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Short Communication

Structure and health status of the sand crab, *Emerita taiwanesis* Hsueh, 2015 from Sangchan Beach, Thailand: The histopathological approach

Sinlapachai Senarat*[1], Natthakitt To-orn[2], Chanyut Sudtongkong[1], Gen Kaneko[3], Natthawut Charoenphon[4], Somrudee Meprasert Jitpraphai[5], Jes Kettratad[5]

[1]Department of Marine Science and Environment, Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang campus, Trang, 92150, Thailand.
[2]Program of Fisheries Science, Faculty of Agricultural Technology and Agro-Industry, Rajamangala University of Technology Suvarnabhumi, Ayutthaya 13000, Thailand.
[3]College of Natural and Applied Science, University of Houston-Victoria, Victoria, Texas 77901, USA.
[4]Department of Anatomy, Faculty of Medical Science, Naresuan University, 65000 Thailand.
[5]Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand.

Abstract: Although the impacts of environmental problems on aquatic organisms have been broadly reported in Thailand, the literature has not covered the sand crab, *Emerita taiwanesis* Hsueh, 2015. In this study, we focused on the structure and health status of *E. taiwanesis*, an economically important crab species, living close to human activity areas in Sangchan Beach, Rayong Province, Thailand. A total of 60 individuals were collected from the conservation and restoration of coastal resource project in Ban Rue Leg Kao Yod-based participatory during December 2016 – January 2017. We identified histopathological changes in the gill structure, but not in other vital organs, including ganglion, stomach, intestine, hepatopancreas and muscular bundles. The histological alterations in the gill include hematocyte infiltration, pyknotic nuclei and degeneration of pillar cells in the gill (50% prevalence), suggesting that the gill is a sensitive organ to environmental changes. Our observation provided a better understanding of *E. taiwanesis* morphology and its overall healthy state on Sangchan Beach. Additionally, we suggest that the sand crab would be a suitable sentinel species for monitoring the environment of coastal areas in Thailand.

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Introduction

Benthic invertebrates such as shrimps, crabs, mussels and sea stars have been documented as useful sentinel marker species in biomonitoring programs, which evaluate the health status of animal populations under a serious threat of environmental illness (Viarengo, 1993; Fowler et al., 2004; Hodkinson and Jackson, 2005; Carew, Pettigrove et al., 2013; El-Gammal, Al-Madan and Fita, 2016; Munroe et al., 2018). These species have suitable characteristics, including small size, common prevalence, relatively sessile behavior, and a strong tendency to bioaccumulate pollutants from environments (Lazorchak et al., 2002; Chiarelli and Roccheri, 2014). The histopathological alterations in these sentinel species can be quantitatively analyzed, enabling the fast and efficient assessment of the environment (Moore et al., 1987; Wedderburn et al., 2000). Multiple fields and laboratory studies have associated the histopathological alteration with the presence of heavy metals and toxic contaminants, demonstrating increased health risks for aquatic organisms in the environment (Sarojini et al., 1993; Victor, 1994; Soegianto et al., 1999a, b; Bhavan and Geraldine, 2000).

The sand crab *Emerita taiwanensis* (Hippoidea) is a newly found species described by Hsueh (2015). It is reported to live on sandy beaches with a grain size of 0.25-1.0 mm or brackish water environment in Taiwan (Hsueh, 2015). Biaklai (2016) also reported the presence of *E. taiwanensis* in Thailand as a dominant species in the Y-shaped Breakwater Area on Sangchan Beach, located close to human communities and industrial areas. The anthropogenic

*Correspondence: Sinlapachai Senarat
E-mail: sinlapachai.s@rmutsv.ac.th
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activities negatively affect aquatic life in general (Silarat et al., 2014; Thai-tourism Thailand, 2015). Indeed, the male to the female sex ratio of *E. taiwanensis* in Sangchan Beach is 1: 1.24, and fecundity ranges from 144-1,293 eggs, indicating the presence of reproductive problems (Biaklai, 2016). The gametogenic maturation and embryonic development of *E. taiwanensis* have been explored (Senarat et al., 2018), but the overall health status of the sand crab is still poorly known. In the present study, we investigated the structure and assessed the health status of *E. taiwanensis* as a sentinel species in Sangchan Beach, Thailand. The histopathological methodology was used for the assessment of health status.

