

## Morphological and histological structure characteristics of the gastrointestinal tract in *Sinogastromyzon szechuanensis*

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**Abstract:** in order to understand the morphological structure characteristics of the stomach and intestinal tract of *Sinogastromyzon szechuanensis*, we observed and studied the morphological and histological structure characteristics of the gastrointestinal tract of *Sinogastromyzon szechuanensis* by means of anatomy, tissue section and light microscopy. The results show that the stomach of *Sinogastromyzon szechuanensis* is U-shaped, and the intestinal tract is composed of three parts: foregut, midgut and hindgut. The coefficient of intestinal tract is  $1.42 \pm 0.20$ . The stomach and intestine consist of mucosa, submucosa, muscle layer and serosa. The gastric glands in the stomach are very developed, the wrinkles in the internal surface of intestine are very developed, the mucosal epithelium is a single-layer columnar epithelium, and on the free surface of it, there is the striated border formed by the dense arrangement of microvilli; the goblet cells are distributed among the epithelial cells, and the number of goblet cells gradually increases from the foregut to the hindgut. The number of wrinkles of intestinal mucosa gradually decreases from the foregut to the hindgut, and the height also decreases, while the thickness of muscle layer gradually increases. The results show that the morphological and histological structure characteristics of the stomach and intestinal tract of *Sinogastromyzon szechuanensis* are adapted to its omnivorous and partial carnivorous nature.

*Sinogastromyzon szechuanensis* is a kind fish belonging to *Balitoridae*, *Sinogastromyzon*. Because of its unique body shape and unique movement mode, it is an ornamental fish with unique style and development value. But they have high environmental requirements and they need clean water and high oxygen environment, so it is difficult to raise them. At present, overfishing and environmental damage have led to a sharp decline in the population resources. Now, they are only found in the middle and upper reaches of the Yangtze River, and have been listed as key protected fish by Sichuan, Yunnan, Guizhou and Chongqing, and there are documents such as the Chongqing Municipal Government Document ([1999] No.65 issued by Chongqing Municipal Government) <sup>[1-3]</sup>.

The stomach and intestinal tract are important digestive and absorption organs for fish to maintain life activities, which have a very important impact on fish growth, development and reproduction. Therefore, studying and discussing the histology and morphology of the stomach and intestines of fish is helpful for us to understand and understand the physiological mechanism of feeding, digestion and absorption of fish <sup>[4]</sup>. There are few published literatures about *Sinogastromyzon szechuanensis*, and they mainly focus on resource investigation, reproductive activity, early development, mitochondrial genome and phylogeny analysis <sup>[5-14]</sup>. We have not seen the morphological and histological studies on the stomach and intestinal tract of *Sinogastromyzon szechuanensis*. In this study, the morphology and tissue structure of stomach and intestinal tract of *Sinogastromyzon szechuanensis* were observed and analyzed by technology of paraffin section and light microscopy, so as to provide histological data for the physiological functions of eating, digestion and absorption of *Sinogastromyzon szechuanensis* and provide reference for the artificial culture of *Sinogastromyzon szechuanensis*.

## 1. Materials and methods

### 1.1 The tested fish

The tested fish were collected from Nei-jiang City, Tuo-jiang River system, Sichuan Province, N 29° 36'41.93", E 105°01'47.67". After one month's indoor aquaculture and domestication with circulating water, we selected 10 healthy *Sinogastromyzon szechuanensis* with uniform specifications, and there was no injury on the body surface. The weight of the sample was  $(2.35 \pm 0.24)$  g, and the body length was  $(5 \pm 0.14)$  cm.

### 1.2 Sample collection and preparation

The samples were taken half an hour after the fish was fed. We quickly fished the fish, weighed the weight of the fish and measured its length. Execute it, dissect it, open the abdominal cavity, observe the position of each organ, and then dissect it and take out the digestive tract, measure the length of the intestine, and calculate the intestine body length index.

Intestine body length index =  $GL/BL$

Where GL represents the length of intestine and BL represents the length of body.

