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Original Article

Esophagogastric region and liver tissue in dog-faced water snake *Cerberus rynchops*: Histology and histochemistry

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ABSTRACT

Observation of the esophagogastric region and liver tissue of the dog-faced water snake, *Cerberus rynchops*, living in Thailand was evaluated using standard histological techniques. The results revealed that the digestive tract of this snake consists of three parts: the oesophagus, stomach and intestine, respectively. From the histological analysis, the longitudinal folds of the oesophagus were lined by pseudostratified columnar epithelia with goblet cells. The goblet cells stained positively with periodic acid-Schiff (PAS) and alcian blue (AB) methods. In the stomach, gastric glands extended into the lamina propria. Each gastric gland had a greater cell size than other regions and also a branched tubular gland was visible in the histological images. The mucous neck cell was positive to PAS and AB reactions, whereas, the oxynticopeptic cells slightly reacted to both PAS and AB staining and were preferentially located in this gland. The liver parenchyma of *C. rynchops* was composed of hepatic sinusoids and hepatocytes. In conclusion, this was the first study on digestive tract and liver tissue in *C. rynchops* from the Paknam Pranburi Estuary, Thailand.

Introduction

The dog-faced water snake (*Cerberus rynchops*) is widely distributed in coastal South Asia, Sri Lanka, Bangladesh, Myanmar, the Andaman Islands, eastward to the coastal areas of the Indo-chinese, Malaysian Peninsulas and Thailand and occurs in several areas with intense human fishing and fish cleaning activities (Murphy, 2010). *Cerberus rynchops* has been listed in Appendix III of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (United Nations Environment Program-World Conservation Monitoring Centre, 2013). Several investigations have been active for this species including on the ecology, population structure and genetic diversity (Lv et al., 2015). However, there appears to be no available information on this species to understand the basic anatomy and histology of the upper important organs, in particular the digestive system and liver tissue.

Therefore, in the present study, a comprehensive study was undertaken of the anatomy and histology of the esophagogastric region (oesophagus and stomach) and liver tissue in this species, *C. rynchops*, using the histological techniques of staining with Harris' haematoxylin and eosin (H&E), alcian blue (AB) and periodic acid-Schiff (PAS).

Materials and methods

Animal collection and histological techniques

The carcasses of three adult *C. rynchops* snakes were collected from fishmongers at two stations (N 12°24′15.8″ E 099°58′25.6″ 2 and N 12°24′21.6″ E 099°58′37.1″) in the Paknam Pranburi Estuary,

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Thailand. The snout-vent length (SVL) measurement was recorded and the animals were dissected. The oesophagus and stomach were measured and then these organs with the liver tissue were fixed in Davidson's fixative (24–36 h) and transferred to 70% ethanol to study their anatomical structures. Sections about 3–4 mm long were cut from the anterior, middle and posterior regions of all organs and subsequently subjected to standard histological techniques (Presnell and Schreibman, 1997; Suvarna et al., 2013). Sections 5–6 μm thick were cut and mounted on separate sides to stain with H&E and AB for detection of mucopolysaccharides and with PAS for detection of glycoproteins (Presnell and Schreibman, 1997; Suvarna et al., 2013). The histological features of the digestive tract and the liver tissue of this species were assessed based on the guidelines provided by Khamas and Reeves (2011) and El-Bakry et al. (2012).

Semi-quantitative analysis

The histochemical reactions in the digestive areas were visually classified into four categories: no (−), weak (+), moderate (++) and strong (+++) reactions.

Results and discussion

Gross anatomy

The average SVL (±SE) of the *Cerberus rynchops* snake sampled was 74 ± 4.06 cm. The oesophagus and stomach average length was 18.05.03 ± 3.09 cm (starting from the caudal end of the mandible) and 7.07 ± 2.30 cm, respectively. The liver and the lung were positioned bellow the caudal oesophagus and the stomach.