Materials and Methods

Sand crab collection: The dead *E. taiwanensis* samples with a mean carapace width of 4-5 cm were collected from December 2016 - January 2017 from the conservation and restoration of coastal resource project in Ban Rue Leg Kao Yod-based participatory, Sangchan Beach, Rayong province, Thailand (12°39'46"N, 101°14'50"E). These samples have been deposited as voucher specimens from the work of Biaklai (2016), and we used a total of 60 fixed samples of them. Species identification was performed according to the decapod taxonomic studies of Boyko and Harvey (1999) and Hsueh et al. (2015).

Morphology and histological techniques: The morphology was observed externally and internally under a stereomicroscope (SZ760B2L). The samples were then processed with standard guidelines of histological techniques (Presnell and Schreibman, 1997). Four-micron-thick sections of each block were stained with Harris's hematoxylin and eosin (H&E) and cresyl violet (CV) (Presnell and Schreibman, 1997; Suvarna et al., 2013). Structural and histopathological features were microscopically evaluated and photographed using a Leica digital 750 light microscope. Each histopathological alteration on each organ was assessed as percent prevalence (%).

Results and Discussion

The examinations of histological sections showed that the central nervous system (CNS), digestive system (DS), and muscular system (MS) did not have any apparent histopathological lesions (Figs. 1-4). On the contrary, the respiratory system (RS) had several histopathological alterations (Fig. 5). The CNS of the sand crab was comprised of the brain (supraesophageal ganglion) located in the head region (Fig. 1A-B) and the ventral nerve cord (VNC) (Fig. 1A-F) as seen in other crabs (Sandeman et al., 1992; Saetan et al., 2013). This system is involved in the endocrine regulation of the growth and reproduction of the crustaceans (Diwan, 2005; Ramachadra, 2018). Stalked compound eyes were present in the anterior part of the head (Fig. 1A, G). The optic nerve connecting the eyes to the brain was observed (Fig. 1G). Longitudinal sections showed that the eye's surface is organized by a layer of facet lenses. A wide crystalline cone layer was visible beneath the lens, and the layer of retinal cells was arranged throughout the rhabdom layer. The pigment cells were widely extended between the crystalline cones and retinal cells (Fig. 1H).

Each part of the brain (Fig. 1C-D) and ganglion (Fig. 1E-F) contained the neuropils (or supporting cells) associated with neuronal cell clusters. A compact arrangement of numerous neurons was observed in the brain, and each cell had a prominent nucleus surrounded by the basophilic nucleoplasm (Fig. 1E-F). The secretory granules were scattered throughout the cytoplasm, which is referred to nissal bodies (also called nissl substances or nissl materials) (Fig. 1F). A previous observation showed that the neurons produce the GnRH-like peptide to control reproductive activity (Saetan et al., 2013). The neuropil contained a single nucleus, which was covered with homogeneously deep basophilic cytoplasm (Fig. 1D-F).

The DS of *E. taiwanesis* consisted of the foregut, midgut and hindgut. The major function of this

