



# Gastrointestinal Tract and Accessory Organs in the Spotted Bent-toed Gecko, *Cyrtodactylus peguensis* (Boulenger, 1893): A Histological and Histochemical Study

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## Abstract

The spotted bent-toed gecko *Cyrtodactylus peguensis* is one of the exploited reptiles in Thailand. In order to provide basic information for the digestive system of this species, we have examined histologically the gastrointestinal and accessory organs of *C. peguensis* using routine methods. The gastrointestinal region of this reptile started from the stomach and the intestine. The stomach was separated into fundic and pyloric regions. In both regions, the stomach wall was formed by four distinct tissue layers, including mucosa, submucosa, muscularis, and serosa layers. Mucous neck cells and oxynticopeptic cells were identified as glycoprotein-producing cells in the stomach by Periodic acid-Schiff (PAS) staining. The small and large intestines shared many histological characteristics, but the former contained more intestinal folds, while the latter had more PAS-positive goblet cells. Histological characteristics of accessory organs, liver and pancreas, were also provided. Overall, the gastrointestinal and accessory organs of *C. peguensis* were largely similar to those from other reptiles, but fine structural information will open up considerable opportunities to further studies related to the endocrinology, the physiology, and the conservation of this species.

## Keywords

- digestive region
- glycoprotein
- gecko reptile
- liver
- Thailand

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## Introduction

The genus *Cyrtodactylus* (Gray, 1827) is the most species-rich genus of the gekkotan lizard,<sup>1</sup> but it is distributed in geographically restricted area of Southeastern Asia. Among > 200 species in this genus, 17 species are considered to have appeared recently in areas of Myanmar,<sup>2,3</sup> of Vietnam<sup>4,5</sup> and of Thailand.<sup>6</sup> The spotted bent-toed gecko *Cyrtodactylus peguensis* is one of the new species that is estimated to have diverged in the Neogene.<sup>7</sup> They usually live in dry evergreen and peninsular monsoonal evergreen forests in western and peninsular areas of Thailand.<sup>8</sup> Unfortunately, *C. peguensis* is continuously captured for ornamental purposes due to their scarcity value and attractive skin colors. The exploitation and habitat loss resulted in a population decline of *C. peguensis*, and this species is now listed on Appendix III of the Convention on International Trade in Endangered Species (CITES) and as a protected species in Thailand.<sup>9</sup>

Although evolutionary relationships have been established for this genus taking advantage of molecular approaches,<sup>7</sup> there is a lack of morphology-based studies, which can provide valuable information about its biological characteristics. In particular, the morphology and histological features of the digestive system, such as the stomach<sup>10</sup> and intestines,<sup>11</sup> are of great importance to understand their eating habits and the diet diversity, which cannot be directly derived from molecular information. In the present study, we aim to provide a basic description of the gross morphology and histological features of the gastrointestinal tract and of its accessory organs to gain in-depth insight into the feeding ecology of *C. peguensis*. Our contribution could be of use for further studies related to the pathology and the physiology of this species, as well as reptile studies from comparative and evolutionary perspectives.

