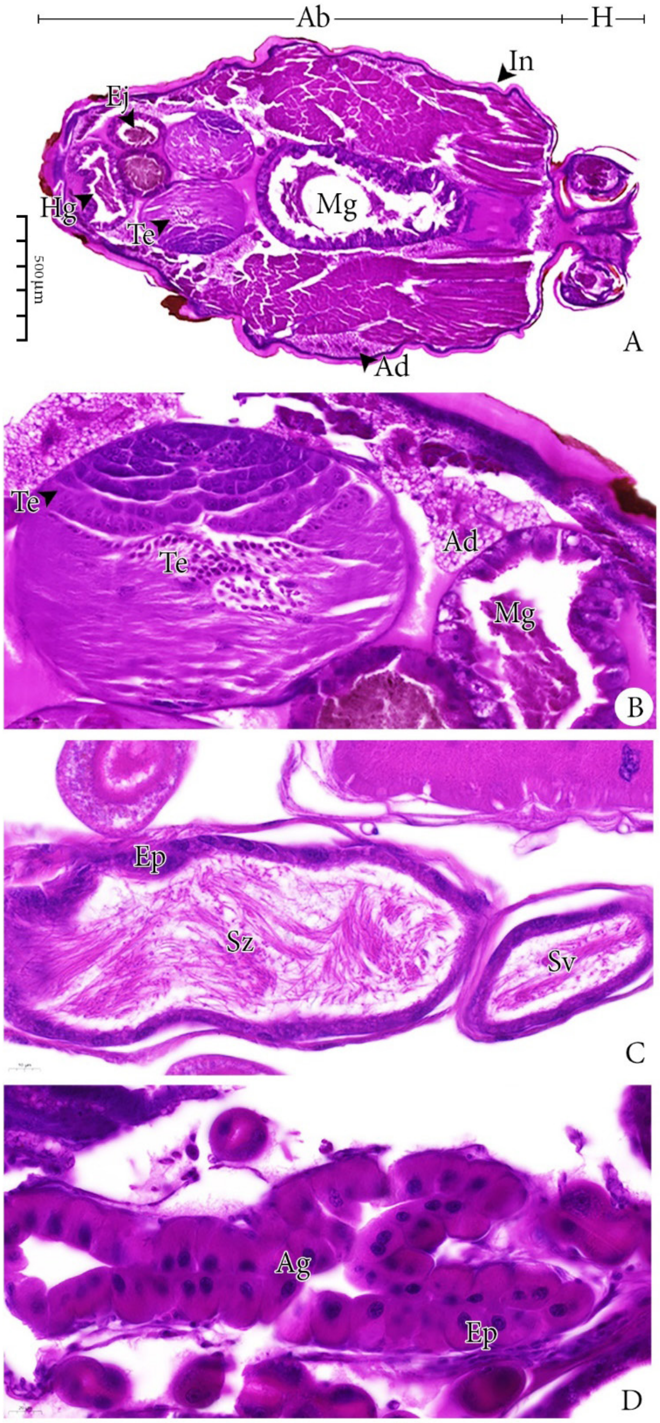

FIGURE 5. Photomicrographs show the male reproductive system of *Halobates hayanus* including the testis (Te) (A-B). The seminal vesicle (Sv) contains packed spermatozoa (Sz) (C). Abbreviations: Ab =abdomen, Ad = adipose cell, Ep = epithelium, H = head, In = integument, Mg = midgut. Staining method: Harris's hematoxylin and eosin (H&E)

type A cells were found at the apical apex of the testis. It contained a small amount of heterochromatin (Figure 6(A)), whereas the spermatogonium type B cell contained strongly structured heterochromatin (Figure 6(B)). This stage was located close to a Sertoli-like cell (Figure 6(B)). Structurally, the spermatogonia were turning into primary spermatocytes after mitosis Figure 6(C)). Spermatogonia contained a large nucleus and were surrounded by a basophilic cytoplasm (Figures 6(C)-6(D)). This stage developed into secondary spermatocytes (Figure 6(E)) in which the nucleus was still visible, but acidophilic cytoplasm was present (Figure 6(E)). The spermatid showed as a small, condensed head and a developing tail (Figure 6(F)). The spermatids stretched to form spermatozoa, seen as a pack of tails among the Sertoli-like cells (Figure 6(G)).

