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ORIGINAL ARTICLE

Structure and Function of the Gastrointestinal Tract of the Green Turtle (*Chelonia mydas*) Hatchling

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Abstract The gastrointestinal tracts of four *Chelonia mydas* hatchlings were examined at the anatomical, histological and ultrastructural level. Our results show that the gastrointestinal tract (GI) is composed by esophagus, stomach, small intestine (SI) and large intestine (LI), and histologically of mucosa, submucosa, muscularis externa (ME) and serosa. The esophagus is marked by conical papillae lined by keratinized stratified squamous epithelium, whereas the remaining GI by simple columnar epithelium; esophageal diverticulum is absent. The stomach covered with mucous granule cells, contains cardia, fundic regions and pylorus, which are separately characterized by cardiac glands, fundic glands and pyloric glands, and have the thickest submucosa and ME of the GI. The ME of the esophagus mainly consist of one layer of circular smooth muscle whereas the rest of GI of two layers, inner circular muscle and outer longitudinal muscle. The SI is slightly longer than the LI and the GI is approximately 5.11 times of the carapace length. The SI is lined with longitudinal zigzag folds and characterized by absorptive cells with longer and denser microvilli, whereas the LI by transversal folds, goblet cells and lymphoid nodules. Only intestinal glands appear in duodenum. Endocrine cells are observed in all sections of the GI and accounted for the largest proportion in duodenum. The results demonstrate a perfect combination of the structure and function of the GI and reveal that the digestion and absorption primarily occurs in the foregut. *C. mydas* hatchling may prefer carnivorous diet.

Keywords anatomy, histology, ultrastructure, digestive tract, diet, sea turtle

1. Introduction

Sea turtles are marine reptiles that contain 2 families, 6 genera and 7 species, all endangered or threatened worldwide (Dijk *et al.*, 2011). There are five species living in China Sea, *Chelonia mydas*, *Eretmochelys imbricata*, *Lepidochelys olivacea*, *Caretta caretta*, and *Dermochelys coriacea*. *C. mydas* (Green Turtle) have the widest distribution as well as the largest population in China. Due to several anthropogenic impacts such as predatory fishing, illegal trade, spawning grounds and habitat destruction, climate warming and sea pollution (Carr 1987, Bugoni *et al.*, 2001), all populations of sea turtles decreased dramatically in the recent decades. Besides, because of longer mature period, about 20–50 years (Morton and Morton, 1983), and low hatchling survival rate in nature, about 1 ‰ (Fosdick and Fosdick, 1994), many turtle populations can not recovery under natural condition. However, hatchling in captivity and releasing is an effective method to restore the turtle population numbers (Zhang and Gu, 2005; Chen *et al.*, 2006). But because people know little about hatchling's, improper management and intestinal diseases often cause mass deaths of turtles.

All knowledge about the biology of the species in question is of great importance for the protection and conservation of sea turtle populations (Magalhães *et al.*, 2012). Biological and ecological studies on sea turtles

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have been carried out to understand their habits. *C. mydas* hatchlings are considered to be pelagic carnivores (Reich *et al.*, 2007), floating in seaweed beds for 3–5 years (Carr, 1987). *C. mydas* juveniles migrate to coastal habitats and consume an omnivorous diet (Bjorndal *et al.*, 1997; Arthur *et al.*, 2008; Nagaoka *et al.*, 2012), and *C. mydas* adults are benthic herbivores (Bjorndal *et al.*, 1997; Brand-Gardner *et al.*, 1999; Melo and Batista, 2008). They mainly feed on algae and sea plants (Bjorndal *et al.*, 1997; Brand-Gardner *et al.*, 1999; Reich *et al.*, 2007).

The morphology of the digestive tract is related to its digestive function (Magalhães *et al.*, 2012, Wyneken 2001; Magalhães *et al.*, 2010). So it is possible to diagnose the diet of a species by identifying its GI features (Magalhães *et al.*, 2012). The gastrointestinal structures and functions of *C. mydas* adults have been provided by many specialists, such as Thompson (1980), Wyneken (2001) , Magalhães *et al.* (2010), Gan *et al.* (2011) and Magalhães *et al.* (2012). Magalhães *et al.* (2012) compared the GI of *C. mydas* adult with those of other marine turtles. Nevertheless, the components and comprehensive descriptions of the GI of the *C. mydas* hatchling were seldom previously indicated. In view of this, the purpose of this study is to characterize the morphological structure of the GI of *C. mydas* hatchlings anatomical, histological and ultrastructural levels. Pointing out the structural and functional differences of the GI in different phases of *C. mydas* will provide essential information for better understanding the feeding habits of *C. mydas* hatchlings, and for sea turtle conservation, clinical diagnosis and treatments.

2. Materials and Methods

During August 2012, four robust newly hatched *C. mydas* (47.54 ± 1.16 mm in curvilinear carapace length), seriously injured by crabs on the beach in National Huidong Nature Reserve of China, were examined from the following viewpoints: anatomy, histology and ultrastructure. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of National Institutes of Health. The protocol was approved by National Huidong Nature Reserve Administration of China. All surgery was performed under ether anesthesia, and all efforts were made to minimize suffering.

Anatomically, the specific methods refer to those of Magalhães *et al.* (2014). The length of the GI was measured (± 1 mm) with the aid of a cotton string and a caliper. The string was positioned along the entire GI, cut at the transition between segments, and then placed on the ruler. The GI was described and photographed with an Olympus DP71 camera under Olympus SZX16 dissecting microscope.

Histologically, three tissues fragments from each GI segment (esophagus, cardia-stomach junction, cardia, fundic region, pylorus, pyloric sphincter, duodenum, jejunum, ileum, cecum, colon and rectum) were removed and fixed immediately in 10% formalin (NBF) for 12 hrs. The tissues were cut transversely except the cardia-stomach junction and pyloric sphincter, which were cut longitudinally. For routine light microscopy, the fragments were dehydrated in ethanol, cleared in xylene, and embedded in paraffin; all cuts were set at a thickness of 7 μm and mounted on glass slides. Sections of the tissues were stained in a Gill's Hematoxylin and Eosin (H&E) (Peng and Yang, 2011) and characterized by Alcian Blue stain (pH 2.5) and Periodic Acid Schiff stain (AB-PAS) (Pearse, 1968).

The structure of the GI was measured (± 1μm) or counted (± 1 SD) by software Image-Pro Plus 6.0 under the microscope. The data were taken evenly from three samples of each segment, each samples contained three tissue sections, five data per section. Values for the data of each segment were compared using software SPSS v. 19 *t*-test ($P = 0.05$).

For scanning electron microscopy (SEM), The tissues were fixed in Karnovsky-solution that contained 2.5% glutaraldehyde and 2% formaldehyde in 0.1 M cacodylate buffer. After rinsing in 0.5% cacodylate buffer, samples were postfixed in buffered 1% osmium tetroxide at 37°C for 2 hrs. Then, samples were treated with 20% HCl at 60°C for 20 min and washed 3 times with ultrasound under 25°C, 2 min per time, to remove the mucus from the surface. Afterward, they were dehydrated in gradient alcohol and dipped into tert-butanol for 3 times, 20 min each time. Then, samples were dried by VFD-21ST-BUOH freeze dryer and plated in the Sputter-coater AGAR B7340. Finally, they were examined and photographed in Philips S-4800 SEM.