## Materials and Methods

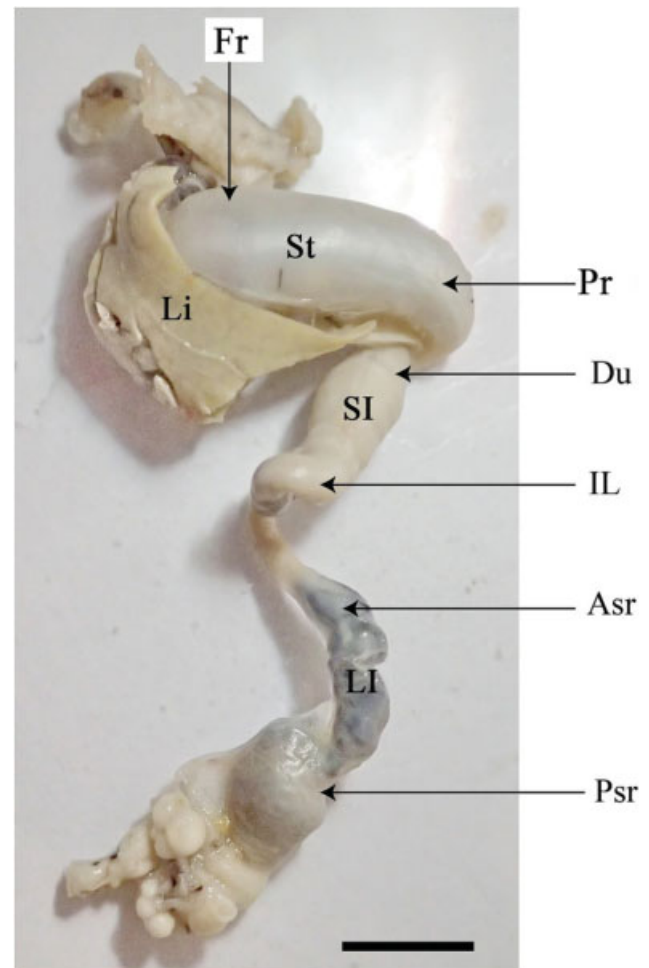
Preserved adult specimens of *C. peguensis* ( $n = 5$ ; PSUZC-REP727, PSUZC-REP154, PSUZC-REP192, PSUZC-REP586, and PSUZC-REP90) were obtained from the National Museum of Department of Biology, Prince of Songkla University, Thailand. All of the specimens were collected from Southern Thailand (Surat Thani, Nakhon Si Thammarat, and Trang provinces). Owing to the unique manner in which these specimens were obtained, ethical approval was not required for the present study. The snout-vent length (SVL) was first determined for all of the specimens. The digestive tract, along with its accessory organs, was then longitudinally dissected out and observed for their anatomical features at the macroscopic level. The total length of the small and large intestines was also determined. All of the digestive tract, except for the esophagus, was subsequently subjected to the standard histological analyses.<sup>12,13</sup> Briefly, using a manual rotating microtome, paraffin blocks were cut at 4  $\mu$ m thickness and stained with Harris hematoxylin and eosin (H&E) to study the basic digestive structure. Periodic acid-Schiff (PAS) staining was also employed to detect glycoprotein production in the mucus-secreting cells.<sup>12,13</sup> These histological

sections were observed and photomicrographed using a light microscope equipped with a TE750-Ua digital camera (Boston Industries, Inc., Walpole, MA, USA).

## Results and Discussion

### Gross Anatomy

The mean SVL of *C. peguensis* was  $54.11 \pm 2.47$  mm (mean  $\pm$  standard deviation [SD],  $n = 5$ ). The length of the digestive tract was  $12.28 \pm 2.52$  cm. All of the five specimens showed similar gross anatomy, as shown in ►Fig. 1. The esophagus, the stomach, and the small intestine were connected to accessory organs (liver and pancreas). The stomach was a wide J-shaped tube and was located in the left antimer. Morphologically, the stomach was composed of two different regions, including fundic and pyloric regions (►Fig. 1). This stomach structure is similar to that of *Hemidactylus mabouia*, which is considered to be carnivorous.<sup>10,14</sup> In contrast, a herbivorous animal, *Iguana iguana*, has a U-shaped stomach.<sup>15</sup> Although we could not find any literature on the



**Fig. 1.** Overall morphology of the digestive system of *Cyrtodactylus peguensis*. The digestive system consisted of the fundic region (Fr) and of the pyloric region (Pr) of the stomach (St), of the duodenum (Du) and of the ileum (IL) of the small intestine (SI), and of the anterior subregion (Asr) and posterior subregion (Psr) of the large intestine (LI). The digestive system was connected to the liver (Li) and to the pancreas. Scale bar = 0.5 cm.

feeding habits of *C. peguensis*, the stomach shape might suggest that *C. peguensis* is carnivorous.

The small and large intestines were separated by a narrow tube and a wall projection (►Fig. 1), as reported in *H. mabouia*.<sup>11</sup> The small intestine was a narrow, coiled tube, and was clearly separated into duodenum and ileum, whereas the large intestine was composed of anterior and posterior subregions, with a thin wall before the opening to the cloaca (►Fig. 1).

## Histology and Histochemistry of the Gastrointestinal Tract

### Stomach

The gastrointestinal tract of this species consisted of two regions, including the stomach and the intestine. The stomach was continued from the esophagus. As for most reptiles,<sup>16–19</sup> the stomach of *C. peguensis* was histologically classified into fundic and pylorus regions.