Histology and histochemistry of the oesophagus

The oesophagus appeared as a simple, tubular structure with tissue layers arranged (Fig. 1A and B), as similarly reported in other snakes (Imai et al., 1991; Abu-Neel, 1999; Cundall et al., 2014) and vertebrates (Imai et al., 1992; Senarat et al., 2014, 2015; Boonyoung et al., 2016). The mucosal layer was thrown into numerous longitudinal folds. The series of its folds were exclusively primary and secondary. The mucosal epithelium was lined by the pseudostratified columnar epithelium, whereas the goblet cells were well observed and arranged within the epithelial layer (Fig. 1A and B). Such observation was similar to that in other reptiles including, *Mauremys caspica* (EL-Taib and Jarrar, 1983), *Coluber florulentus* (Dehlawi and Zaher, 1989), *Chalcides levitoni* and *Mauremyes caspica* (El-Taib et al., 1982; EL-Taib and Jarrar, 1983; Kozaric et al., 2011) and *Pituophis catenifer* (Khamas and Reeves, 2011). The histochemical techniques revealed that the cytoplasm of the goblet cells showed a strong positive reaction to the AB and PAS methods, indicating that they secreted both mucopolysaccharides and glycoproteins (Fig. 1C–F) as in *P. catenifer* (Khamas and Reeves, 2011) and other reptiles (Elliott, 2007; Ahmed et al., 2009) and fish species (Parillo et al., 2004; Senarat et al., 2014, 2015; Boonyoung et al., 2016). The mucous helps in lubricating the surface to facilitate the movement of food items (Khamas and Reeves, 2011). Basal cells had an elongated shape and were observed close to the basement membrane. The lamina propria was made up of loose connective tissue, collagen fibres, small blood vessels and capillaries but no esophageal glands were found throughout the oesophageal region (Fig. 1A and D). This was similar to the condition described for some amphibian reptilian esophageal lamina propria such as in the salamander, *Triturus carnifex* (Liquori et al., 2005). The muscularis mucosa was observed along the mucosa layer. The submucosal layer was rarely