3. Results

The GI of *C. mydas* hatchlings in situ are shown in Figure 1. GI length is 5.11 times of the curvilinear carapace length (CCL), composed by esophagus (26.14 ± 1.81 mm), stomach (26.38 ± 1.47 mm), small intestine (SI) (105.60 ± 5.21 mm) and large intestine (LI) (85.18 ± 4.48 mm), except the oropharyngeal cavity and cloaca.

3.1 Esophagus Anatomically, the esophagus is a

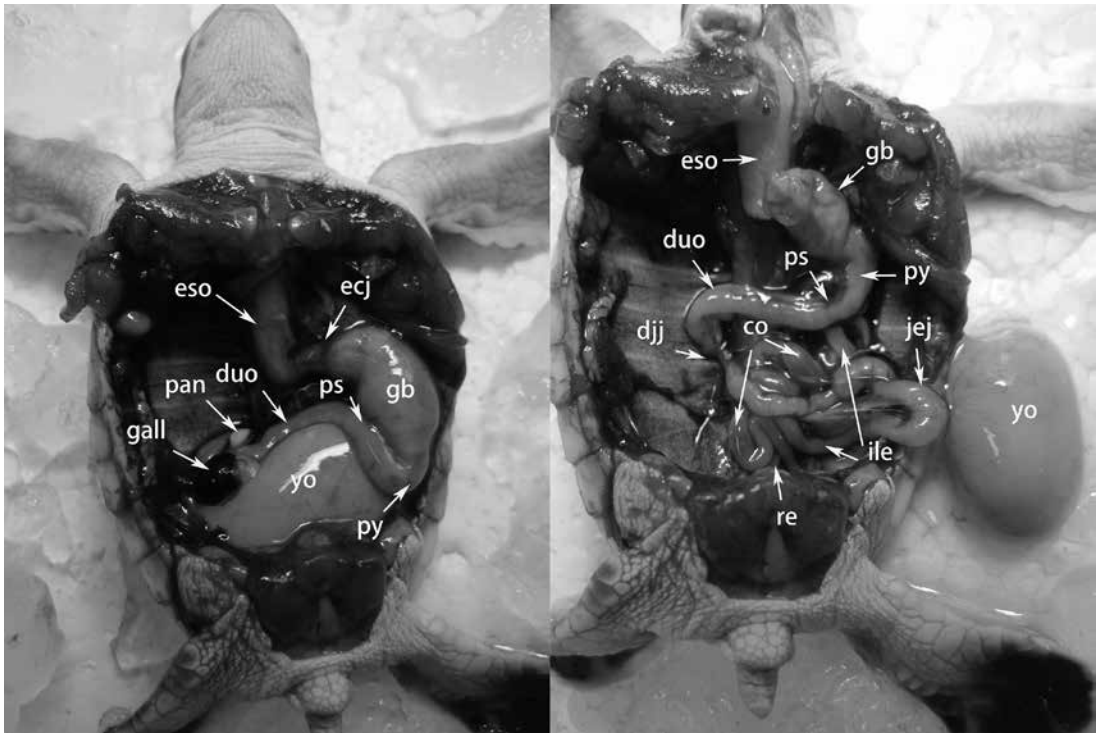

Figure 1 The gastrointestinal tract in situ of the green turtle hatchling. Esophagus (eso); esophagus-cardia junction (ecj); fundic region (gb); pylorus (py); pyloric sphincter (ps); duodenum (duo); pancreas (pan); gallbladder (gall); yolk (yo); duodenum-jejunum junction (djj); jejunum (jej); ileum (ile); colon (co); rectum (re).

L-shaped tubular muscle organ, tapering backward. It is located medially within the cervical region and its caudal region turns to the left where it meets the cardia (Figure 1). The esophagus is marked by conical papillae, which orienting to the stomach, gradually becoming larger toward its distal end (Figure 2 A1). In the esophagus-cardia transition, the conical papillae become short and blunt, and are replaced by 8 longitudinal folds in cardia (Figure 2 A2). Interestingly, the peripheral surface of this region is intertwined with abundant vascular network. Esophageal diverticulum is absent.

Histologically, the esophageal mucosa is overlaid by keratinized stratified squamous epithelium (KSS) (Figure 3 B1). In esophagus-cardia transition, the epithelium transforming from KSS to stratified columnar epithelium (MC) (Figure 3 B2), and finally to simple columnar epithelium (SC), where abundance epithelial basal cells transfer to the outermost layer of the epithelium and then develop into cuboidal, exhibiting distinct plump and crystal clear, stained red and blue separately in AB-PAS (Figure 3 B3). The esophageal papillae are also covered by KSS. They are formed by the extension of lamina propria, rich with collagen fiber and elastic fiber. The papillae invaginated at root where one or two small papillae are generally located within, forming numerous ω-shaped grooves (Figure 3 B1). Esophageal gland is absent.

The esophageal muscularis mucosa (MM) is composed of thin longitudinal muscle, very close to the muscularis externa (ME), so that the esophageal submucosa is difficult to be distinguished in this section. The MM of the esophagus-cardia transition is the thickest of the GI (Table 1) and forms the cardiac sphincter together with connective tissue in cardia (Figure 3 B3).

The esophageal ME is the thickest of the GI. The upper one-third ME of the esophagus consists of skeletal muscle, whereas the posterior is mainly composed of a layer of circular smooth muscle lining somewhat in a spiral way and its outermost region is always arranged in alternately with loose connective tissue (Figure 3 B1). While in the esophagus-cardia transition, the ME begins to appear two layers, inner circular smooth muscle (CSM) and outer longitudinal smooth muscle (LSM). The inner CSM is relatively compact and thicker; whereas the outer LSM is very thin and usually arranged alternately with loose connective tissue, in which the blood vessels are usually visible. The esophageal serosa is composed of loose connective tissue and accounted for the thickest of the GI (Table 1).

In SEM, the esophagus is covered by numerous pentagon/hexagonal disc shaped keratinocytes, which have many irregular ripples on their surface，but villi,

Tabble 1 The comparisons of various compositions of gastrointestinal tracts in *Chelonia mydas* hatchlings. The blanks: the structures are absent or too tiny to be measured.

	Folds (um)	Epithelium thickness (um)	Epithelium Cells Num.	Goblet Cells Num.	Muscularis Mucosa (um)	Submucosa (um)	Circular Muscle (um)	Outer longitudinal Muscle (um)	Serosa (um)
Esoghagus	1856.94±254.13	20.23±6.19			6.61±2.33		166.62±46.16	243.13±79.25	77.95±32.67
Cardiac sphincter			13.8±1.75	13.8±1.75	90.80±17.51	303.56±52.03	95.96±7.25	33.28±4.87	47.91±13.12
Fundic region	605.92±160.72	47.27±8.60	22.5±4.08		26.88±8.14	58.80±25.76	95.59±35.66	34.89±14.39	14.33±5.21
Pylorus	658.57±216.36	36.67±6.14	21.7±2.11		20.00±5.42	30.84±10.87	77.74±24.37	40.55±19.04	77.44±43.29
Pyloric sphincter						56.04±14.90	368.35±87.66	50.52±16.83	77.32±41.27
Duodenum	324.79±111.71	31.74±7.37	30.1±2.68	4.7±1.88	7.81±1.88	14.85±5.24	51.49±11.74	17.85±6.27	6.81±2.34
Jejunum	203.96±66.28	43.72±7.12	29.4±2.54	4.4±1.17	9.11±1.60	10.57±4.68	18.26±3.38	15.62±4.76	9.16±1.76
Ileum	169.06±41.53	65.56±12.97	21.7±2.49	10.7±2.26	13.58±1.97	11.77±3.12	38.56±12.27	23.80±6.48	12.99±3.93
Cecum	209.31±39.61	83.55±14.43	30.8±3.04	9.6±0.96	15.89±2.65	18.25±5.76	36.18±10.54	41.49±15.58	10.73±2.00
Colon		38.06±3.54	30.5±3.20	8.2±2.04	8.45±1.09	5.57±2.12	18.06±3.34	11.90±2.76	4.31±1.14
Rectum	144.34±27.13	54.92±19.93	28.4±2.01	6.9±1.28	11.45±3.56	7.19±1.47	36.61±7.88	26.92±7.14	11.22±3.16
n	45	45	45	45	45	45	45	45	45