In the fundic region, the stomach wall was thick and formed by four distinct tissue layers (from inside to outside), including mucosa, submucosa, muscularis layers, and serosa (►Fig. 2A), as found in other vertebrates.<sup>19</sup> Several longitudinal folds (or gastric rugae) were observed in the mucosa and submucosa layers. The submucosa was comprised of loose connective tissue and blood vessels, as found in those of other vertebrates.<sup>19,20</sup> Under the submucosa, there was a thick muscularis layer consisting of two layers: an inner circular muscle layer and an outer longitudinal muscle layer (►Fig. 2A). The serosa was a thin layer of connective tissue covered by the mesothelium.

The invagination of the mucosal surface formed several furrows, the structure also called gastric pit (►Fig. 2B). The mucosal layer was clearly composed of two sublayers, including the epithelial layer and the lamina propria (►Fig. 2B). The lamina propria was a part of the mucosal layer consisted of vascularized loose connective tissues with several simple tubular gastric glands (►Fig. 2C).

The PAS staining identified several types of glycoprotein-producing cells in the fundic region. The epithelial layer of the mucosa was covered by columnar epithelium cells, which were strongly reacted with PAS (►Fig. 2D; simple mucus-secreting cell) as previously reported in *Siphonops annulatus*.<sup>21</sup> These glycoproteins are reported to protect the epithelial lining.<sup>22</sup> Mucous neck cells were also reacted with PAS (►Fig. 2D), whereas PAS-positive oxynticopeptic cells had a polymorphic shape and concentric spherical nucleus. The existence of glycoproteins in mucous neck cells was consistent with previous reports on *Hemidactylus mabouia*,<sup>10</sup> *Natrix natrix*,<sup>23</sup> and some other vertebrates.<sup>18</sup> The roles of mucous neck cells and oxynticopeptic cells in vertebrates are to secrete pepsinogen and hydrochloric acid (HCl),<sup>24</sup> although, in mammals, the chief cell secretes pepsinogen and the parietal cell secrete HCl.<sup>21</sup>

The histological structures of the pyloric region were similar to those of the fundic region (mucosa to serosa). Minor differences include that the fundic region constituted more prominent longitudinal fold than the pyloric region

(►Figs. 2E–2H), and that few gastric glands were observed in the pyloric region (►Figs. 2E–2H).

### Small Intestine

The intestine was divided into two regions: duodenum and ileum. The transitional area between the stomach and duodenum was histologically different from the stomach, with fewer mucosal folds (►Fig. 3A). The muscular sphincter was also observed at the transitional area (►Fig. 3A).

The duodenum region contained mucosa, submucosa, and muscularis layers, but there were fewer mucosal folds compared with the stomach (►Fig. 3B). No submucosal (Brunner) gland was observed. The inner circular muscle and outer longitudinal muscle were thicker than those of the stomach (►Fig. 3B). This duodenum structure was similar to those of other reptiles.<sup>25,26</sup>

The duodenum region also contained goblet cells. Remarkably, these goblet cells were stained by PAS (►Fig. 3C), but not by H&E (►Fig. 3D), indicating that these cells actually contain mucosa. The mucosal layer was covered by a ciliated simple columnar epithelium (►Fig. 3D). This characteristic has been reported in some reptiles, including *Kinosternon scopioides*<sup>20</sup> and *Xerobates agassizii*.<sup>27</sup> Several intraepithelial lymphocytes were localized in the lower portion of the epithelium (►Fig. 3D), as also found in some reptiles (*Lacerta hispanica* and *Natrix maura*).<sup>28</sup> The structure of the ileum was very similar to that of the duodenum, but with fewer goblet cells (►Figs. 3E–3H).

