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# Digestive System of *Anourosorex squamipes* - Appearance and Morphological Features

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**ABSTRACT**

***Background*:** *Anourosorex squamipes* have a wide range of feeding habits, which is a kind of omnivorous animal. As an indispensable part of organisms, the differentiation degree of digestive system indicates the evolution degree of species. The higher the evolution degree of animals, the higher the differentiation of digestive system. At present, the research on *Anourosorex squamipes* digestive system mainly focuses on its feeding habits and mainly depends on the direct observation of gastric contents with the naked eye, but the research on the morphology of digestive system has not been reported.
***Materials, Methods & Results*:** Twenty adult *Anourosorex squamipes* were selected to investigate the characteristics of the appearance, including the contour parameters (body weight, body length, forefoot length, posterior foot length, nasal length, tail length), digestive system parameters (length of esophagus, intestinal length, intestinal weight, stomach weight and liver weight) and morphological structure of the small intestine by histological methods. The results showed that there were no significant differences in body weight, body length, forefoot length, posterior foot length, nasal length and tail length between males and females in *Anourosorex squamipes*, and no significant differences were observed in parameters of digestive system in length of esophagus, intestinal length, intestinal weight, stomach weight and liver weight. There were no significant differences in villus height, muscular layer thickness and villus height/recess depth (V/C) between males and females, but only ileal crypt depth decreased significantly in males.
***Discussion*:** Animal morphological characteristics are closely related to their life habits, unique organs and lifestyles ensure that they thrive on earth. In our study, we found that unique characters are found by observing the appearance of *Anourosorex squamipes*, the snout is blunt and short, the eyes are degenerated, and the tail is very short and covered with scales, which may be related to its feeding habits, the living environment-long-term nocturnal life in underground with minimal exposure to sunlight, and the unique tail can reduce the friction between tail and soil during the movement. There are certain differences in the appearance parameters of male and female *Anourosorex squamipes*, but are not significant. The esophagus, stomach, intestine, liver and other parameters of *Anourosorex squamipes* have little difference between males and females, indicating that *Anourosorex squamipes* don't have obvious distinctions between males and females in food intake. However, the liver weight of male *Anourosorex squamipes* is larger than female, indicating that the physiological metabolic capacity of male is larger than female. It is speculated that this may be related to the difference in the amount of activity between males and females in daily life. Males are more active and require more energy, but their digestion and absorption abilities are weaker than females. Therefore, the male *Anourosorex squamipes* needs more food to provide energy to meet the daily physiological metabolism, and the larger stomach capacity provides the essential conditions for the male to store more food. In addition, we found that the crypt depth of male *Anourosorex squamipes* is generally greater than that of females, especially in the ileal crypt depth, indicating that the female's cell maturation rate is greater than that of the male. It is speculated that the female has a greater demand for nutrient absorption and better digestion and absorption of food, because females need to give birth to offspring and require greater nutrition.

**Keywords:** *Anourosorex squamipes*, contour characteristics, digestive system, small intestine.

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## INTRODUCTION

*Anourosorex squamipes* is a kind of small beast, which has a wide range of feeding habits, but they would not eat artificial diet [31]. *Anourosorex squamipes* mainly distributed in Sichuan province in China, which is the dominant species in Sichuan. It mainly lives underground with high humidity and often more active at night [24].

At present, researches on *Anourosorex squamipes* or Soricidae focuses on the development and phenotypic correlations [34,44], determination of sex [37], genetic structure of populations and genetic diversity and structure [23,44], reproduction [3,16,40], geographic differences in karyotypes [27], habitat selection and the response to human disturbances [54], the chromosomes and genome [4,8,20,35,42], viruses and parasites and so on [9,10,29,43], however, few reports on its digestive system. The higher the evolution degree of animals, the higher the differentiation of digestive system [11]. The research on the morphology of digestive system has not been reported.