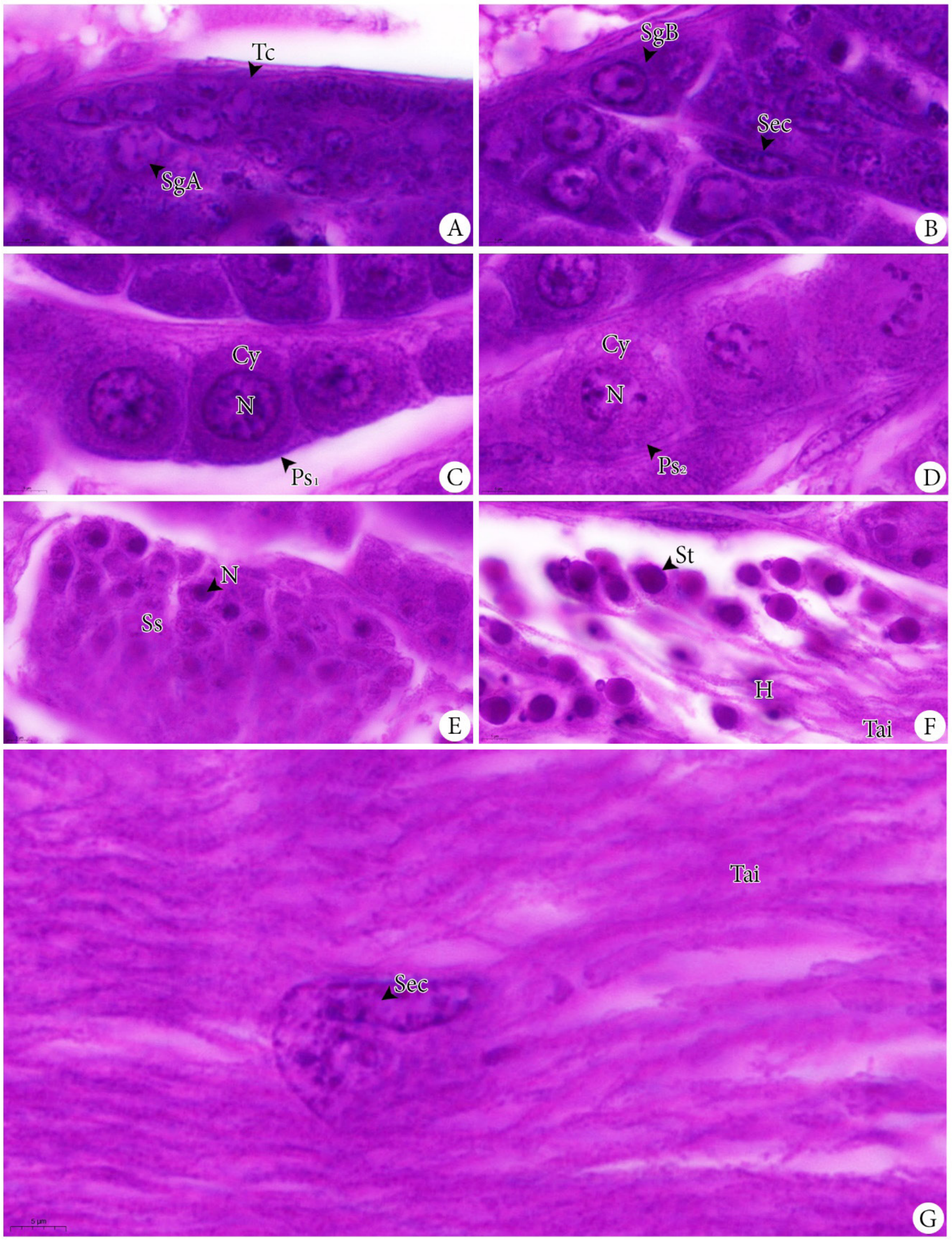

FIGURE 6. High magnification photomicrographs show the spermatogenesis of *Halobates hayanus* including spermatogonia type A (SgA, A), spermatogonia type B (SgB, B), early stage primary spermatocyte (Ps1, C), late stage primary spermatocyte (Ps2, D), secondary spermatocyte (Ss, E), spermatid (St, F) and spermatozoa (G). Abbreviations: Cy = cytoplasm, H = head, N = nucleus, Sec = Sertoli-like cell, Tai = tail, Tc = testicular capsule. Staining method: Harris's hematoxylin and eosin (H&E)

The female reproductive system comprised two components: A pair of ovaries, located along the midgut in the digestive tract (Figure 7(A)), and an oviduct. Oocytes at different stages of development were visible in the vitellarium (Figure 7(B)) close to nurse cells (Figure 7(C)), which were considered to be 'telotrophic ovarioles'. The oocytes were divided into three stages: the previtellogenic stage (Ps), vitellogenic stage (Vs), and mature stage (Ms) (Figures 7(D)-7(H)). In the Ps stage, cells expand. Ps oocytes had a distinctly basophilic cytoplasm (Figure 7(D)). Follicle cells formed a single layer around Ps oocytes (Figure 7(D)). The magnitude of Vs oocytes set them apart from Ps oocytes. At the Vs, the cytoplasm of oocytes contained tiny, developing spherical yolk granules (Figure 7(E)). Each granule of acidophilic yolk responded favourably to H&E staining (Figure 7(F)). The height and gradual transition of the cytoplasm to basophilicity at this stage made the follicular cells very apparent (Figure 7(F)). The Ms oocytes were the biggest and had an irregular form (Figure 7(G)). Expanded yolk granules were scattered throughout the ooplasm and varied in size. Even though the follicular cells in Ms oocytes were not as tall as those in Vs oocytes, the Ms follicular cells were larger and thicker (Figure 7(H)).