Cut about 0.5cm tissue blocks from the central part of the stomach, foregut, midgut and hindgut of *Sinogastromyzon szechuanensis* respectively, and then rapidly fix them in Bouin's solution. When preparing the sample, take it out, wash it with water according to the routine preparation procedure for tissue section, then dehydrate it with 30%, 50%, 70%, 80%, 90%, 95% and 100% ethanol gradients, put it in xylene to make it transparent, and finally embed it in paraffin with low melting point. Then, use YD-202 microtome to section it transversely, and the thickness is 5  $\mu$ m. Then, place the section on a clean glass slide, and stain it with HE staining, and seal the section with neutral balsam. Observe and film it with OLYMPUS DP72 optical microscope. The evaluation parameters of intestinal morphology include: the number of mucosa wrinkles on the cross section of digestive tract; the height of mucosa wrinkles; the width of lamina propria in wrinkles; the thickness of submucosa; the degree of infiltration of basophils into the lamina propria and submucosa; the presence and the number of vacuoles on the nucleus; the thickness of muscle layer.

### 1.3 Data statistics

The test results are expressed as "average value  $\pm$  standard deviation". For all statistical analysis, we used SPSS 17.0 software. After the data was analyzed by ANOVA, we used Turkey's multiple comparisons to analyze the significance of the differences between tissues, and the significance level is 0.05.

## 2. Test result

### 2.1 Morphological structure of stomach and intestinal tract of *Sinogastromyzon szechuanensis*

From the point of view of morphology (Fig. 1), the stomach and intestinal tract of *Sinogastromyzon szechuanensis* hover in the abdominal cavity, which can be divided into four parts: stomach, foregut, midgut and hindgut. The stomach is dilated and saclike, and it is in the shape of "U", which is divided into the cardiac part, the blind sac and the pyloric part. The length of the intestine is  $(7.08 \pm 0.90)$  cm, and the intestine body length index is  $1.42 \pm 0.20$ . The whole length of the intestinal tract is longer than that of the body, and the shape of the intestine is not straight but curved. The part from the connection with the stomach to the first turning point is the foregut, the part from the first turning point to the last turning point is the midgut, and the part from the last turning point to the anus is the hindgut. From the foregut to the midgut, the diameter becomes smaller and the dividing point is obvious, but there is little difference between the diameters of midgut and hindgut

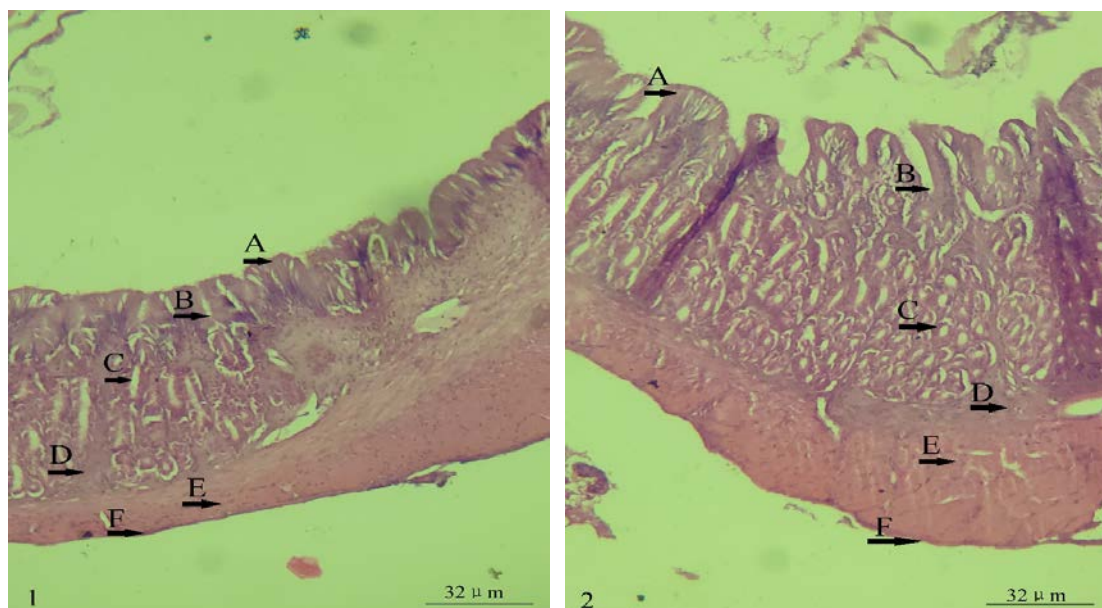


Fig.1 Anatomical form of stomach and intestinal tract of *Sinogastromyzon szechuanensis*  
ST: stomach; FG: foregut; MG: midgut; HG: hindgut