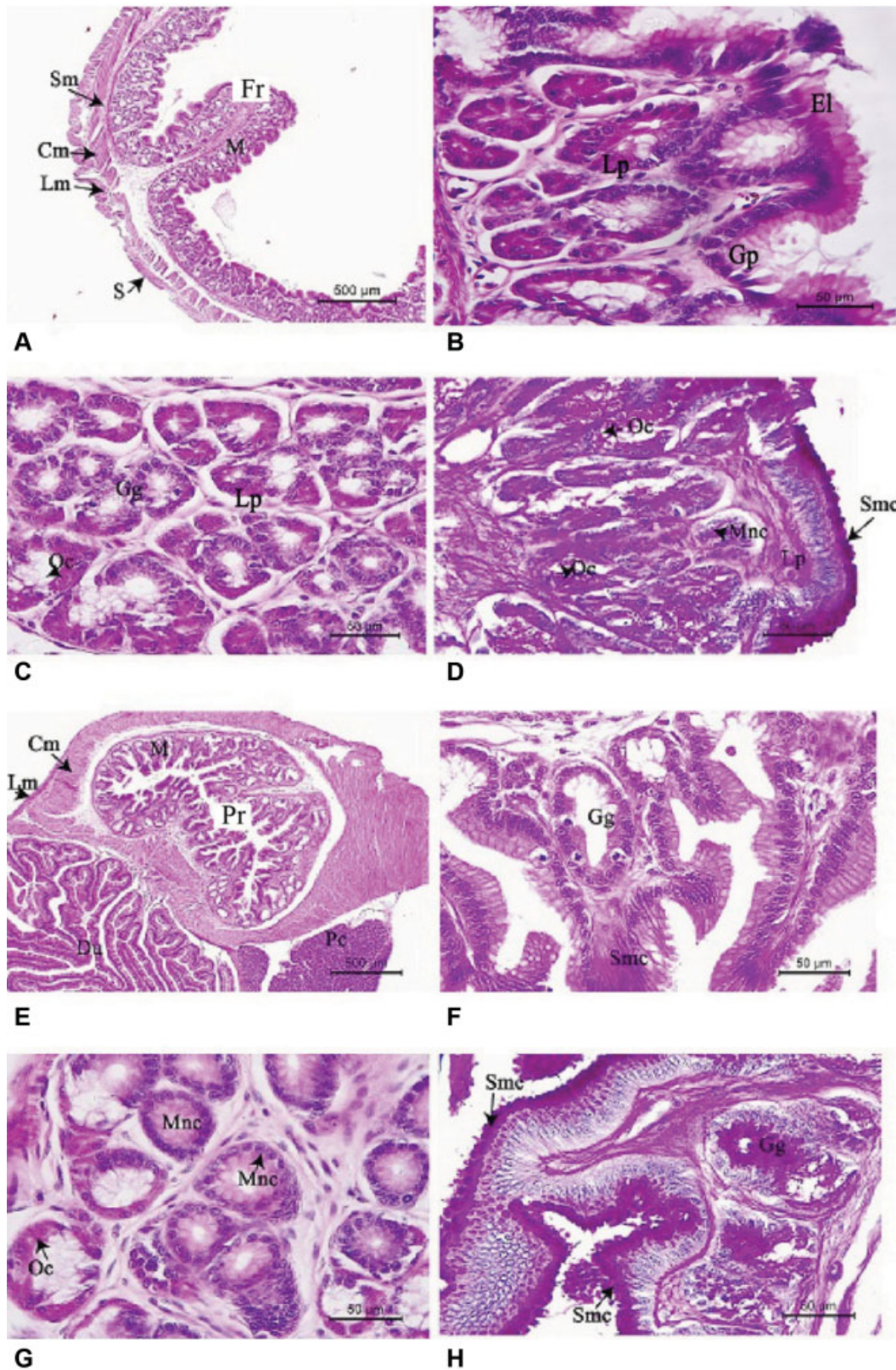
### Large Intestine

The ileum was connected to the large intestine, which was divided into the anterior and posterior subregions. The histological features of the wall of the large intestine were similar to those of the small intestine, but the number of intestinal folds of the anterior large intestine was much lower than that of the small intestine, and no microvilli were observed (►Fig. 4A). The muscularis layer was classified into two layers, the outer longitudinal and the inner circular layers (►Fig. 4A). The serosa was also observed (►Fig. 4A).

The epithelial cells of the large intestine had a columnar, long and narrow shape (►Fig. 4B). Cells on the apical surface of the large intestine were strongly reacted with PAS (►Figs. 4C–4D) as and *Diplometopon zarudnyi*.<sup>29</sup> Moreover, the large intestine had more goblet cells than the small intestine, as revealed by the PAS method (►Fig. 4D), which is consistent with the observations in *Caiman crocodilus yacare*,<sup>25</sup> and in *Xenodon merremii*.<sup>30</sup> However, the role of goblet cells in the large intestine remains unclear, although these cells probably lubricate the food, facilitating its passage along the alimentary tract, preventing mechanical damage to the mucosa.<sup>31</sup> It is also reported that these cells facilitate the passage of feces.<sup>19</sup>