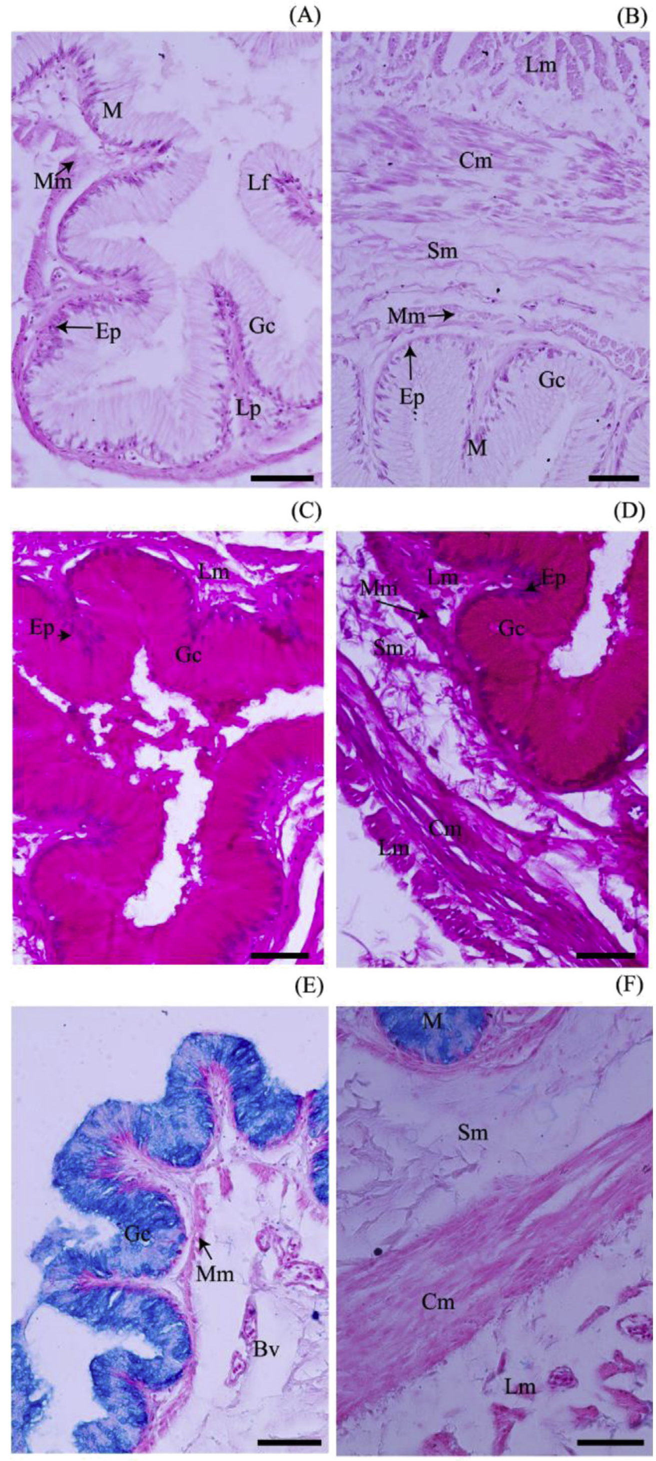

Fig. 1. Light micrograph of the oesophagus in *Cerberus rynchops* snake treated with methods using: (A, B) Harris's haematoxylin and eosin; (C, D) periodic acid-Schiff; (E, F) alcian blue, where scale bar = 50 μm and Bv = blood vessel, Cm = circular muscle layer, Ep = epithelium, Gc = globelt cell, Lf = longitudinal fold, Lm = longitudinal muscle layer, M = mucosa, Mm = muscularis mucosae, Sm = submucosa.

composed of loose connective tissue and several blood vessels. The muscularis externa layer was a thick layer of an inner circular and outer longitudinal layer of smooth muscle (Fig. 1B and F). Lastly, the serosal layer was present and surrounded by a simple squamous epithelium.

Morphology, histology and histochemistry of the stomach

The stomach was easily differentiated from the oesophagus. The length of the stomach was about half the length of the oesophagus and had four distinct layers, in the same pattern as the oesophagus. It has been similarly described in other vertebrates (Rovira et al., 1993; Patt and Patt, 1969). In the gastric mucosal layer of the fundic region, shallow gastric pits of the stomach were observed in both anatomy and histology (Fig. 2A and B). A layer of the mucosal longitudinal fold showed many and large portions of fingerlike projections. The epithelial layer of these folds was covered by a simple columnar epithelium (Fig. 2C). After using histochemical methods, strong PAS and AB positive reactions were clearly detected on the surface of the epithelium (Fig. 2D–G, Table 1), which confirmed that these cells produced both glycoprotein and mucopolysaccharides, similar to those of the stomach of *Siphonops annulatus* (Carvalho and Junqueira, 1999). It is possible that these chemical components protected the lining epithelial layer, as they are present in many animals and humans (Derrien et al., 2010). However, no