## 2.2 Tissue structure of stomach and intestinal tract of *Sinogastromyzon szechuanensis*

### 2.2.1 Stomach

The tissue structure of the stomach can be divided into mucosa, submucosa, muscle layer and serosa (Chart I-1). The mucosa epithelium of the stomach is composed of a single layer of columnar cells, which are arranged orderly and closely, with goblet cells distributed among them; the nucleus is long oval and located at the cell base, with deep staining; free lymphocytes can also be seen in the epithelial layer. The wrinkles of mucosa are dense, but there is no branching phenomenon and the height is  $(11.65 \pm 2.95) \mu\text{m}$ . The epithelium is sunken downward, forming a gastric pit (Chart I-1). Under the epithelial cells, there is the lamina propria, and the lamina propria of the cardiac stomach contains the gastric gland tissue. Most of them are composed of several to more than ten layers of round or oval acinus, which opens at the gastric pit, and they are arranged in a single layer and are long and narrow (Chart I-1); the gastric glands in the blind sac are arranged in multiple layers, and most of them are round, and the bottom reaches the submucosa (Chart I-2). During HE staining, the HE staining of gastric gland cells was deep, and there was only one kind of low columnar cells that formed the gastric gland. The connective tissue between the gastric glands can reach the columnar cells of the mucosa. The gastric gland tissue in the pylorus is undeveloped, and the gastric gland tissue near the intestine disappears. No mucosa muscle was observed in the stomach of *Sinogastromyzon szechuanensis*. The connective tissue of lamina propria and the connective tissue submucosa are continuous, and there is no obvious demarcation line, and there are a large number of dispersedly-distributed circular smooth muscle fibers. There are large blood vessels, lymphatics and nerves in the submucosa. The muscle layer is developed, and it is composed of smooth muscle. The serosa is thin.



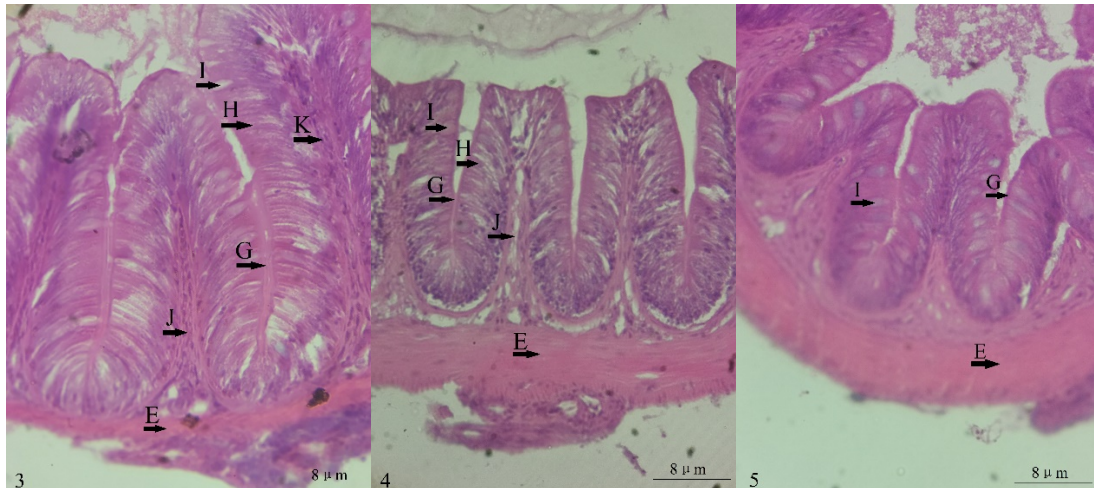


Chart I

1: Cardiac stomach; 2: Blind sac; 3: Foregut; 4: Midgut; 5: Hindgut

Note: “↑” refers to the A mucosa layer; B gastric pit; C gastric gland; D submucosa; E muscle layer; F serosa; G brush border; H absorptive cell; I goblet cell; J lamina propria

### 2.2.2 Intestinal tract

Under the light microscope, the tissue structure of each section of intestinal wall of *Pelteobagrus fulvidraco* is basically the same. The walls of foregut, midgut and hindgut are composed of four layers: mucosa, submucosa, muscle layer and serosa (Chart I-3). The mucosa is composed of epithelium and lamina propria. There is no muscularis mucosa, which makes the distinction between lamina propria and submucosa not obvious. The mucosa epithelium is mainly composed of high columnar absorptive cells and goblet cells, mainly absorptive cells. The nucleus is oval and it is close to the cell base. The staining of the free surface of the cells is deep, and it is a regular band structure, and it is a striated border, and this phenomenon is obvious on each segment of the intestine. The number of goblet cells is relatively small, scattered among the absorbing cells, and the HE staining is vacuolar. Under the epithelium, the lamina propria is composed of dense connective tissue. No intestinal gland was observed in any segment of the intestine. The submucosa is undeveloped, and the muscle layer is divided into two layers: the inner circular muscle and the outer longitudinal muscle. The circular muscle layer is relatively more developed. The serosa is composed of a thin layer of connective tissue and its surrounding mesothelium (Chart I-3,4,5).