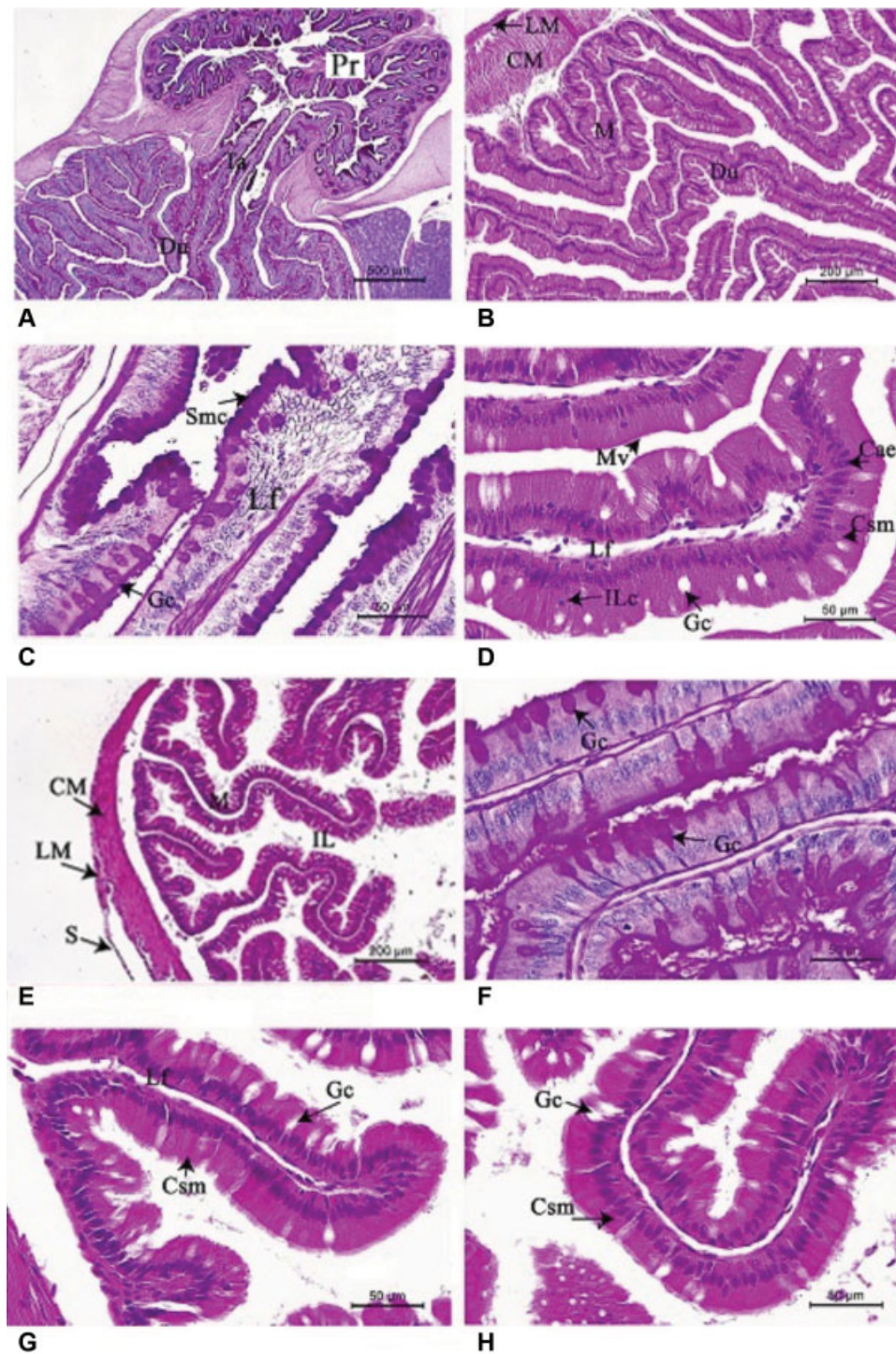
The histological characteristics of the posterior large intestine were largely similar to those of the anterior large intestine, except for the reduced number of intestinal folds (►Figs. 4E–4G).