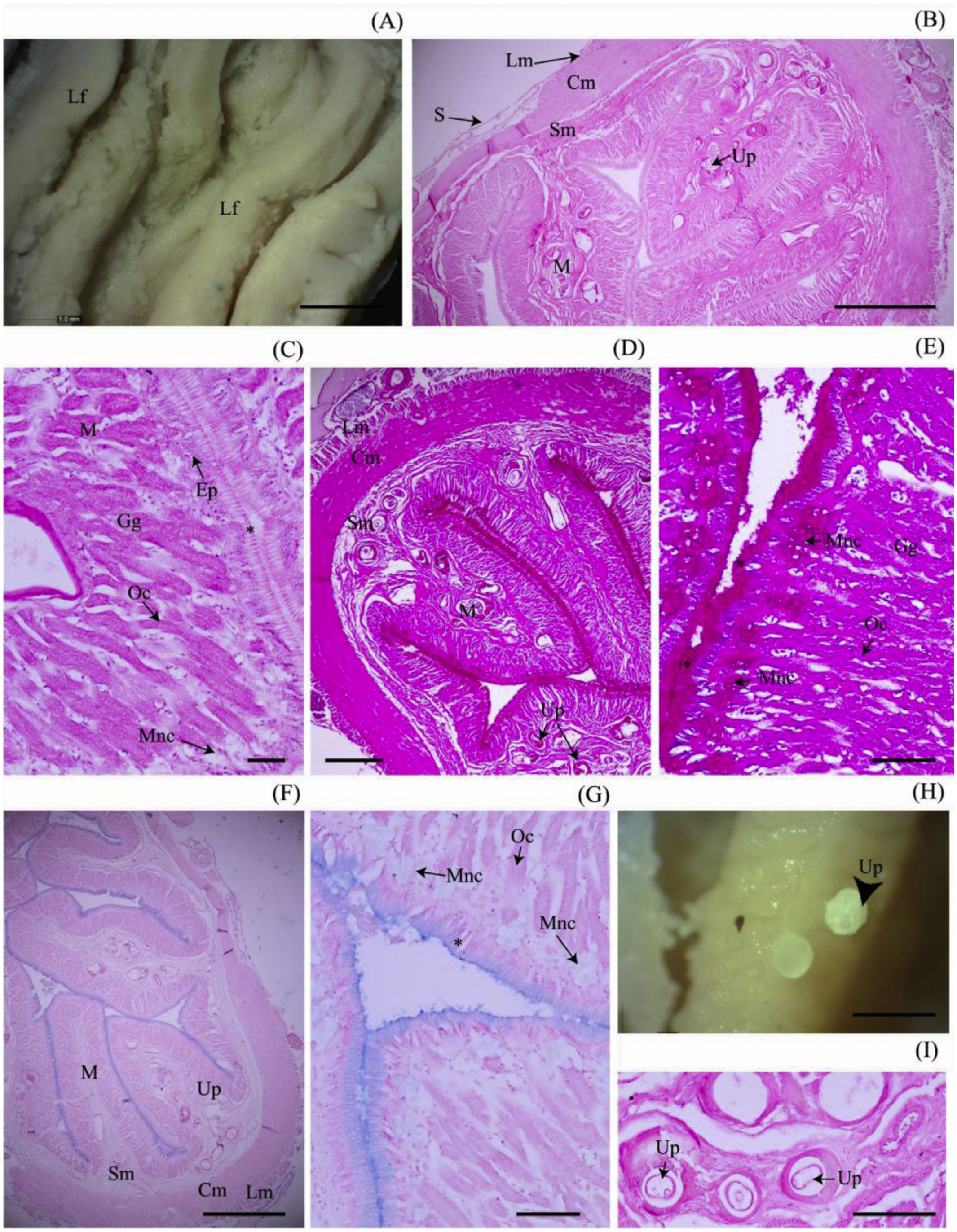

Fig. 2. Light micrograph of the stomach in *Cerberus rynchops* snake treated with methods using (B, C, I) Harris's haematoxylin and eosin; (D, E), periodic acid-Schiff; (F, G) alcian blue, where scale bar = 1.5 mm (A), 100 μm (B, D, F, H), 20 μm (C, E, G, I) and Bv = blood vessel, Cm = circular muscle layer, Ep = epithelium, Gg = gastric gland, Lf = longitudinal fold, Lm = longitudinal muscle layer, M = mucosa, Mm = muscularis mucosae, Mnc = mucous neck cell, Oc = oxynticopeptic cell, S = serosa, Sm = submucosa, Up = unidentified parasites, * = histochemical reactions.

Table 1
Results of semi-quantitative analysis of histological and histochemical methods.

Histology and histochemistry	Goblet cells (oesophagus)	Surface epithelial cells (stomach)	Neck cells (stomach)	Oxynticopeptic cells (stomach)	Goblet cells (intestines)
H&E	–[a]	–	–	–	–
PAS	+++	+++	+++	+	+++
AB	+++	+++	+++	+	+++

[a] – = no reaction; + = weak reaction; ++ = moderate reaction; +++ = strong reaction.