The difference of the histological structure of the foregut, midgut and hindgut of *Sinogastromyzon szechuanensis* is mainly seen in the mucosa (Table 1). The intestinal mucosa protrudes to the intestinal cavity and forms a wrinkle wall, and the wrinkle walls of the foregut are high and many. The average wrinkle height of mucosa is 8.59 μm; the number of wrinkles of midgut mucosa is generally 16, and the height is lower than that of foregut, which is 6.63 μm averagely; the top of hindgut wrinkle is sometimes blunt, and the wrinkle number of mucosa is significantly reduced, which is generally 12, which is 7.85 μm averagely, which is slightly higher than that of midgut, but still lower than that of foregut. The thicknesses of muscle layers are about 0.27 μm, 0.36 μm and 0.41 μm respectively. In the foregut and midgut, we can observe more goblet cells. In the hindgut, goblet cells are densely distributed. The thickness of the submucosa in the foregut, midgut and hindgut are about 1.38 μm, 1.38 μm and 1.33 μm respectively, and the difference is not significant.

Table 1. Measurement parameters of tissue structure of stomach and intestinal tract of *Sinogastromyzon szechuanensis*

Tissue	Diameter of the intestinal circle/ $\mu\text{m}$	Number of folds in mucosa	Height of folds in mucosa/ $\mu\text{m}$	Height of submucosa/ $\mu\text{m}$	Thickness of muscle layer/ $\mu\text{m}$
Stomach		16.00 $\pm$ 1.26 <sup>a</sup>	11.65 $\pm$ 2.95 <sup>a</sup>	7.45 $\pm$ 1.71 <sup>a</sup>	2.69 $\pm$ 0.98 <sup>a</sup>
Foregut	66.50 $\pm$ 8.17	16.80 $\pm$ 1.48 <sup>a</sup>	8.59 $\pm$ 2.76 <sup>ab</sup>	1.38 $\pm$ 0.13 <sup>b</sup>	0.27 $\pm$ 0.04 <sup>b</sup>
Midgut	49.17 $\pm$ 5.75	16.33 $\pm$ 2.33 <sup>a</sup>	6.63 $\pm$ 1.69 <sup>b</sup>	1.38 $\pm$ 0.37 <sup>b</sup>	0.36 $\pm$ 0.09 <sup>b</sup>
Hindgut	49.50 $\pm$ 3.39	12.83 $\pm$ 1.72 <sup>b</sup>	7.85 $\pm$ 1.65 <sup>ab</sup>	1.33 $\pm$ 0.33 <sup>b</sup>	0.41 $\pm$ 0.17 <sup>b</sup>

Note: That the shoulder mark of the data in the same column does not contain the same letter indicates that the difference is significant ( $P < 0.05$ ).

### 3. Discussion

#### 3.1 Adaptability between the morphological characteristics of stomach and intestinal tract and the eating habits

The intestinal tract coefficient of fish is closely related to its feeding habits<sup>[15]</sup>. Generally, the intestine of phytophagous fish is more complex and the relative gut length is larger. In general, the intestines of carnivorous fish are straight, no bending or individual bending, and the relative gut length is the smallest<sup>[16]</sup>, while that of omnivorous fish is generally between the two above. The intestine length of fish will change with the change of feeding conditions. At the same time, during the development of individual fish, the length of intestine will change significantly. Moreover, the intestine length of omnivorous fish also depends on the proportion change of the components of the food they eat<sup>[17]</sup>. The specific relative gut length of *S. nukiangensis* and *ptychobarbus kaznakovi* are  $2.96 \pm 0.92$  and  $1.46 \pm 0.39$  respectively, and that of *schizophylgopsis thermalis* is  $3.41 \pm 1.26$ , the former two are omnivorous and partially carnivorous, the latter is omnivorous and partially phytophagous<sup>[18]</sup>. The intestinal coefficient of *Sinogastromyzon szechuanensis* is  $1.42 \pm 0.20$ , and although they are all omnivorous fish, the specific food preferences of different fish are different, and the feeding habits are different from *Schizopygopsis thermalis*, but similar to *S. nukiangensis*, they are omnivorous and carnivorous.