**Fig. 2.** Fundic (A-D) and pyloric (E-H) regions of stomach. (A) Overall histology of the fundic region of the stomach. (B) The mucosal layer of the fundic region was composed of epithelial layer (EL) and lamina propria (Lp). (C) Gastric glands (Gg) and oxynticopeptic cell (Oc) were observed in the lamina propria (Lp). (D) Periodic acid-Schiff (PAS) staining of the mucosal layer of fundic region. (E) Overall histology of the pyloric region (Pr). (F-H) The oxynticopeptic cell (Oc) and the mucous neck cell (Mnc) in the gastric gland of the pyloric region. Cm = circular muscle layer, Du = duodenum, Lf = longitudinal fold, Lm = longitudinal muscle layer, M = mucosa, Msc = mucosal neck cell, S = serosa, Sm = submucosa, Smc = simple mucus-secreting cells. Note: A-C, E-G = Harris hematoxylin and eosin (H&E) staining; D, H = periodic acid-Schiff (PAS) staining.





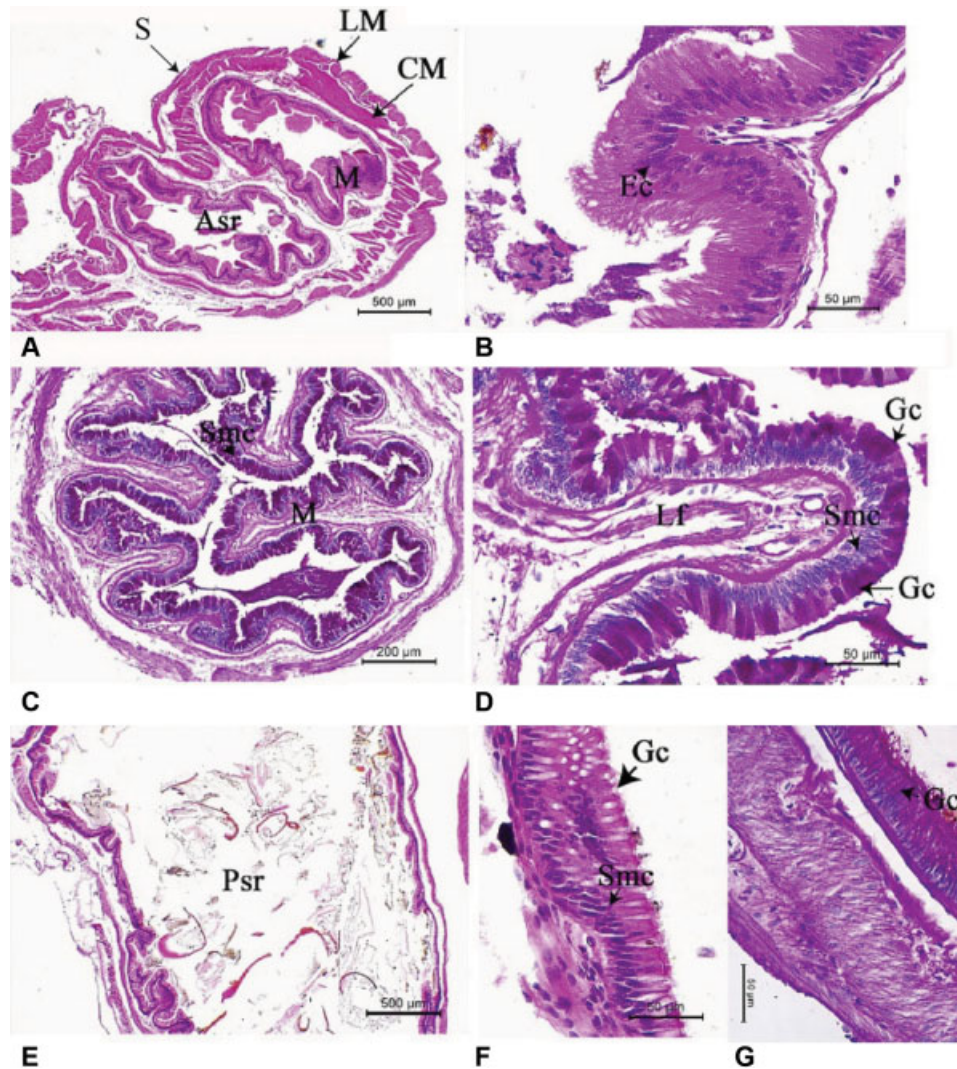
**Fig. 3.** Light photomicrograph of the small intestine. (A) The transitional area (Ta) between pyloric region (Pr) and duodenum (Du). (B) Overall histology of the duodenum. (C-D) Duodenum with several longitudinal folds (Lf) and goblet cells (Gc). (E) Overall histology of the ileum (IL). (F-H) The ileum (IL) contained a ciliated simple columnar epithelium (Csm) and goblet cells (Gc). CM = circular muscle layer, ILc = intraepithelial lymphocyte, LM = longitudinal muscle layer, M = mucosa, Mv = microvilli, S = serosa, Sm = submucosa, Smc = simple mucus-secreting cells. Note: A-C, D, E, G-H = Harris hematoxylin and eosin (H&E); C, F = periodic acid-Schiff (PAS).