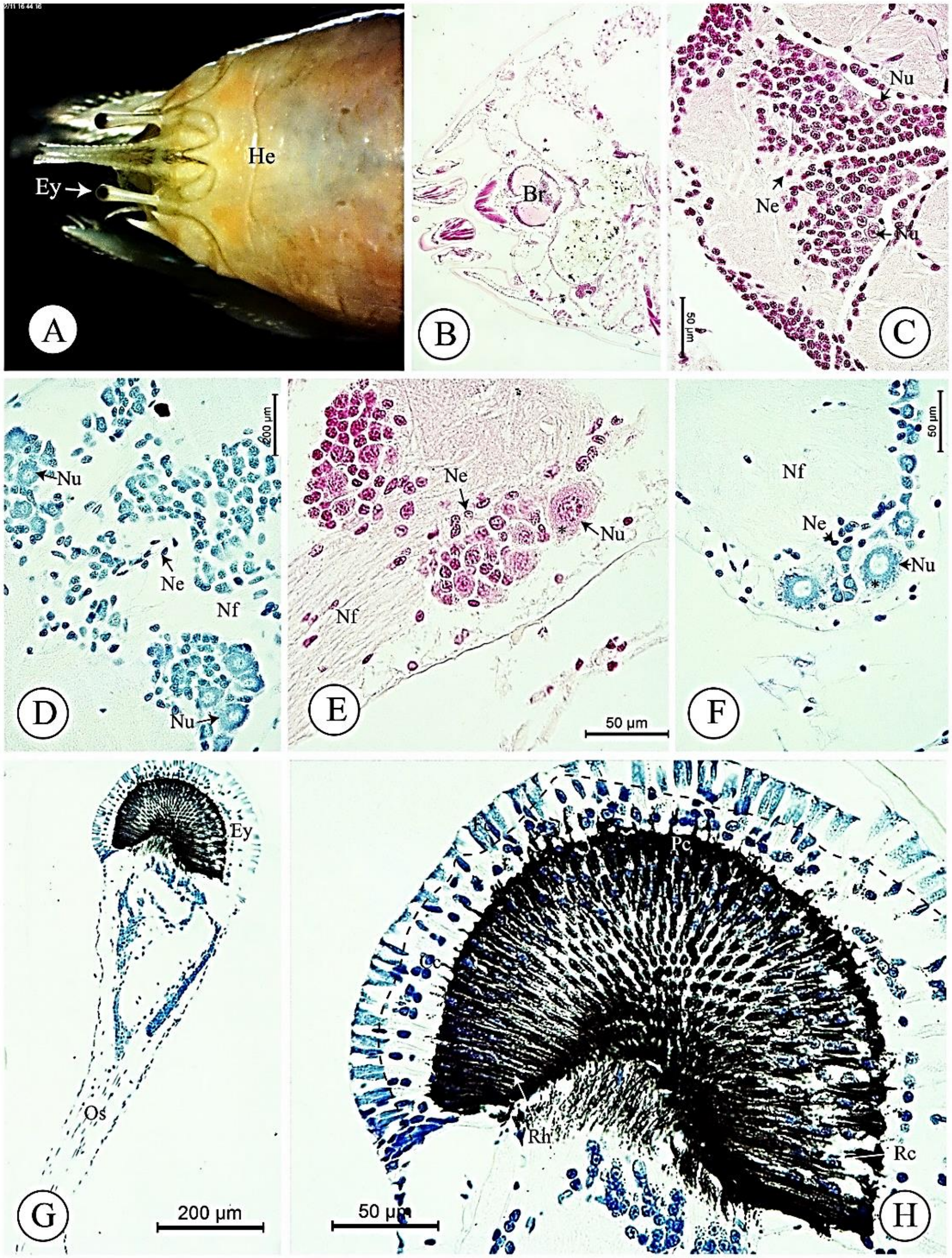

Figure 1. Morphology and light microscope of the central nervous system (CNS) and eye structure (Ey) of *Emerita taiwanesis* A: Head (He) connected to compound eyes. B-D: The brain (Br) contained neuropils (Ne) and clusters of neurons (Nu). Neuronal fibers (Nf) were also observed. E-F: A representative ganglion in the brain. Ganglions are contained in both neuropils (Ne) and the cluster of neurons (Nu). The nissal bodies (asterisk) were seen in the neucleoplasm. Each ganglion was connected to the nerve tract with neuronal fibers (Nf). G: Longitudinal section showing the eye (Ey) connected to the optic stalk (Os). H: The detailed structure of the eye is composed of muti-cellular layers including a layer of facet lenses (Fc), crystalline cone layer (Cc), retinula cell (Rc), and rhabdom layer (Rh). The pigment cells (Pc) were broadly scatted among retinula cells and the rhabdom layer. B, C, E = Harris's hematoxylin and eosin (H&E), D, F, G-H = cresyl violet (CV).

system includes the ingestion, transit of nutriments and mechanical digestion (Ceccaldi, 1989). In the cardiac stomach, various folds projecting into the lumen were observed (Fig. 2A-B). The epithelium of