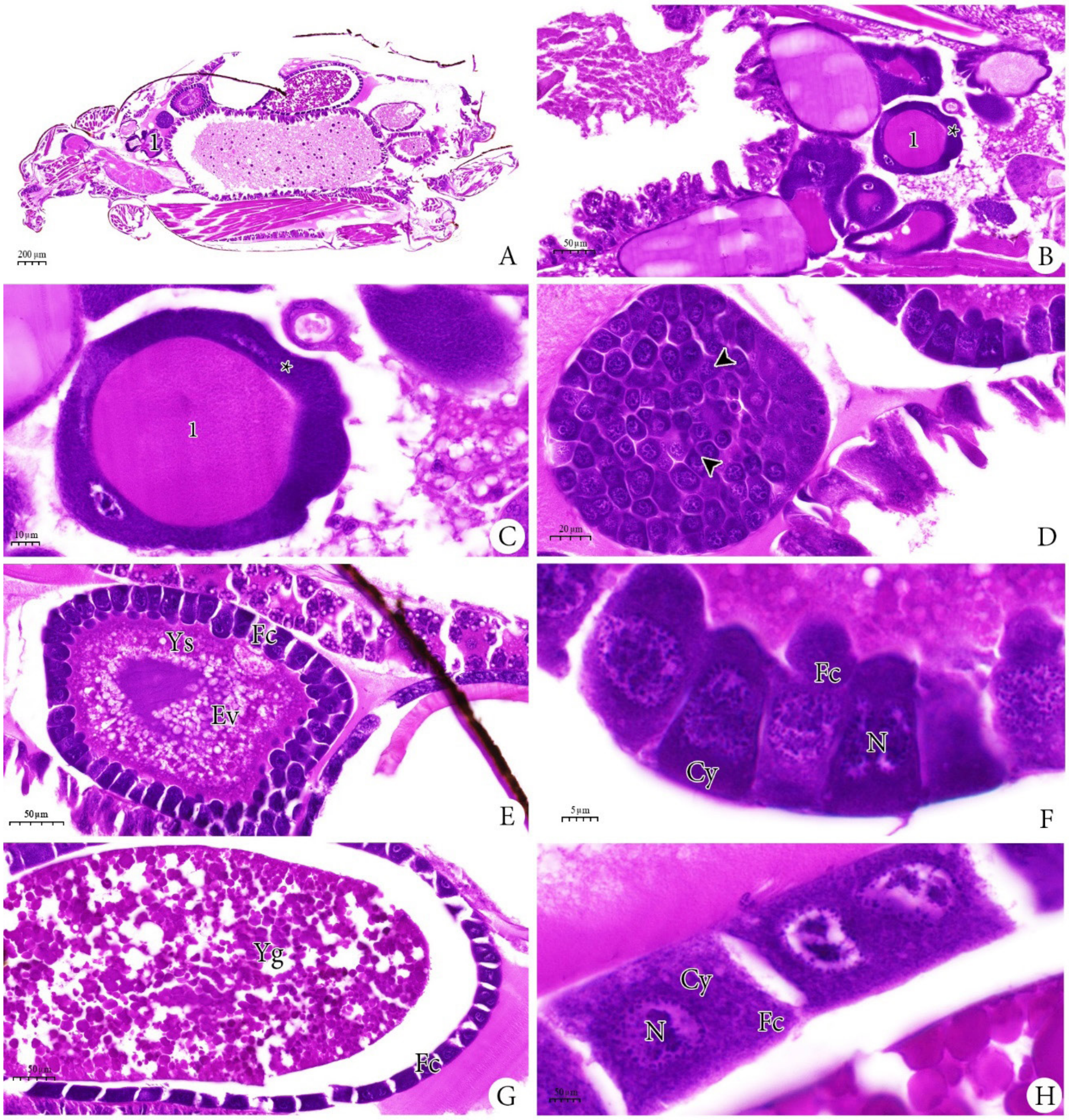

FIGURE 7. Light photomicrographs of the female reproductive system of *Halobates hayanus* shows several oocytes in the ovary (Ov) (A-B) including oogonia (C), previtellogenic stage (Ps) with acidophilic cytoplasm (1) and follicular cell (asterisk), vitellogenic stage (Vs) showing the initial composition of yolk granule (Yg) (E-F) and well-developed follicular cell (Fc) and mature stage (Ms) (G-H). Abbreviations: Cy = cytoplasm, N = nucleus. Staining method: Harris's hematoxylin and eosin (H&E)

DISCUSSION

The histological overview of some insects has been described (Barbosa, Berry & Kary 2015; Billen & Wilson 2008; Chapman 1998; Klowden 2013), in which their system could be classified into five systems including the integument, nervous, muscle, digestive, excretory, and reproductive systems. It is similarly found in the Gerridae including the female reproductive features (Koçakoğlu, Candan & Suludere 2019), and overall systems in both *A. annandalei* (Poolprasert et al. 2022) and *H. hayanus* (present study). We found that the basement membrane, a layer of live epidermal cells, and a cuticle produced by the epidermis constitute the integument in *H. hayanus*, as was similarly reported in the study of general insects (Klowden 2013). Typically, an insect's simple cuticle has three layers, epicuticle, exocuticle and endocuticle (Barbosa, Berry & Kary 2015). Histologically, the exocuticle was eosinophilic and had a pink tint, suggesting the presence of glycoproteins. Glycoproteins are proteins with oligosaccharide chains (glycans) covalently linked to side chains of amino acids. They are associated in all insect species with a wide variety of biological activities and molecular processes (Scheys et al. 2018; Vandenborre et al. 2011).