## Histology and Histochemistry of the Accessory Organs

### Liver

The liver lobes were surrounded by loose connective tissues. The hepatic parenchyma was comprised of hepatocytes and

the hepatic sinusoid, a unique vascular structure commonly observed in the liver (►Fig. 5A), as in the case of *Nerodia fasciata fasciata*.<sup>32</sup> Each hepatocyte had a polygonal shape and a spherical nucleus (►Fig. 5A). Being strongly reacted with PAS, hepatocytes contained glycoproteins (►Fig. 5B). This observation is similar to those from *T. compressicauda*,<sup>33</sup>



**Fig. 4.** Light photomicrograph of the large intestine. (A) The anterior subregion (Asr) of the large intestine with many longitudinal folds. (B) Epithelial cells of the anterior large intestine. (C, D) PAS staining of the anterior large intestine, detecting Smc and Gc. (E) The posterior subregion (Psr) of the large intestine with less longitudinal folds. (F) Epithelial cells of the posterior large intestine. (D) PAS staining of the posterior large intestine. Ec = epithelium cell, CM = circular muscle layer, Gc = goblet cell, Lm = longitudinal muscle layer, M = mucosa, S = serosa, Sm = submucosa, Smc = simple mucus-secreting cells. Note: A-B, E-F = Harris hematoxylin and eosin (H&E); C-D, G = periodic acid-Schiff (PAS).

some anurans and urodeles.<sup>34–38</sup> The liver of *C. peguensis* is thus considered to be a carbohydrate/protein storage organ. Extracutaneous pigment cells were also observed among hepatic cells (►Fig. 5A). This feature was also observed in the liver of other vertebrates, such as *Sparus aurata* and *Dicentrarchus labrax*,<sup>39</sup> *Prochilodus argenteus*,<sup>40</sup> and *Kinosternon flavescens*.<sup>41</sup> It is believed that the extracutaneous pigment cells arise from Kupffer cells that belong to a mononuclear phagocytic system of the mesodermal origin.<sup>42,43</sup> The pigment cells have protective roles against cytotoxic agents.<sup>44–47</sup> The hepatic sinusoid varied in size and contained simple endothelial cells on the basement membrane (►Fig. 5A). The portal area of the hepatic parenchyma contained the hepatic vein.