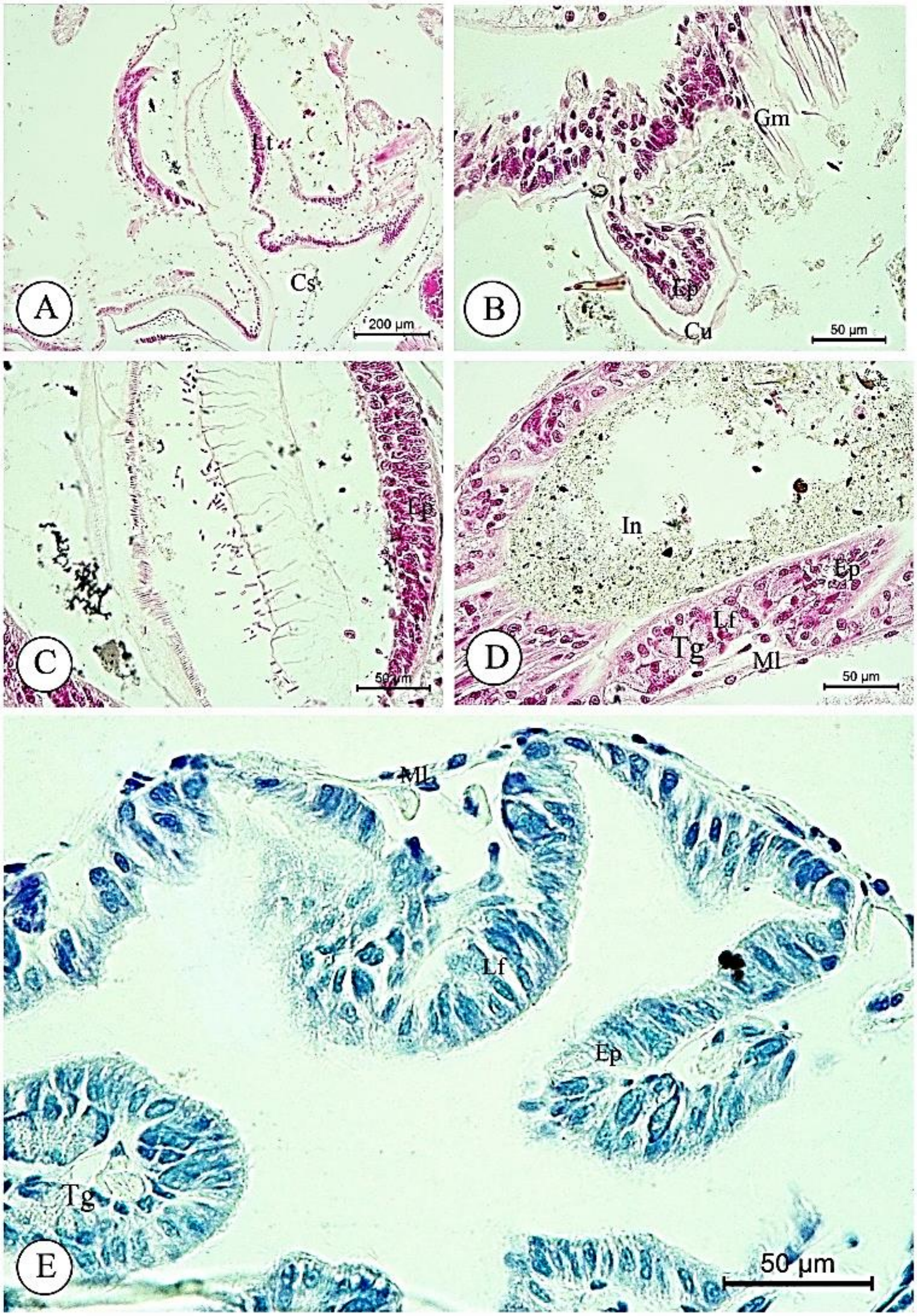

Figure 2. Light microscope showing the structure of stomach and intestine of *Emerita taiwanesis***.** A: The representative histology of cardiac stomach (Cs) and the lateral teeth (Lt). B: High magnification showing longitudinal folds projecting into the lumen that was lined by cuticle (Cu) and a simple culumnar epithelium (Em). The gastric mill teeth (Gm) were also observed. C: High magnification of lateral teeth (LT) containing the thick epithelium (Ep). D-E: Deep longitudinal fold (Lf) of intestine (In) lined with the simple columnar epithelium (Ep) and tegumental glands (Tg). The thin muscular layer (Ml) surrounded of the intestinal wall.