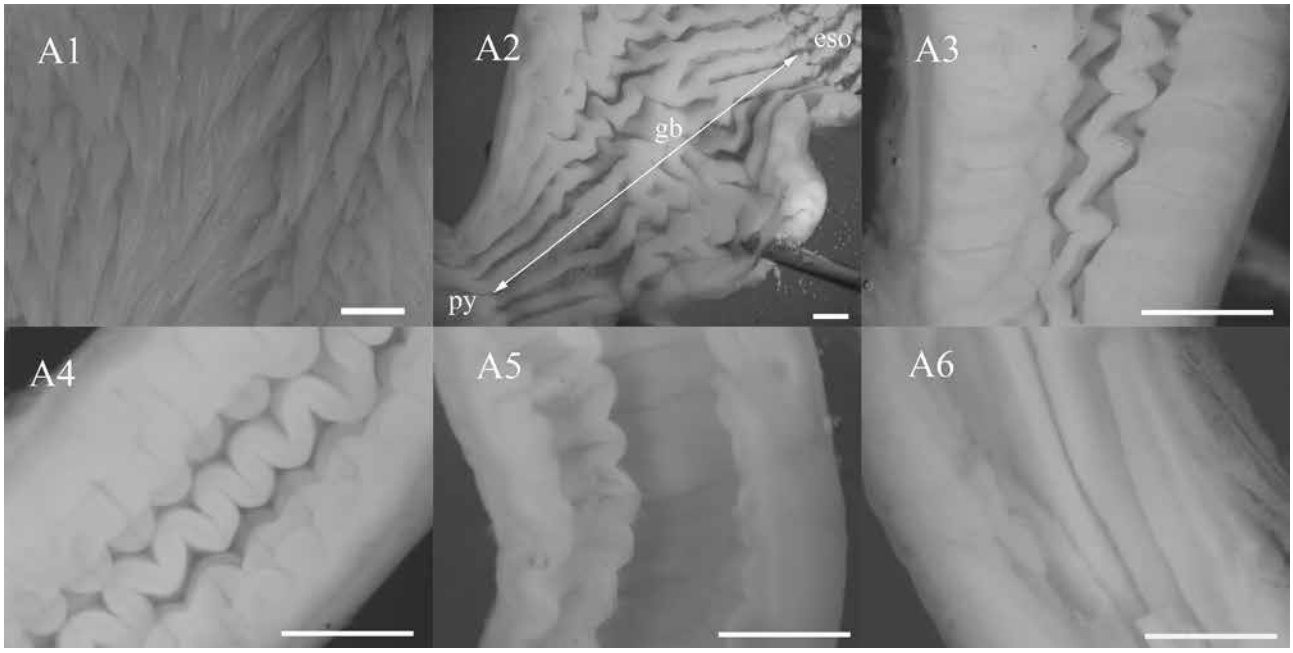

Figure 2 Anatomical characteristics of the gastrointestinal tract of *Chelonia mydas* hatchlings. A1: esophageal papillae; A2: distribution of the gastric folds; A3: longitudinal lightning-like folds in the duodenum; A4: longitudinal zigzag folds in the jejunum; A5: transverse folds in the colon; A6: rectilinear folds in the rectum. esophagus (eso); fundic region (gb); pylorus (py). Scale bar = 1 mm.

microvilli and goblet cells are absent (Figure 4 C1).

3.2 Stomach Anatomically, the stomach is J-shaped and lies to the left of the midsagittal plane. The transition between esophagus and stomach is somewhat S-shaped, in which the stomach bent to the left (Figure 1). The stomach contains three regions: the anterior cardia (2.49 ± 0.33 mm, n = 4), the middle saccular fundic region (16.31 ± 1.50 mm, n = 4) with a greater curvature on its left and a lesser curvature on its right, both bending to the left, and the posterior U-shaped tubular pylorus (7.52 ± 0.61 mm, n = 4), soon ascending to the right (Figure 1). Subsequently, the pyloric sphincter shrinks greatly at the pylorus-duodenum junction, left top of the U-shaped pylorus (Figure 1).

According to the arrangement of the folds, the fundic region can be divided into three sections: 8 parallel and rectilinear folds (4.12 ± 0.43 mm, n = 8) characterize the anterior section ; shortly after that, the parallel folds