### Pancreas

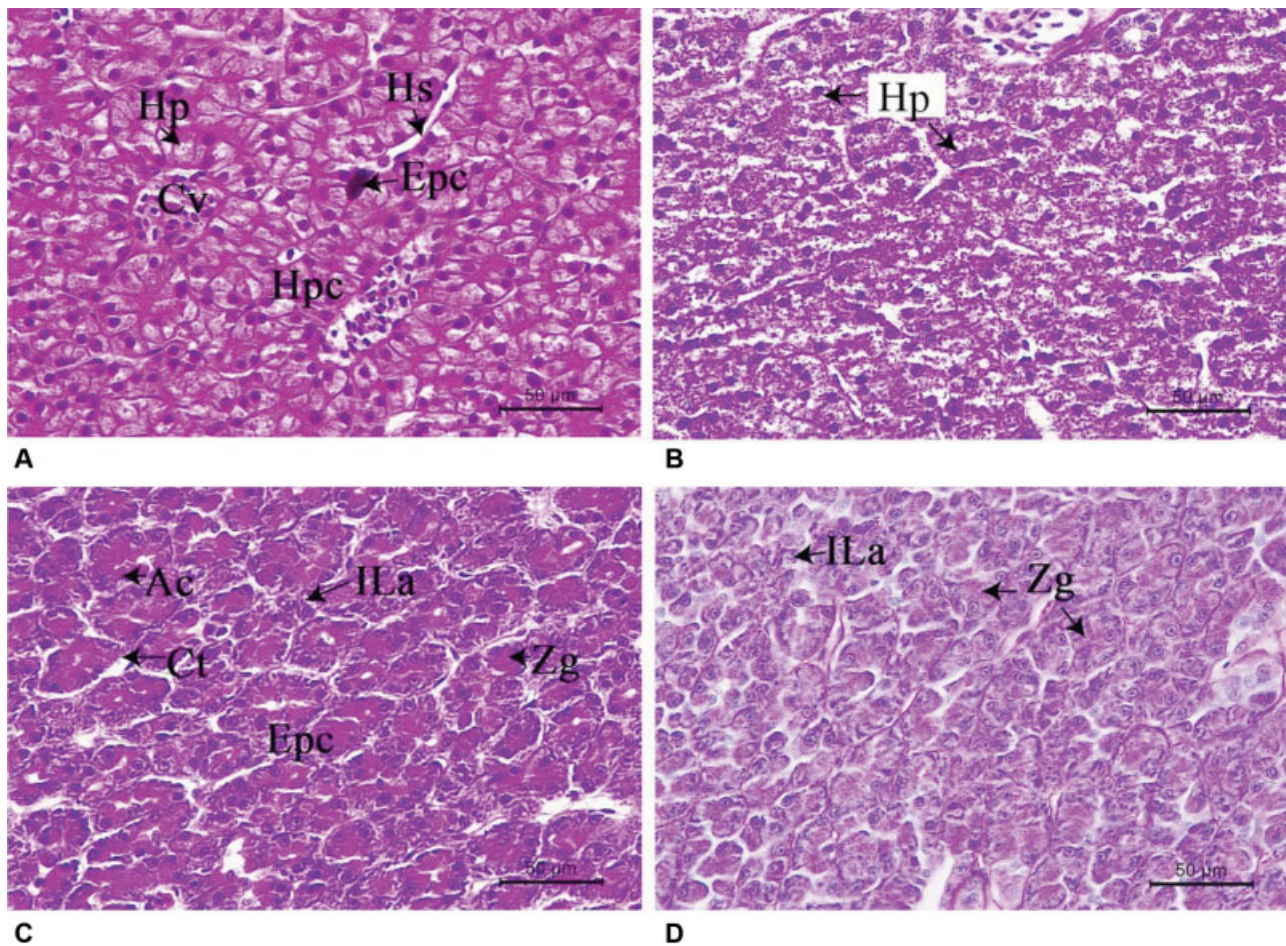
The pancreas of *C. peguensis* was small colonies of two types of pancreatic cells, exocrine and endocrine cells, distributed

along the intestine. The exocrine pancreatic cells formed acini surrounded by loose connective tissues (►Fig. 5C). Each exocrine pancreatic cell had a pyramidal shape and ellipsoid nucleus at the basal region of the cell (►Fig. 5C). Several eosinophilic zymogen granules were also found in the cytoplasm (►Fig. 5C). The pancreatic duct was covered by simple cuboidal epithelium, the apical surface of which reacted with PAS (►Fig. 5D). The endocrine pancreatic cells, as also called islets of Langerhans, were a small cluster surrounding the exocrine pancreatic cell. Several blood vessels were observed near the endocrine pancreatic cells.

### Conclusion

This is the first study that reports the histological and histochemical properties of the digestive tract and accessory organs of *C. peguensis*, a protected reptile species in Thailand. Although the information from our study was largely similar





**Fig. 5.** Light photomicrograph of accessory organs. (A) H&E staining of the liver. (B) PAS staining of the liver. (C) H&E staining of the pancreas. (D) PAS staining of the pancreas. Ac = acinar cell, Ct = connective tissue, Cv = central vein, Epc = extracutaneous pigment cells, Hp = hepatocytes, Hpc = hepatic parenchyma, Hs = hepatic sinusoid, Ila = islets of Langerhans, Epc = exocrine pancreatic cell, Zg = zymogen granules.

to that from other reptiles, it will open up future opportunities to study the fine structure, the endocrinology, the physiology, and the conservation of this species.

#### Conflicts of Interests

The authors have no conflicts of interests to declare.

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