#### 3.2 Adaptability between the tissue structure characteristics of stomach and intestinal tract and the eating habits

The developed “U” shaped stomach of *Sinogastromyzon szechuanensis* can hold a lot of food and prolong the digestion time; the mucosa of the stomach forms high and bulging mucosa wrinkles, indicating that the stomach has a very large range of expansion and contraction, which is very beneficial to increase the volume of stomach, the surface area of mucosa and the digestion of food. Under the epithelial cells, there is gastric gland tissue, which is the same as it that *S. canaliculatus*, *R. sarba* and *L. crocea*<sup>[19-21]</sup> and other fishes have developed gastric glands. The pepsin secreted by gastric glands is in full contact with the food in the stomach cavity, which is helpful for the digestion of animal protein in the acid environment of the cardia and blind sac. Therefore, the main function of the stomach is to digest the protein in the food. At the pylorus of the stomach, there is a tissue structure as the transition from the stomach to the intestine, and the gastric gland tissue is undeveloped or even just disappears, which indicates that the pylorus is the place that continuously digests the food mixed with digestive enzymes from the cardia and blind sac. The developed muscle layer in the stomach wall strengthens the peristalsis of the stomach, which is conducive to the full contact of food and digestive fluid. In addition, there are goblet cells in the stomach, which can not only play a lubrication function, but also prevent acid gastric juice from damaging the mucosa.

There are many blood capillaries in the mucosa, and we can see that many blood cells are distributed in the lamina propria, which can rapidly transport nutrients. The loose connective tissue in the submucosa can buffer the extrusion of chyme and can give a good protection.

The surface structure and secretion ability of the intestinal mucosa are related to the digestive and absorptive capacity of the intestine<sup>[22,23]</sup>. The foregut of *Sinogastromyzon szechuanensis* receives food from the stomach cavity, and many wrinkles are formed in the mucosa, which prolongs the residence time of the food in the intestine; moreover, the height of the mucosa wrinkles in the foregut is the highest, increasing the contact area with the food and making the food be fully digested and absorbed. From the foregut to the hindgut, the density of goblet cells gradually increased, which is the same as that of most bony fishes<sup>[24-26]</sup>, indicating that the intestinal secretion ability gradually strengthens from the front to the back. Food is gradually digested and absorbed from the front to the back in the intestine. The increase of goblet cell density is convenient for the passage of food residues, fecal formation and fecal surface lubrication, which is conducive to defecation. At the same time, the trend of increasing of the wrinkles of the mucosa in the hindgut is conducive to increasing the contact area between the intestine and the chyme or food residue, and promoting the reabsorption of nutrients; in addition, the gradual thickening of the muscle layer from the foregut to the hindgut can also enhance the contractility and peristalsis ability of the intestine tract, and further promote the formation of feces and excretion of food residue. The significant differences in the structure of the foregut, the midgut and the hindgut indicate that there are differences in the digestion and absorption of nutrients in different segments of the intestine tract. Therefore, it can be inferred that the main part of the absorption of nutrients in *Sinogastromyzon szechuanensis* may be in the foregut.

There is a striated border structure on the free surface of the columnar epithelial cells in each segment of the intestine. On the one hand, the microvilli greatly increases the contact between food and intestinal tract, expands the effective area of digestion and absorption, and extends the retention time of food in intestinal tract to the greatest extent; on the other hand, the striated border structure is also related to the distribution of peptidase, disaccharidase and other digestive enzymes, which is conducive to the full digestion of food and the full absorption of nutrients; in addition, the microvilli can also retain the secretion of goblet cells, which has a buffering effect and can alleviate the mechanical damage of food on the intestinal wall<sup>[27-29]</sup>.

#### 4. Conclusion

The morphology and tissue structure of the stomach and intestinal tract of *Sinogastromyzon szechuanensis* determine that each part of the stomach and intestines will perform different functions, showing a high degree of consistency with its omnivorous and partially-carnivorous characteristics. Therefore, we suggest that in the breeding process, animal-protein-based compound feed should be used. Due to the short digestive tract of this fish, we suggest that we should adopt a feeding method of “small feeding amount but multiple feeding times” when breeding *Sinogastromyzon szechuanensis*, which is good for its growth.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: “All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.”

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