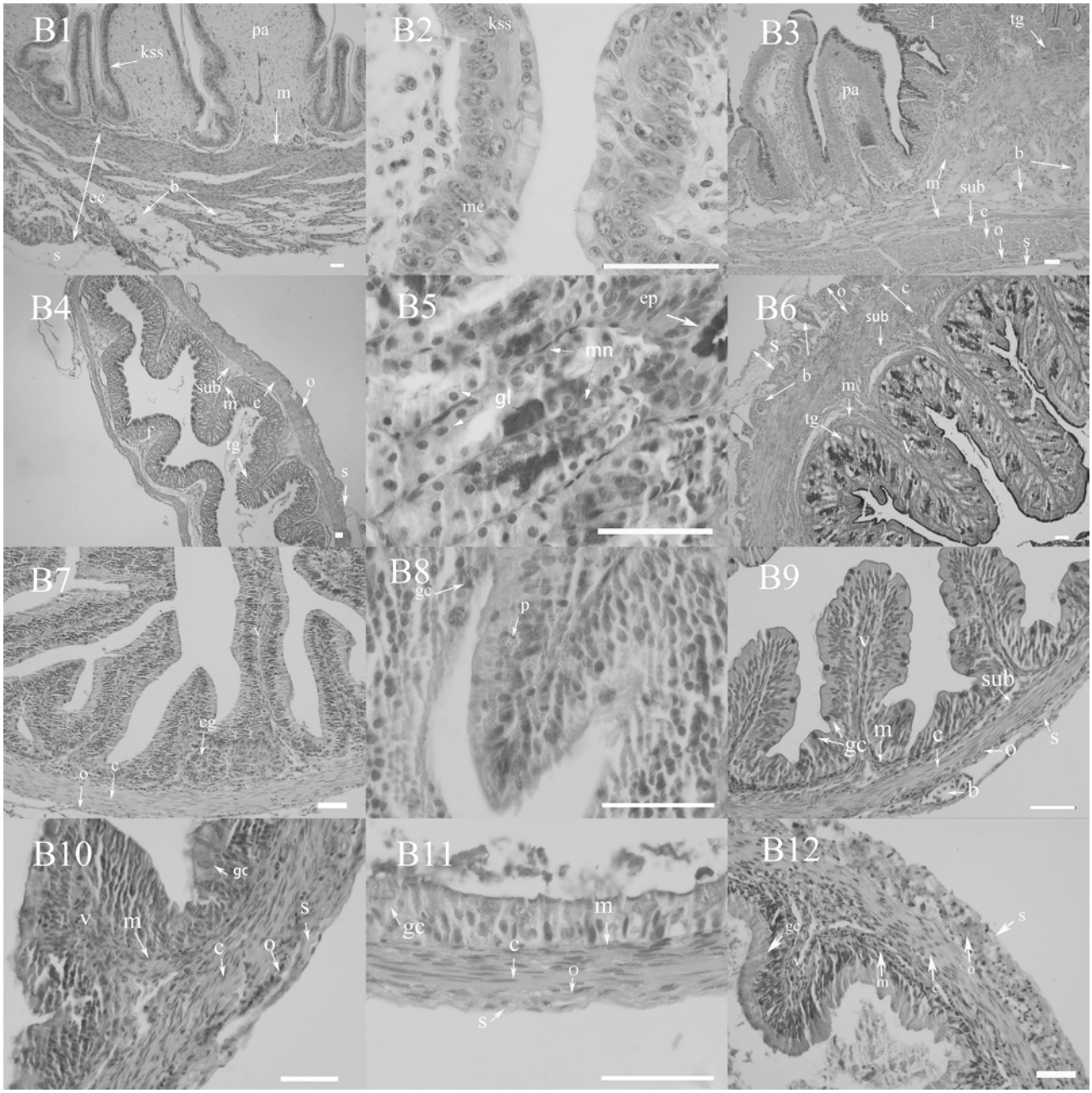

Figure 3 Histological characteristics of the gastrointestinal tract of *Chelonia mydas* hatchlings. B1: esophagus; B2, B3: the longitudinal cut of the esophagus-cardia transition; B4: fundic region; B5: fundic glands; B6: pylorus; B7, B8: duodenum; B9: jejunum; B10: ileum; B11: colon; B12: rectum. Keratinized stratified squamous epithelium (kss); papilla (pa); muscularis mucosa (m); esophageal circular muscle (ec); blood (b); serosa (s); stratified columnar epithelium (mc); lymph node (l); gastric gland (tg); submucosa (sub); inner circular muscle (c); outer longitudinal muscle (o); fold (f); gland cells (gl); mucous neck cells (mn); epithelium (ep); villi (v); crypt gland (cg); goblet cell (gc); endocrine cell (p). B1, B2, B4 and B12 are H&E staining; B3, B5, B6, B7, B8, B9, B10 and B11 are AB-PAS staining. Scale bar = 50 um.

begin weaving, branching and converging, and finally give way to the transverse folds (4.04 ± 0.51 mm, n = 14) in the middle section; the posterior section is 5.60 ± 1.29 mm (n = 21) long and its folds restore their parallel and rectilinear pattern and have same quantity as in the anterior region, but smaller in size (Figure 2 A2). After that, a sphincter separates the fundic region from the pylorus. In the pylorus, the folds branch again, and soon become smaller in size but enlarge quantity posteriorly.

Histologically, the gastric wall is composed of mucosa, submucosa, ME and serosa. Gastric mucosa can distinguish mucous epithelium, lamina propria and MM of 3 levels. The mucous epithelium is SC, and its epithelial columnar cells, are measured 0.05 ± 0.002 mm

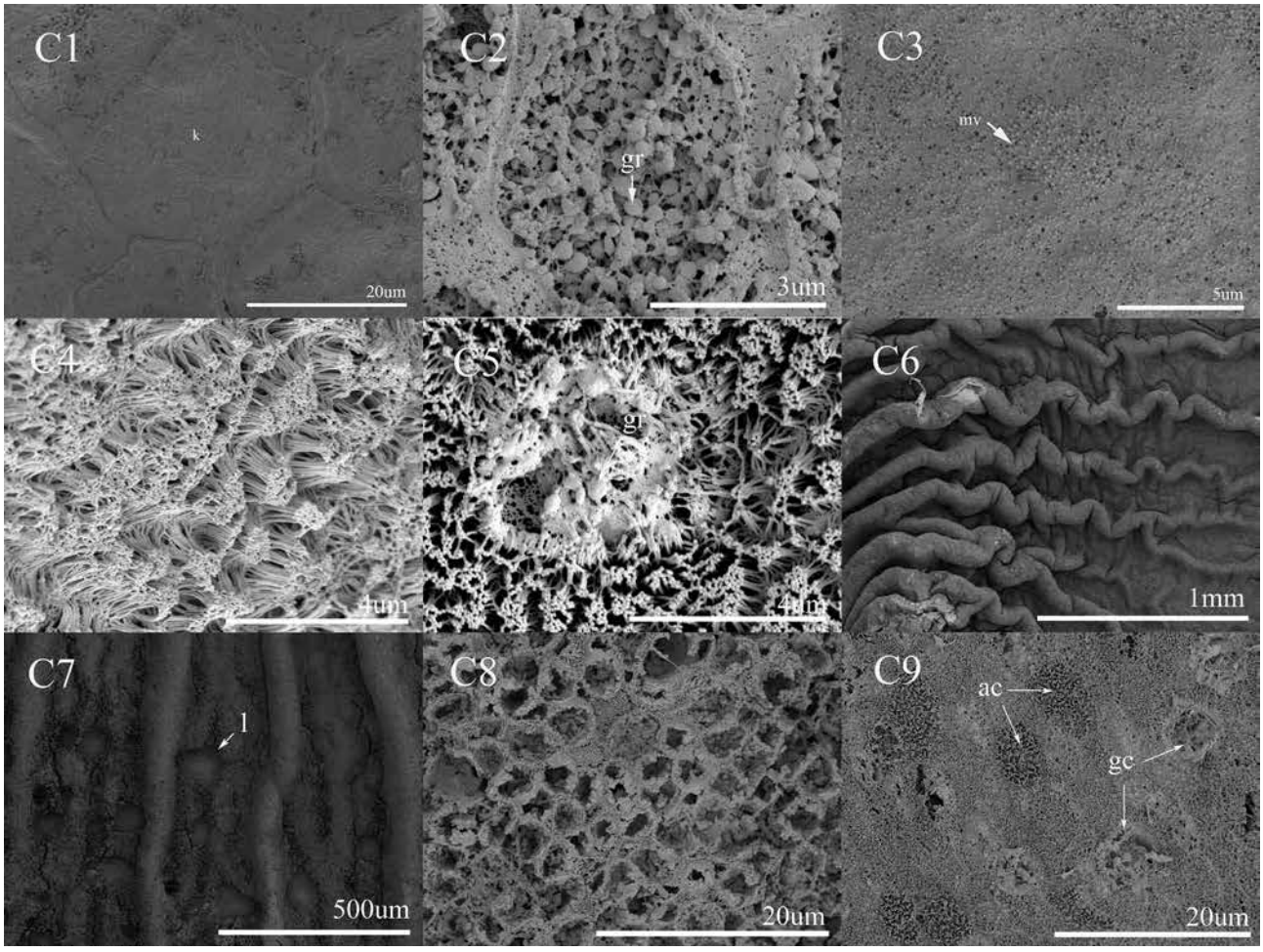

Figure 4 Scanning electron microscope characteristics of the gastrointestinal tract of *Chelonia mydas* hatchlings. C1: inner surface of the esophagus; C2: epithelial cells of the stomach; C3: microvilli in the duodenum; C4: microvilli in the jejunum; C5: microvilli and goblet cells in the ileum; C6: folds in the cecum-colon junction; C7: fold and lymph nodes in the colon; C8: goblet cells in the colon; C9: the absorptive cell and goblet cells in the rectum. Keratinocyte cells (k); granules (gr); microvilli (mv); lymph node (l); absorptive cells (ac); goblet cells (gc).