Muscular bundles responded well to H&E staining, and longitudinal sections demonstrated that they comprised multiple muscle fibers (or muscle cells). An oval heterochromatic nucleus was seen at the periphery of muscle cells. These findings were consistent with those from the striped blister beetle *Epicauta waterhousei* (Langkawong et al. 2013), the webspinner *Oligotoma saundersii* (Poolprasert & Senarat 2014), and the marine water-strider *Asclepios annanadalei* (Poolprasert et al. 2022).

Insects have excretory organs in the abdominal cavity that empty into the intersection of the midgut and the hindgut which are referred to as Malpighian tubules (MTs). MT cells often have long microvilli on their apical side (Özyurt et al. 2017). In this regard, the epithelial characteristics in MTs of this *Halobates* species resembled those of *A. annanadalei* (Poolprasert et al. 2022) and other insects, such as coleopterans (Coleoptera) (Areekul 1957), lapidopterans (Lepidoptera) (Standlea & Yonke 1968), hemipterans (Hemiptera) (Özyurt et al. 2017), and hymenopterans (Hymenoptera) (Arab & Caetano 2002). It has been proposed that protein synthesis in MTs might enhance the function of the excretory system by, for instance, collecting water and nitrogenous waste (uric acid) from the hemolymph and eliminating the remains of the diet through the anus. The MTs are the primary osmoregulatory organ, and according to some studies, they are analogous to the kidneys of vertebrates and the nephridia of annelids (Berridge & Oschman 1969; Gullan & Cranston 2014; Liu & Hua 2018). As similar to previous in the Malpighian tubules of the wood ant, *Formica polyctena* (Hymenoptera), an experimental recording of the activity of a co-transport system and an H$^+$ pump was attempted (Nocelli et al. 2016). It is intriguing to speculate that the development of the Malpighian tubules of *H. hayanus* might be correlated to the adaptation of the species to an estuarine environment, to decrease the loss of water and metabolites in *Asclepios annandalei* as mentioned as by Poolprasert et al. (2022).

The CNS and the peripheral nervous system make up the majority of an insect's nervous system. The brain (frontal ganglion), ventral nerve cord, and ventral ganglia make up the CNS. Since their CNS is aligned ventrally along the length of their body, insects, like humans, have a ventral nerve cord. The peripheral nerve system contains sensory and motor nerves, as well as a stomatogastric ganglion. The insect nervous system also comprises the pars intercerebralis, corpus cardiacum, and corpus allatum, as well as a neuro-endocrine system with organs for storing and releasing substances (Barbosa, Berry & Kary 2015; Gullan & Cranston 2014; Poolprasert et al. 2022). The protocerebrum, deutocerebrum, and tritocerebrum are three pairs of lobes that form the brain structure. The lobes consist of fused ganglia, which are neural cell formations that process sensory inputs. Different actions and processes are managed by each lobe. The number of neurons varies amongst insect brains. A honeybee has one million neurons compared to the 100,000 or less that the typical fruit fly possesses (Eichler et al. 2017; Gullan & Cranston 2014; Howard et al. 2018). Polilov and Makarova (2020), Poolprasert et al. (2022, 2020), and Stöckl and Heinze (2015), have all carried out studies of the brains of insects. It is accepted that all brain regions play a part in the synthesis of neurotransmitters and neuromodulators. These two chemical peptides may be crucial to the processes that regulate reproduction and food intakee (Barbosa, Berry & Kary 2015; Gullan & Cranston 2014; Triplehorn & Johnson 2005).