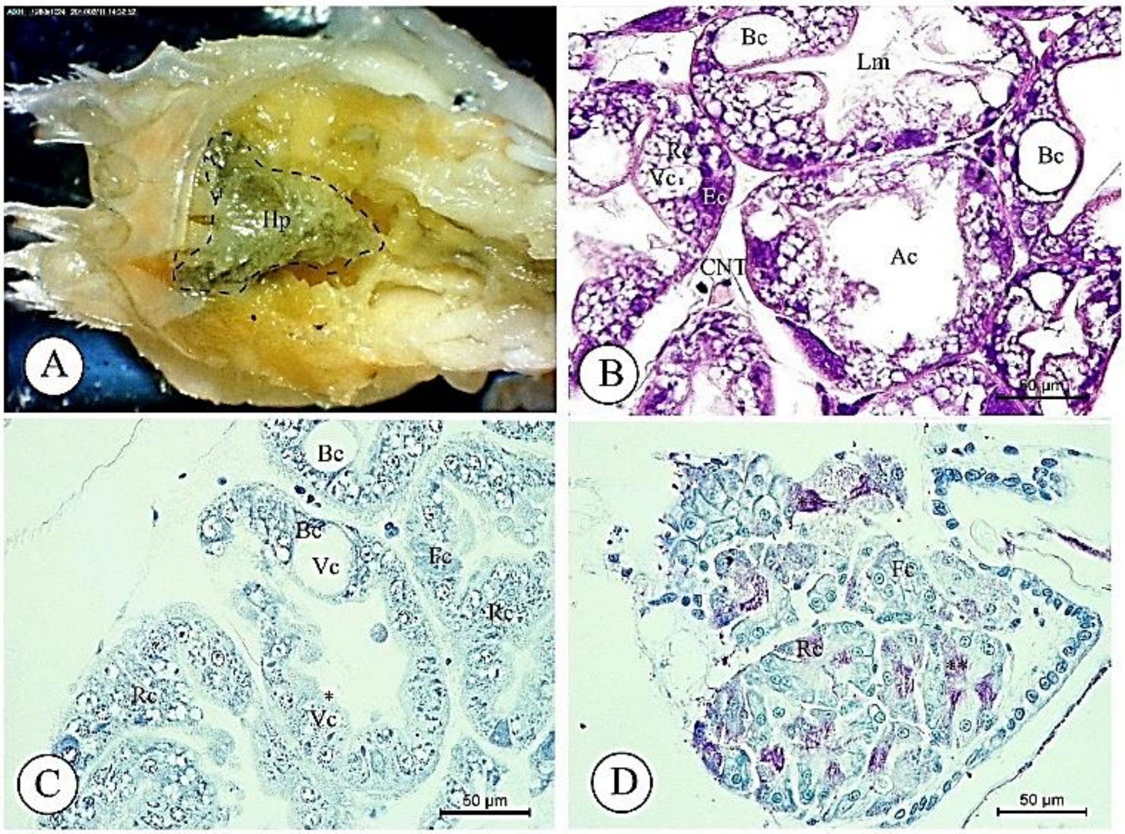

Figure 3. Morphology and light microscope showing the hepatopancreas of *Emerita taiwanesis*. A: The yellowish-brown hepatopancreas (Hp) was observed in the cephalothoracic cavity. B-C: Circular acini (Ac) were separated from neighboring structures by a thin sheet of connective tissue (CNT). The lumen (Lm) of the acinus had a star-like shape. The epithelium of this organ was composed of four main types of cells, including embryonalzellen cell (E-cell), restzellen cell (R-cell), blasenzellen cell (B-cell), and fibrillenyellen (F-cell). Abbreviations: arterisk = an apical microvillar border, Vc = vacuoles.

the mucosal layer in the cardiac stomach was lined with a simple columnar epithelium and covered by a thin cuticle (Fig. 2B). The ossicle system was composed of gastric mill teeth (Fig. 2B) that comprised two lateral teeth (LT) (Fig. 2A), the thickened cuticle and epithelium of the teeth (Fig. 2C) in line with the observation of other crustaceans (Jantrarotai et al., 2005; Lumasag et al., 2007; Melo et al., 2006). The gastric mill teeth are considered to help the digestion of the hard-shelled prey (Anger, 2001). Meanwhile, the intestine was a tube with deep longitudinal folds (Fig. 2D-E) lined by a simple columnar epithelium (Fig. 2F). Numerous tegumental glands were scattered throughout the intestinal epithelium (Fig. 2D-E). It was also covered by a thin muscular layer (Fig. 2D-E).