(n = 45) long, characterized by oval nucleus at their base, and the mucous granules on their top zone at thickness of 0.018 mm in average, red in AB-PAS stain. Gastric pits, tiny funnel shape, are usually appearing in the epithelial depressions and prevailing in the fundic region and pyloric region.

The gastric gland is single tubular gland and its gland cells are cuboidal or low columnar. Their nucleus is global or oval, located at the bottom of them, and usually have more than one nucleolus. According to their locations, gastric glands can be divided into the cardiac glands, fundic glands and pyloric glands.

The cardiac glands are short-duct, located in the lamina propria of the cardia, mainly overlaid with mucous cells, secreting neutral mucins. It is arranged discretely and each one of them is encased in connective tissue septum, which usually embraces abundant blood vessels and nerve fibers. The fundic glands dominate the fundic region and can be divided into 2 zones (Figure 3 B5). Zone 1 is the upper 1/2 portion of the gland, composed of columnar epithelial cells and mucous neck cells, secreting neutral mucin; zone 2 is the rest 1/2 portion of the gland, took over by a kind of gland cells, negative in AB-PAS，but they can not subdivided into parietal cells and chief cells. On the other hand, the interstitial space of the gastric glands is heaped by abundance muscle fibers, which extending from the MM and companied with a few slim nerves and capillaries. The pyloric glands are overlaid with abundant mucous cells secreting neutral mucin and a few gland cells which negative in AB-PAS, and usually coiled at endpieces.

The gastric MM is composed of smooth muscle and its thickness ranks second only to those of the esophagus (Table 1). The submucosa of the GI only can be easy

defined in the stomach. The gastric submucosa consists of loose connective tissue, in which a large number of blood vessels are embedded (Figure 3 B4; B6). Especially within the root of the folds, blood vessels and nerves are very common.

The gastric ME contains two layers: inner CSM and outer LSM. The superficial layer of the LSM is alternated with abundant connective tissue, where myenteric nerves and large blood vessels are also common. According to Table 1, the thickness of the CSM is over twice than those of the LSM; but the thickness of the ME are similar in the cardia, fundic region and pylorus. However, the ME and CSM of the pyloric sphincter is the thickest of the GI. The gastric serosa is composed of lose connective tissue. The thickness of the fundic serosa is 1/3 of the cardiac serosa, and 1/5 the pyloric serosa (Table 1).

In SEM, gastric pits, sulcus, gyrus and mucous granules cells characterize the stomach. The gastric pits, funnel shape, are visible on the top of the folds, while the sulcus and gyrus are located at the base of the folds. Only one kind of regular pentagonal/hexagonal epithelium cells with abundant flat mucous granules occurs in the stomach (Figure 4 C2).

3.3 Small Intestine The SI consists of duodenum (28.89 ± 4.15 mm, $n = 4$), jejunum (30.21 ± 2.41 mm, $n = 4$) and ileum (46.50 ± 8.09 mm, $n = 4$). Anatomically, according to the winding degree and size of the folds, the duodenum has two subdivisions. The anterior part starts at the pyloric sphincter and end at the corner where it connects with the pancreas. This section of the duodenum is smooth on the periphery surface, marked by 26 longitudinal lightning-like folds (Figure 2 A3). However, the posterior part is the C-shape loop of ampulla duodenum where the pancreas is enclosed in and marked by 21 longitudinal zigzag folds.
The jejunum is separated from duodenum by a sphincter in jejunum-duodenum junction. Different from duodenum, the tube of the jejunum undulates greatly and circle blood vessels frequently emerge on its outer surface. In its inner surface, there are 13–21 longitudinal zigzag folds, running parallel to each other (Figure 2 A4).

There are no obvious boundaries between the jejunum and ileum. The size and shape of folds in the ileum are similar to those in the jejunum, but reduce their number to 8–13.

Histologically, the SI mucosa is characterized by SC, which is composed of absorptive cells and goblet cells. Vast majority of the epithelium cells are absorptive cells and they are negative in AB-PAS. The SI goblet cells are similar, each one of them is first stained red, followed by blue. The goblet cells of the jejunum and ileum have similar size and proportion, secreting in merocrine methods; whereas those of the ileum are larger in size and increase their proportion to 1/3 of the epithelial cells in the ileum (Table 1), secreting in apocrine method. Only glands emerged in the duodenum. Its gland cells are usually stained blue in AB-PAS.

Beside, the submucosa bumps to the lumen forming the villi of the SI. Each of the villi contains a large number of capillaries, but devoid of lacteals. There are many tiny finger-like villi emerging on their surface, especially at root. In spite of this, the villi have distinctions in duodenum, jejunum and ileum. The villi of duodenum are filiform and have a flexural appearance with sharp angular edges (Figure 3 B7; B8). In contrast, the villi of the jejunum are low tree shape that they become relatively symmetric and the angular edges disappear in this portion (Figure 3 B9). The villi of the ileum are low slope shape and lower and smaller than those of the jejunum (Figure 3 B10). In addition, abundant nerves and blood vessels as well as endocrine cells are located in SI' mucosa and submucosa. Nevertheless, the largest numbers of the endocrine cells are presented in the SI, especially in the posterior segment of duodenum (Table 2). A global or oval nucleus is located on one side of the endocrine cell and huge number of tiny granules on its other side, negative in AB-PAS (Figure 3 B8). More interesting is that they are also crowded in the pancreas. Nevertheless, lymphocytes are not common in this region.

The MM is obvious, but the submucosa is thin in this portion. The ME of the SI consists of two layers, inner CSM and outer LSM. In contrast, the ME of the duodenum and ileum is similar in thickness, and their CSM is thicker than LSM, but about twice thicker than those of the jejunum. In jejunum, the CSM reduces size and keeps relative uniform with outer LSM. The serosa is thin, composed of lose connective tissue.

In SEM, there are two kinds of epithelial cells, absorptive cells and goblet cells, emerged in the SI. The absorptive cells are characterized with short fungiform microvilli in the anterior duodenum (Figure 4 C3), but the microvilli of absorptive cells become longer, denser and fascicular in the posterior duodenum and the jejunum (Figure 4 C4), and then gradually get shorter in the ileum. By contrast, the goblet cells are small knobs in the duodenum and the jejunum but crater shape in ileum (Figure 4 C5) and the secretive particles start to present apparent on their surface.

3.4 Large Intestine The LI is approximately 0.2 times shorter than the SI. It can be subdivided into three portions: cecum (5.40 ± 5.88 mm, $n = 4$), colon (50.36

Table 2 Gastrointestinal characteristics of the *Chelonia mydas* hatchlings. Keratinized stratified squamous epithelium (KSS); stratified squamous epithelium (SS); stratified columnar epithelium (MC); simple columnar epithelium (SC); single tubular gland (ST); blue (B); purple (P); red (R); red inside and blue outside (RB); parentheses indicate the stain of the goblet cells; merocrine secretion (MS); apocrine secretion (AS); independent existence (/); relative frequencies: +++++ (high), ++++ (relative high), +++ (moderate), ++ (relative low), + (low), – (not detected or absent).