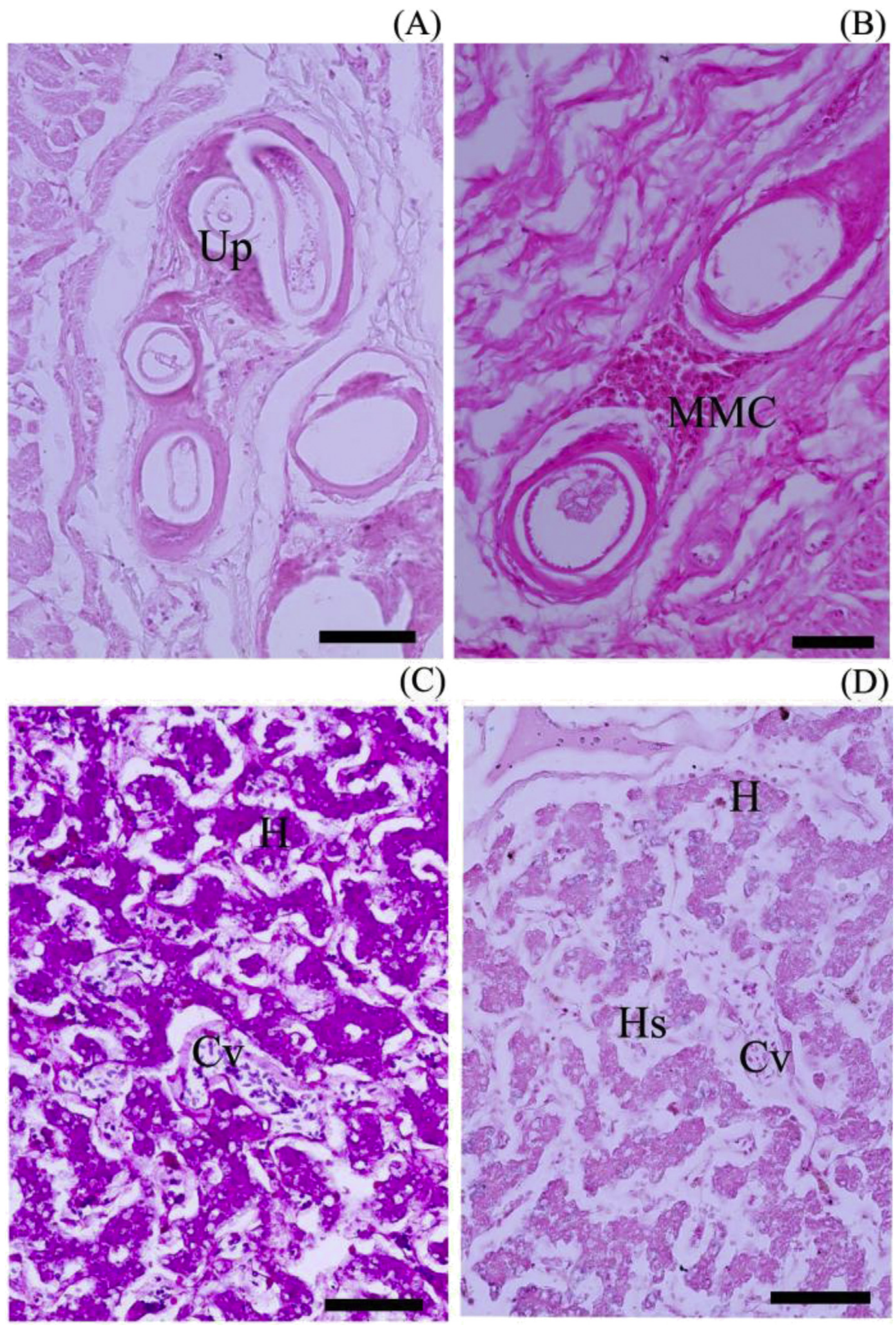

Fig. 3. Light micrograph of the stomach and liver tissue in *Cerberus rynchops* snake, treated using methods of (A, B) Harris's haematoxylin and eosin; (C) periodic acid-Schiff; (D) alcian blue, where scale bar = 20 μm (A, B), 30 μm (C–D) and Cv = central vein, Ep = epithelium, Gg = gastric gland, H = hepatocytes, Hs = hepatic sinus, M = mucosa, MMC = melanomacrophage centre, Up = unidentified parasites, * = histochemical reaction.