The hepatopancreas (or the digestive gland) of *E. taiwanesis* was morphologically visible as yellowish-brown tissue within the cephalothoracic cavity (Fig. 3A). This organ has a key role in metabolism and xenobiotic detoxification in crustaceans (Johnston et al., 1998; Sousa and Petriella, 2001). It is constituted by a great number of oval or circular acini (tubules) (Fig. 3B-D). Each acinus was covered with simple epithelial cells, which were prominently separated from the neighboring ones by a thin sheet of connective tissue (Fig. 2B). The cross-sectional observation found that the acinus lumen has a star-like shape (Fig. 2B). The acinus was classified into four main cell types (E, R, B, and F cells) (Fig. 3B-D) based on the detailed features reported in other crustaceans (Ceccaldi, 1989; Maharajan et al., 2015). The embryonic or embryonalzellen cell (E-cell) was the first cell close to the basement membrane (Fig. 3B). A round to oval nucleus was found in the middle region of the cytoplasm of this cell. Restzellen cell (R-cell) was a tall columnar cell with an apical microvillar border

Figure 4. Light microscope images showing the muscular buddles of *Emerita taiwanesis*. A: The muscle (M) was parallel to the gill structure (Gi). B: High magnification showing the tightly packed myomeres (My) that formed the striated muscle. The presence of multi-nucleate cells (cycles) of the skeletal muscle fibers (asterisks) was observed.

(Fig. 3C) with a basal nucleus (Fig. 3C). The numerous small lipid vacuoles were easily found in this cell (Fig. 3C). Blasenzellen cell (B-cell) was the large cell containing a giant, single secretory granule (Fig. 3C). In addition, a spindle-shaped fibrillenyellen cell (F-cell) was identified between B-cells and F-cells (Fig. 3C). The nucleus was centrally located in this cell. The cytoplasm of this cell had a non-vacuolated structure (Fig. 3C-D).

The muscular bundles were widely scattered along the body (Fig. 4A) and tightly packed as the muscle segments known as “myomeres” (Fig. 4B). It was formed by striated muscle and mainly contained skeletal muscle fibers. Each myofibril comprises several myofilaments. The presence of multi-nucleate cells was observed in the skeletal muscle fibers, where the flattened nuclei were seen in the periphery in parallel to each other for the whole length of the fiber (Fig. 4B).

The gills of *E. taiwanesis* were organized along the body (Fig. 4A) with several normal lamellae structures (Fig. 5A). It could be classified into primary and secondary lamellae (Fig. 5A). The surface of the secondary lamella was covered with a thin layer of the cuticle (Fig. 5B). Irregular intervals of pillar cells were observed in the primary lamellae parallel to the surface (Fig. 5B). The secondary lamella had uniform interlamellar and normal haemocoelic spaces with an optimum number of haemocytes (Fig. 5B). However, a few types of histopathological alterations were present: the hemocytes infiltration with 10 percent prevalence (Fig. 5C) and degeneration of hemocytes and both pyknotic nuclei and degeneration of pillar cells (50% prevalence) (Fig. 5C-D).

The gills are the primary respiratory organ in crabs, which are also responsible for many physiological functions such as excretion of nitrogenous wastes, regulation of acid-base balance and ion regulation (Wilkens, 1981; Redmond, 1995). The alteration of gill structure is related to the functional impairment of homeostasis often caused by environmental pollutants (Alazemi et al., 1996; Kumar and Tembhre, 2010). The observed histopathologies in gills are minor and can normally be found in healthy *E. taiwanesis*. However, these lesions might be the defense responses to some pollutants such as nickel (Abraham and Radhakrushnanm 2002), a combination of chlorpyrifos and cypermethrin (Maharajan et al., 2015) and industrial effluent environment (Jerome and Chukuka, 2016).