	Epithelium	Muscular Layer	Glands and stain	Goblet Cell	Mucosal Cell Stain	Endocrine Cells	Folds	Lymphocytic Nodules
Esophagus	KSS	1	–	–	–	+	–	–
Cardia	SS/MC/SC	2	ST(R)		– R/B	++	–	+
Fundic region	SC	2	ST(R/B)	–	P	+	8	–
Pylorus	SC	2	ST(R)	–	P	+	11–14	–
Duodenum	SC	2	ST(B)	RB (MS)	–	+++++	21–26	–
Jejunum	SC	2	–	RB (MS)	–	++++	13–21	–
Ileum	SC	2	–	RB (AS)	–	++	8–13	–
Cecum	SC	2	–	RB (AS)	–	++	6–8	–
Colon	SC	2	–	RB (AS)	–	++	–	++++
Rectum	SC	2	–		R	+++	10–11	+

± 0.88 mm, n = 4) and rectum (29.42 ± 5.36 mm, n = 4). Anatomically, the whole segment of the LI is attached by developed mesentery, but fatty tissue is rarely found. The cecum is sac like, lying on the left hemidiaphragm. It has a similar structure as the posterior ileum, lined by rectilinear folds. But the folds detour drastically all of a sudden in the cecum-colon junction, forming ileocecal valve (Figure 4 C6). However, in colon, the longitudinal rectilinear folds give way to the transverse folds (Figure 2 A5), but there are also several folds running longitudinally across the transverse folds. Even though, haustra coli, teniae coli and appendices epiploicae are absent in this portion. Posterior to the colon, rectilinear folds replace the transverse folds and characterize the entire rectum (Figure 2 A6).

Histologically, the LI is lined by SC and dominated by goblet cells, which secreting a kind of compound mucin (Figure 3 B11), so there is far more mucus coating the digesta in the large intestine than any other part of GI. Besides, a few endocrine cells are located but gland is absent in this region. In addition, diffuse lymphocytic infiltration is obviously detected throughout the mucosa of the rectum, especially in the lamina propria.

The MM of the LI is very thin, but the MM of the cecum is little thicker than those of the colon and rectum. The ME of the LI also contains two layers, inner CSM and outer LSM. There are also many myenteric nerve plexus and blood vessels distributed between the muscle layers. However, the CSM contains relatively compact muscle fibers and has a similar thickness as the outer LSM in cecum; but little thicker than the LSM in the colon and rectum. According to Table 1, the cecum has the thickest ME of the whole intestine, whereas those of the colon is the thinnest of the whole intestine, but the ME become thicker again in the rectum, approximately twice of the colonic ME. The serosa is thin, comprised of lost connective tissue.

In SEM, the cecum is covered with 1/3 of the goblet cells and 2/3 of the absorptive cells, whereas the colon dominated by goblet cells which microvilli shorter on their periphery, and hence shapes its honeycombed appearance (Figure 4 C8). Between the colonic folds, there are a large number of lymphoid nodules spotted on the surface (Figure 4 C7). Contrast to the colon, the rectal absorptive cells are larger in quantity and their microvilli are longer (Figure 4 C9). However, lymphoid nodules are difficult to locate in this region.

4. Discussions

4.1 Esophagus The esophagus of *C. mydas* hatchlings is located medially within the cervical region, a little different from those of the *C. mydas* juveniles and adults mentioned by Magalhães *et al.* (2012) that their esophagus deviates laterally to the left. It is a thick tube, wide in anterior region and narrow in posterior region that also different from the esophagus of the *Podocnemis expansa*, *P. unifilis*, *P. sextuberculata*, *P. erythrocephala*, and *Peltocephalus dumerilianus*, which are slender tubes, uniform in diameter (Magalhães *et al.*, 2014).

In addition, the entire esophageal wall of the *Testudo horsfieldi* (Xu and Liu, 1996) is characterized by folds, whereas the anterior esophageal wall of the *P. expansa*, *P. unifilis*, *P. sextuberculata*, *P. erythrocephala and Peltocephalus dumerilianus* (Magalhães *et al.*, 2014) lined by conical papillae and the posterior by folds. Even though, there no sphincter found in all their esophagus-stomach junctions. In contrast, in *C. mydas* hatchlings are

formed by conical papillae of entire region and have a sphincter in its esophagus-stomach junction that is similar to those of the *C. mydas* juveniles and adults, and all other four marine species such as *E. imbricate*, *L. olivacea*, *C. caretta* and *D. coriacea* (Magalhães *et al.*, 2012). However, different from the tortoise and freshwater turtles mentioned above. Therefore, the structure of the esophagus of the *C. mydas* are probably related to the food filtration as Bleakney (1965) suggested in the paper.

The broad esophagus can provide more space to accommodate more food. However, due to the *C. mydas* needs to squeeze out the excess water out of its esophagus when it feeding, thus, the papillae are important for implementing such functionality because the papillae are capable of facilitating intake and avoiding regurgitation of food (Bleakney, 1965; Wyneken, 2001). In order to cater to this ability, the invaginations at the root of papillae act as pipes that are conducive to expelling water. In addition, the cardiac sphincter is located in the acute angle of the esophagus-stomach junction that, such a specialized structure, does help reducing the outside pressure to avoid the water getting into the stomach when turtle feeding; and prevent gastric juice refluxing from the stomach to the esophagus that cause inflammation as Cheng *et al.* (2003) indicated in human.

The esophageal mucosa of *C. mydas* differs greatly from those overlaid by SS (stratified squamous epithelium) and MC with goblet cells of the *P. expansa*, *P. unifilis*, *P. sextuberculata*, *P. erythrocephala and Peltocephalus dumerilianus* (Magalhães *et al.*, 2014), instead, it is overlaid with KSS without goblet cells. In sea water, the KSS is in favor of water proofing and mechanical resistance (Reynolds and Rommel, 1996; Silva, 2005), and capable of relieving friction resulting from the passage of food through the esophagus and keeping hydrated and protecting from sea water dehydration.

Moreover, the entire esophagus of the *P. expansa*, *P. unifilis*, *P. sextuberculata*, *P. erythrocephala* and *Peltocephalus dumerilianus* (Magalhães *et al.*, 2014) are mainly composed of smooth muscles; by contrast, the anterior 1/3 esophagus of the *C. mydas* hatchlings composed of skeletal muscle, whereas the rest 2/3 of smooth muscles. This may greatly improve esophageal expansion and contraction. Therefore, the esophagus can resist water pressure and eliminate excess water from the esophagus in the depths when *C. mydas* feeding (Wyneken, 2001; Magalhães *et al.*, 2012).

Additionally, diverticulum is mainly used for storing food for long migrations (Balazs *et al.*, 1998) and for better digestion of the algae and sea grass (Wyneken, 2001; Magalhães *et al.*, 2012). It was observed in some *C. mydas* juveniles and adults (Wyneken, 2001; Magalhães *et al.*, 2012), nevertheless, it is absent in *C. mydas* hatchling, and in *E. imbricate*, *L. olivacea*, *C. caretta* and *D. coriacea* (Magalhães *et al.*, 2012), that must relate to the diet and habitat of *C. mydas* in different stages.

4.2 Stomach To an animal without teeth, the stomach is particularly important; the relationship between the shape and structure of the stomach and the diets also appears to be close (Romer and Parsons, 1995; Work, 2000; Wyneken, 2001). In *C. mydas*, the stomach relative length increases with age. The stomach length of the *C. mydas* hatchling in present study is 2.63 ± 0.15 cm (n = 4), 0.55 times of the CCL. Those of the *C. mydas* juveniles are 22.37 ± 9.08 cm (n = 9), 0.60 times of the CCL (Magalhães *et al.*, 2012); and those of *C. mydas* adults is 78.50 cm (n = 1), 0.72 times of the CCL (Magalhães *et al.*, 2010). *C. caretta* eats benthic snails, bivalves decapod; *L. olivacea* mainly eats jellyfish, tunicates, sea urchins, moss, bivalves, snails, prawns, crabs and worms (Ernst *et al.*,1994); and *D. coriacea* mainly feeds on jellyfish (Mrosovsky *et al.*,2009). But their stomachs appear differently in relative length. In Magalhães et al (2012), the stomach length is 0.47 times of the CCL (90.65 ± 3.32 cm, n = 2) in the *C. caretta*, is 0.81 times of the CCL (45.07 ± 7.18 cm, n = 6) in the *L. olivacea*, and 1.20 times of the CCL (135 cm, n = 1) in *D. coriacea*.