mucous cells were found in the epithelial layer. The lamina propria was composed of loose connective tissue and several blood vessels, whereas many gastric glands were well developed through the longitudinal fold. However, it was different from *P. catenifer* in which the longitudinal fold of the stomach was composed of two regions consisting of the non-glandular and glandular portions with unclear reasons for this being so (Khamas and Reeves, 2011). Each gastric gland had a larger cell size than other regions and was also seen as a branched tubular gland. In the neck regions, the mucous neck cell was not stained differently with H & E (Fig. 2C) On the other hand, a strong positive reaction to PAS and AB was clearly detected in this cell (Fig. 2E and G, respectively), whereas the oxynticopeptic cell slightly reacted with PAS and AB and was preferentially located in this gland (Fig. 2E and G, respectively). Hence, this study confirmed that the mucous neck cells of the stomach produced neutral glycoprotein, similar to those of *Natrix natrix* (Scillitani et al., 2012) and another amphibian, *Ceratophrys ornate* (Fry and Kaltenbach, 1999). The function of the mucous neck cells and oxynticopeptic cells was to secrete pepsinogen and hydrochloric acid (HCl), similar to previous reports (Liquori et al., 2002). However, in mammals, this is different because the chief cell secretes pepsinogen and the parietal cell secretes the HCl (Carvalho and Junqueira, 1999). The muscularis mucosa was well detected. The submucosal layer was made up of connective tissue and various blood vessels. The muscularis layer had an extremely thick muscle layer which was comprised of two layers being inner, circular and an outer, longitudinal muscle layers. Occasionally, between the muscular layers myenteric plexus and ganglia were also present between the two muscular layers (data not shown). Finally, a thin serosal layer was seen. In the pyloric region, the basic structure was similar to the previous region. The gastric glands were present throughout the lamina propria; however, the muscularis was a thinner layer than those layers in the stomach (Data not shown).

The main histopathological changes of the stomach sections were exclusively detected in a special unidentified parasite, which was found in both the lamina propria and submucosa (Figs. 1H–I and 2A–B). A possible explanation is that it may resemble *Trichinella* spp., as described by several investigators within some other animals (Shirazian et al., 1984; Despommier, 1998; Pozio et al., 2002; Pozio and Zarlenga, 2005; Pozio and Murrell, 2006).

Histology and histochemistry of the liver tissue

Based on histological and histochemical methods, the liver parenchyma was composed of hepatic sinusoids and hepatocytes, similar to those of *Nerodia fasciata fasciata* (Ganser et al., 2003). At higher magnification, each hepatocyte cell was irregularly shaped with a prominent central nucleus. The cell had a strongly positive reaction with PAS, indicating the presence of glycoproteins (Fig. 3C). However, it slightly reacted with AB, indicating the loss of mucopolysaccharides (Fig. 1D). The sinusoid was lined with a simple endothelial cell resting on a basement membrane. The space between their hepatocytes was made up of intersinusoidal cords. In addition, among the liver lobes, the hepatic triad (hepatic portal vein, hepatic artery and bile duct) was detected.

In conclusion, the histological and histochemical properties were described of the digestive tract and liver tissue in *C. rynchops*, as a common and important species in Thailand. Although, the information from this study was quite similar to that reported for other vertebrates, it provides new, additional insight into the anatomy and histology of the digestive tract and liver tissue, which will be applied or used for other purposes.

Conflict of interest

The authors declare that there are no conflicts of interest.

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