Conclusions

Although Sangchan Beach, Rayong province, Thailand, is a major human activity area, many

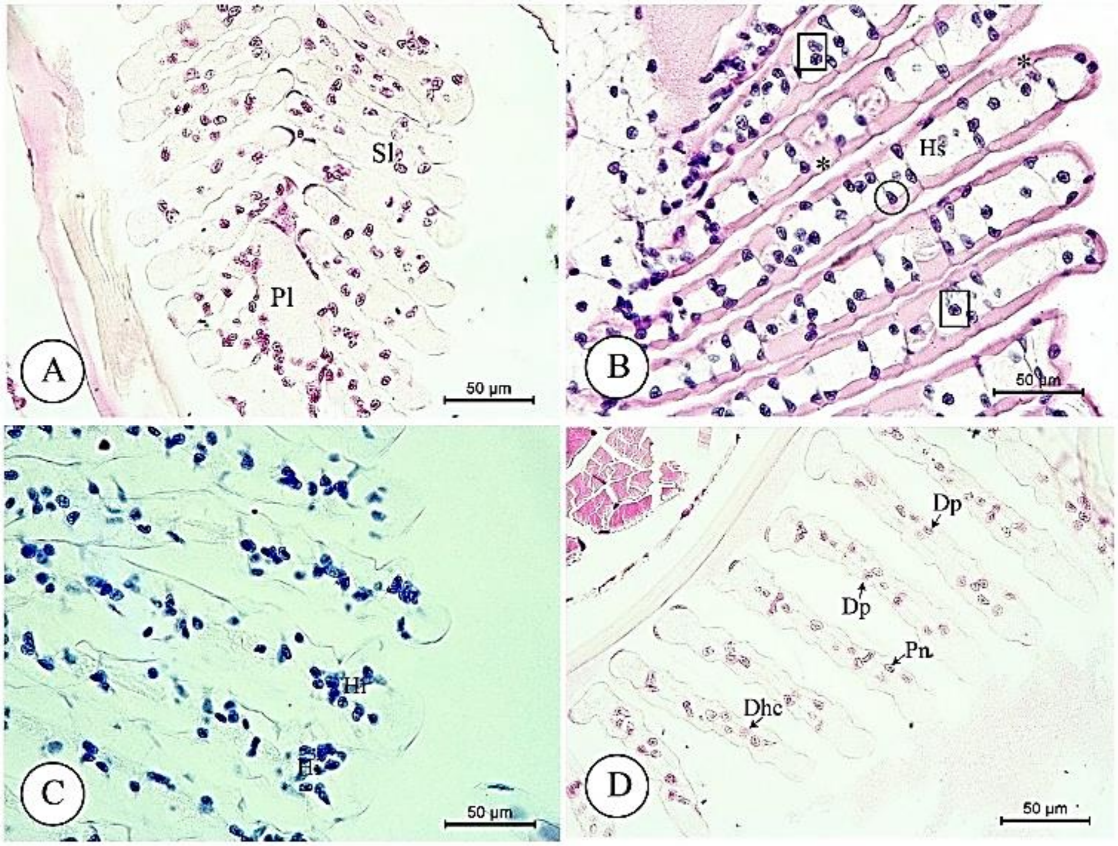

Figure 5. Light microscope showing the gills of *Emerita taiwanesis*. A: The normal gill are composed of several normal lamellae structure that were divided into primary (PI) and secondary lamellae (SI). B: The cuticle of the secondary lamella was lined with the outer layer of cuticle (arterisk). Pillar cells (cycle) and hemocytes (square) were found within the haemocoelic space (Hs). B: Hemocytes infiltrations (HI) were observed in the secondary lamellae. D: Lesions included degeneration of hemocyte (Dhc), pyknotic nuclei of pillar cell (Pn) and degeneration of pillar cells (Dp).

organs of *E. taiwanesis* had no histopathological lesions except for the gill. These data suggest that the sand crab is in healthy condition, which conveys positive evaluations of the current conservation strategies in this area.

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