In the same species in different development phases, the stomach becomes greater and can provide more space for food accommodation as to provides plenty of energy for development. On the other hand, the relative length varies in different species that must relate to the available nutrient content of the food. The stomach of turtles may adapt to the change of foods and the contradiction between the growth and development needs.

In the herbivores, *C. mydas* juveniles and adults, and the omnivorous, *L. olivacea*, *E. imbricate*, *C. caretta* (Parsons and Cameron 1977; Magalhães *et al.*, 2012), *P. sextuberculata* and *Peltocephalus dumerilianus* (Magalhães *et al.*, 2014), the entire region of the stomach is characterized by longitudinal folds, whereas, in the herbivores *P. expansa*, *P. unifilis*, and *P. erythrocephala* (Magalhães *et al.*, 2014), the cardiac region and fundic region are marked by transverse folds and the pyloric region by longitudinal folds. By contrast, in the *C. mydas* hatchling, the cardiac region and pyloric region are marked by longitudinal folds, whereas the anterior and posterior fundic region by longitudinal folds and the middle by transverse folds, that resemble those in the *D.*

coriacea (Magalhães *et al.*, 2012). However, the folds are absent in the posterior fundic region of the *D. Coriacea*.

Additionally, the longitudinal folds probably facilitate food items through the lumen, where it can be restricted by transversal folds (Parsons and Cameron, 1977). Therefore, the pattern and distribution of the gastric folds various from species may associate with the gastric dynamic and diet of animals, but that still need further researches.

For the stomach folds created by the bumping of the submucosa, a layer of flexible connective tissue, the longitudinal folds to contribute to the gastric expansion and the transverse folds to benefit the extension of the stomach that can provide more place for food storage and promote the gastric peristalsis as to churn the food for better digestion under the actions of muscles.

The entire region of the stomach is characterized by glands in the present study that resembles those of *C. mydas*, *(*Magalhães *et al.*, 2010), *P. sextuberculata*, *Peltocephalus dumerilianus* (Magalhães *et al.*, 2014), *Mauremys mutica* and *Chinemys reevesii* (Li and Tang, 2014), but somewhat different from those of the *P. expansa* and *P. erythrocephala* (Magalhães *et al.*, 2014), that the glands are absent in the cardiac region and fundic region but present in the pyloric region. Thus, the distribution of gastric glands of may reflect the adaptation to their eating habits.

The nature of the gastric gland is also one of the important factors that reflect the animal habits. The gastric glands are important for digestion. They can release proteases and hydrochloric acid, which kills or inhibits bacteria and provides the acidic pH of two for the proteases to work (Gore and Levine, 2007). However, few people deeply research the composition of the turtle gastric glands. In our present study, we found that the *C. mydas* hatchling has similar gastric glands to those in *Alligator sinensis* (Wu *et al.*, 2001) and *Trionyx Sinensis* (Wang and Du, 1996), that their gastric gland cells cannot be subdivided into chief cells and parietal cells. So the gastric gland cells of the *C. mydas* may possess both functions of chief cell and parietal cell as Wu *et al.* (2001) and Wang and Du (1996) indicated in their studies. But it still needs further researches.

The thicker MM makes it possible to subdivide a considerable part of them to surround the glandular cavities and tract of the epithelium cells for a constant state of gentle agitation that can keep strong acids and protein-digesting enzymes expelling to the lumen for food digestion. Additionally, the thick gastric submucosa is capable of alleviating pressure from the epithelium generated by food and preventing the wall from damage under friction. All that once again significantly reflect the perfect combination of gastric structure and function. To tailor to gastric functions, both the gastric MM and gastric submucosa of the *C. mydas* hatchling become thickest than any other regions of the GI (Table 1). The MM may play an important role for gastric glands secretion.

Similar to the gastric ME of the *C. mydas* juvenile and adult described in the papers of Magalhães *et al.* (2010) and Gan *et al.* (2011), the gastric ME of *C. mydas* hatchling is also composed of two layers, but it is very thick. This may be consistent with the gastric functions, biochemical and physical digestion (Wyneken, 2001), because the thicker muscle layer can make for efficient mixture of digesta.

To tailor to the gastric functions of storage and digestion, the muscular layer and serosa of the pylorus resembles those of the cardiac region, more thicker than those of the fundic region that hold the pylorus usually in a tubular shape and make the stomach saclike. Besides, under the synergy of pyloric sphincter, the pyloric region is capable of preventing not well digested digesta into the hindgut. However, due to authors rarely detailing the submucosa ME and MM of stomach in previous studies, so whether they are various from species and closely related to the diet of animal that need to be confirmed in the future.

4.3 Intestine It is possible to identify each section of the digestive tube according to the morphology and distribution of the fold, absorptive cell, goblet cell and gland. They must be closely related to the intestinal digestion and absorption.

In *C. mydas* juveniles and adults, *L. olivacea*, *C. caretta* and *E. imbricata* (Magalhães *et al.* 2012), mucosa of the duodenum are characterized by reticular folds, whereas the jejunum/ileum by rectilinear longitudinal folds. The distribution of reticular folds and longitudinal folds are just the opposite in the *Testudo horsfieldi* (Xu and Liu, 1996). In *Mauremys nutica* and *Chinemys reevesii* (Li and Tang, 2014), transverse folds are appeared in the duodenum, longitudinal folds in jejunum/ileum. In *P. unifilis*, *P. expansa*, *P. erythrocephala* and *Peltocephalus dumerilianus* (Magalhães *et al.* 2014), bearing reticulate folds was identified in the duodenum, longitudinal zigzag folds in the jejunum/ileum. In present study, longitudinal zigzag folds mark the entire SI.

The small intestine is regionally specialized to absorb amino acids, carbohydrates, sugars, water, fatty acids, and minerals (particularly calcium and phosphorus) (Wyneken 2001). The presence of folds considerably enlarges the

surface of this organ, thus increasing the area available for absorbing nutrients (Romer and Parsons 1985; Wyneken 2001). Therefore, the distribution and pattern of the intestinal mucosal folds presented in different species demonstrate that the pattern of intestinal folds is likely to be associated with what kind of the food and how much of the available nutrients contained in it. But it remains to be confirmed further.

Intestinal glands of *C. mydas* hatchlings are only confined to the duodenum, but those of the *C. mydas* juvenile and adult (Magalhães *et al.*, 2010) to the LI. In *Ocadia sinensis* (Fu *et al.*, 2014), *Mauremys nutica*, *Chinemys reevesii* (Li and Tang, 2014) there are glands appeared in the duodenum, jejunum/ileum, but absent in LI. In the *Trionyx sinensis wiegmann* (Wang and Du, 1996), *P. expansa*, *P. unifilis*, *P. sextuberculata*, *P. erythrocephala*, *and Peltocephalus dumerilianus* (Magalhães *et al.*, 2014), there are no gland found in SI and LI. The intestinal glands can secret digestive enzymes to break down the proteins and complex carbohydrates of food, and hormone to regulate the intestinal functions. Therefore, the distribution and characteristics of the intestinal glands appeared differently in different species that may be closely related to their intestinal functions and the adaptation to their diet.