The microstructure of the digestive system of *H. hayanus* is comparable to that observed in the marine water-strider *A. annandalei* (Poolprasert et al. 2022). The histological difference in the structures of the mucosal linings of the midgut and hindgut suggests that they serve separate purposes. A study of insects (Triplehorn & Johnson 2005) concluded that the hindgut is mainly employed for absorption, whereas the midgut is primarily used for digestion. The midgut epithelium of insects can present four cell types: principal, goblet, regenerative, and endocrine (Chapman 1998), although goblet cells are found only in the midguts of certain insects (Chapman 1998; Happ 1984). Our observations showed three types of epithelial cells in the midgut of *H. hayanus*: principal, regenerative, and endocrine cells. These findings are consistent with previous reports of other hemipterans (Dai et al. 2019; Fialho et al. 2009; Utiyama, Terra & Ribeiro 2016). Principal cells play an important role as secretory cells. We observed differences in the characteristics of principal cells in different regions of the midgut of *H. hayanus*. These regional differences in midgut principal cells have also been observed in other insects (Li et al. 2018; Marana et al. 1997; Roelfstra et al. 2010). Regenerative cells give rise to principal cells and are found near the base of the principal cells (Li et al. 2018). Endocrine cells in the insect midgut play essential roles in many critical physiological functions by releasing various peptides, and the type and distribution of regulatory peptides differ between species (Rothman & Orci 1992). More research in this area is required.

The male reproductive system of *H. hayanus* consists of three major organs: the testes, seminal vesicle and accessory glands. The number of testicular follicles differs among heteropterans (Candan, Özyurt Koçakoğlu & Suludere 2018). Seven follicles per testis are commonly observed, and variations in this number were suggested to be derived states (Candan, Özyurt Koçakoğlu & Suludere 2018; Munhoz et al. 2021). Our observations of the testicular follicles in *H. hayanus* implied that the development of each component occurs in a specific area. The development progresses from the apex to the base as follows: spermatogonia form in the germarium; spermatogonia divide and transform into spermatocytes in the growth zone; spermatocytes undergo two meiotic divisions to become spermatids in the maturation and reduction zone; spermatids transform into spermatozoa in the transformation zone (Candan, Özyurt Koçakoğlu & Suludere 2018; Munhoz et al. 2021; Novais, Dias & Lino-Neto 2017). The presence of spermatogenic cells at different stages suggests that *H. hayanus* produces spermatozoa continuously, allowing more than one mating during adulthood (Bushchini 2007; Moreira et al. 2008; Munhoz et al. 2021). The arrangement of germ cells in cysts is a common feature of insects (da Cruz-Landim 2001; Dallai, Gottardo & Beutel 2016). The number of spermatozoa per bundle varies according to the number of cells that develop synchronously into each cyst (Lino-Neto et al. 2008). The variations in the number of cells in each cyst are useful in hemipteran systematics (da Cruz-Landim 2001; Munhoz et al. 2021). *H. hayanus* also exhibits two pairs of tubular accessory glands covered by squamous epithelium. Some hemipterans lack this organ and encase spermatozoa in amorphous material produced by cells in deferent ducts (Munhoz et al. 2021). Therefore, the presence of accessory glands suggests a vital role in producing a secretion that affects sperm activation and nourishment, female behaviour, and antibiotic activity (Himuro & Fujisaki 2008; King et al. 2011).

The female reproductive system in insects consists of paired ovaries, lateral oviducts, a common oviduct, a spermatheca, and accessory glands (Barbosa, Berry & Kary 2015; Gullan & Cranston 2014). Insect ovaries can be separated into two types: panoistic and meroistic, which differ in how nutrients are made available to oocytes (Barbosa, Berry & Kary 2015). Recently, Poolprasert et al. (2022) comprehensively detailed the structure of the reproductive system of the female *A. annandalei*, which is comparable to the system we observed in *H. hayanus*. Since both marine gerrid species are hemipterans, our observation that hemipteran ovaries are composed of a variable number of telotrophic ovarioles is consistent with the findings of Poolprasert et al. (2022) and is supported by earlier investigations (Elelimy et al. 2017; Grodowitz et al. 2019).

CONCLUSION

By showing the essential mechanisms of *H. hayanus*, this histological analysis provided new information to the corpus of knowledge. Along with the well-developed integument system and Malpighian tubule, the abundance of the respiratory organ may be an adaptation to the estuarine environment that enables *H. hayanus* to halt the loss of water and metabolites. The results of this study may have an impact on further studies of this species and the genus *Halobates*.

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