In mammals, the duodenum may be the principal site for iron absorption, (Latunde-Dada *et al.*, 2002) and chemical digestion (Gray and Lewis, 2000), so the same functions taking pace in the duodenum of the *C. mydas* hatchling is also possible. The green turtle has similar feeding habits and the same basic GI tract subdivisions as manatee (Bjorndal, 1979). Additionally, the glands in the duodenum of the *C. mydas* resemble those of the manatee mentioned by Reynolds and Rommel (1996), for the producing acid mucin. Thus, the duodenal glands of the *C. mydas* may be associated with digestion and absorption of algae, but it still needs further study.

However, the absorption is given priority in the jejunum and ileum. Despite their folds being less than those of the duodenum and a unit of epithelial absorptive cells are similar in each section of the SI, the total length of the jejunum and ileum are over 2 times of the duodenum and possess longer and denser microvilli than those of the duodenum that provide a larger area for absorption.

The cecum and rectum of the *C. mydas* are probably the place for volatile fatty acid (VFA) absorption. In these two regions, the absorptive cells are more than goblet cells and a unit of them is also similar to those of the SI. Base on Bjorndal (1979), organic acids produced in the cecum provide 15.2% of the green turtle's daily energy balance and the higher absorbing proportion of volatile VFA is observed carrying out in the cecum. This phenomenon is also found in the manatee which has similar feeding habits and the same basic GI subdivisions as *C. mydas* (Reynolds and Rommel, 1996). On the other hand, the total VFA concentration in the *C. mydas* increases dramatically in the hindgut, especially in colon (Bjorndal, 1979), but it tapers in rectum that just correspond to our suggestion that the rectum is also the specific place for VFA absorption.

In *P. expansa*, *P. unifilis*, and *P. erythrocephala* (Magalhães *et al.*, 2014), a dilatation was located in the anterior region of colon, followed by a straight and tubular region of smaller diameter. In *P. sextuberculata* and *Peltocephalus dumerilianus* (Magalhães *et al.*, 2014), the dilatation is absent and the colon is tubular and of uniform diameter along its entire length. In *L. olivacea*, *C. caretta*, *E. imbricata*, *C. mydas juvenile* and adult (Magalhães *et al.*, 2012), an alternation of saccular without fold and narrow regions with longitudinal folds were located in colon. The exception is that the colon of *D. coriacea*, characterized with irregularly distributed folds.

Different from other intestinal portions, transverse folds mark the colon of the *C. mydas* hatchling. This structure is also different from those of the colons of the *C. mydas* juvenile and adult (Magalhães *et al.*, 2010) and other species mentioned previously. Hence, arched grooves exists between the colonic transversal folds of the *C. mydas* hatchling are probably related to the colonic arched areas of the *C. mydas* juvenile and adult, because both can play a role of colonic haustra as in humans despite only the LI of mammals develop into a true colon (Romer and Parsons, 1977). This particular structure of the colon may slow the contents to the next section that promotes the propagation of anaerobic bacteria which take part in the high efficiency of digestion of cellulose, sugar, lipids and protein, and contributes to the absorption of nutrients, especially vitamin B, vitamin K and water as Cheng *et al.* (2003) mentioned in human. However, the colonic alternation of saccular and narrow regions of the *C. mydas* juvenile and adult that seems more efficient in those two functions. Besides, the colonic glands, which present in the *C. mydas* juvenile and adult noted by Magalhães *et al.* (2010) are absent in *C. mydas* hatchling. Therefore, the colon showed different features in *C. mydas* of different age is accord with their diet.

To tailor to the LI is the place of bacterial digestion of cellulose and other carbohydrates from plant materials

(Hildebrand and Goslow, 2006), the ME became the thinnest of the GI in *C. mydas* hatchling will reduce the intestinal peristalsis as to slow the passage of the digesta and contributed to the fermentation of food.

In order to adapt to this harsh environment of the colon, the epithelial cells made corresponding changes. So the *C. mydas* resembling the *P. expansa*, *P. unifilis*, *P. sextuberculata*, *P. erythrocephala*, and *Peltocephalus dumerilianus* (Magalhães *et al.*, 2014), goblet cells dominate the colon. That is because the goblet cells playing an effective role in intestinal lubrication and protection, and avoiding infringement of bacteria. It is also why the apparently greater mucus coating the digesta in the LI than any other parts of the GI in present study. Furthermore, the lymphonodus are closely related to intestinal immune and can effectively inhibit the growth of bacteria (Junqueira and Carneiro, 1999), the colon is abundant in lymphonodus that is also devoted to the GI defense.

The length of the gut is related to the animal's diet (Wyneken, 2001). Generally, the small intestine tends to be longer in carnivores and shorter in herbivores (Stevens and Hume, 1998) and the ratio of GI to CCL in herbivorous is relatively larger than those of the omnivorous and carnivorous (Ye *et al.*, 2012). In *C. mydas* hatchling, the SI longer than the LI similar to those in the *C. caretta*, *L. olivacea*, *E. imbricate* and *D. coriacea* (Magalhães *et al.*2012). Whereas, the SI (145.13 ± 46,52 cm, n = 9) is 0.64 times shorter than the LI (238.43 ± 54.99 cm, n = 9) in *C. mydas* juveniles (Magalhães *et al.* 2010), and the SI (333.20 cm, n = 1) (Magalhães *et al.* 2010) is 2.09 times shorter than the LI (1032.30 cm, n = 1) in *C. mydas* adult. On another hand, the ratio (GI/SCL) is 5.11 in the *C. mydas* hatchling, less than the 7.53 (n = 2) of the *C. caretta*,7.99 (n = 1) of the *D. coriacea*, 10.12 (n = 6) of the *L. olivacea* , 10.25 (n = 1) of the *E. imbricate*, 11.52 (n = 9) of the *C. mydas* juvenile and 13.57 (n = 1) of the *C. mydas* adult, all that calculating from the datum in paper of Magalhães *et al.* (2010) . Obviously, the length of LI becomes longer along the age of *C. mydas* that is also accorded with its diet of different stages.

Adult *C. mydas* and manatee are marine herbivores, they having similar feeding habits and basic GI subdivisions (Bjorndal, 1979). The manatee had been confirmed as a hindgut digestive animal, since the SI is dominated by goblet cells whereas very abundant of absorptive cells are contained in the large intestine (Reynolds and Rommel, 1996), but these is just contrary to the GI of *C. mydas* hatchling in present study. Hence, *C. mydas* may be a foregut digestive animal.

4.4 Endocrine Cell Endocrine cells are located in the whole GI of *C. mydas* hatchling, and also crowded in the pancreas. They are accounted for the largest proportion in the duodenum (Table 2), that correspond with the distribution and the characteristics of the endocrine cells in freshwater turtle mentioned by Gençer tarakçi *et al.* (2005).

Gastrointestinal endocrine cells synthesize various kinds of gastrointestinal hormones and play an important role in the physiological functions of the alimentary tract (Bell, 1979). In freshwater turtle, the endocrine cells were determined to secret serotonin, gastrin and insulin (Gençer tarakçi *et al.*, 2005), so those of the *C. mydas* hatchling produce similar hormones to control the physiological functions of GI is also possible, but need further researches.

In conclusion, the present result demonstrates the perfect combination of the GI structure and function and supports the statement of Wyneken (2001) that different sections of the GI vary greatly in structure and function. The characteristics of the GI in the *C. mydas* of different ages coincide with the change of their diet. *C. mydas* hatchling is probable foregut digestive animal and more likely to be carnivorous.

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