The intestine has the functions of digesting and absorbing nutrients, electrolytes and water, and can also secrete some enzymes and transporters [13,22,47], the intestinal absorption function is closely related to its structure, and any morphological change will lead to the impairment of intestinal function [7,12,51]. Therefore, in the present study the contour parameters and morphological parameters of digestive system of *Anourosorex squamipes* were investigated. We hope provide scientific data for the research of *A. squamipes*, and also provides some basic data for its ecological prevention and control by studying the characteristics of their digestive system.

## MATERIALS AND METHODS

*Animals*

Twenty healthy adult *Anourosorex squamipes* (10♂10♀) which captured from the campus of China West Normal University in Shunqing District, Nanchong City, Sihcuan Province of China were used as experimental materials in this experiment.

*Gross observation*

After anesthesia, the appearance parameters were measured with a cursor caliper. 4% paraformaldehyde was then perfused into the left ventricle, followed by overall immersion and fixation for more than 24 h. After that, *Anourosorex squamipes* were dissected by anatomical methods of small animals. After observing the characteristics of each site, the digestive tract was separated from the body, the parameters were measured, weighed and recorded. The gut was then fixed in a 4% paraformaldehyde solution for at least 24 h.

*Histological observations*

Tissue samples of about 1 cm in the middle duodenum, jejunum and ileum were taken, fixed in 4% paraformaldehyde, rinsed through running water, dehydrated with 75%, 85%, 95% and 100% alcohol at all levels, then transparent with toluene, finally samples were wax, embedded, cut into 5 μM thick sections, followed by hematoxylin-eosin (H.E) staining (H9627-25G, HT110132-1L)[1], observed and photographed under an optical microscope[2], selected 10 typical visual fields (complete villi, straight) from tissue sections to measure the small intestinal villus height, crypt depth and myolayer thickness, and the average was used as the measurement data.

*Appearance parameter measurement*

Body length, front foot length, rear foot length, snout length and tail length are measured using a cursor caliper (500-164-30 Mitutoyo)[3] and weight was measured using an electronic scales (Quintix 124-1CN)[4] [Figure 1].

*Detection of the digestive system parameter*

The length of the esophagus and digestive tract were measured with cotton thread, and then the length of the cotton thread was measured by a steel ruler. The weight of each digestive tract was weighed with an electronic scale, and the liver was isolated and weighed.

*Measurement of small intestinal villus height, crypt depth, and muscular thickness*

After the small intestine staining seals, they were observed and photographed under an optical microscope. Ten typical visual fields (the villi were complete and straight) were selected from tissue sections to measure the height of the villus, crypt depth and muscular thickness of the small intestine (Figure 2). Its average was used as the determination data.

*Data analysis*

Data analysis was performed using SPSS20.0 software for this experiment. The obtained data were performed by performing a paired-sample T test, and the results are indicated as the mean ± standard deviation (Mean ± SD). The measurement parameters such as body weight and body length were averaged between female and male. *P* values were obtained using the t-test that were used to compare the degree of difference between female and male. ($P < 0.05$ indicates significant difference between females and males, and $P < 0.01$ indicates extremely significant difference between females and males)

## RESULTS

*Appearance parameters*

No obvious differences were found between males and females *Anourosorex squamipes* (Table 1), the cheeks are long and has an ochreous spot, dark gray or black brown back, light gray or slightly yellow abdomen (Figure 3 A & D); the back of the 4 feet is gray black, fingers (toes), claws are white, and dark brown tail (Figure 3 C). Muzzle is blunt and short, eye degenerate, about rapeseed size; no obvious ears are found (Figure 3 B). The fur is fine, charcoal gray of the whole body, hip hair is close to red; the tail is extremely short, scaly, naked and little fluff on the top of the tip (Figure 3 D). The forefeet are short and blunt and slightly robust, as showed in Figure 3.

The average weight of male *Anourosorex squamipes* is 29.03 ± 2.44 g and the average body length is 110.64 ± 7.65 mm; the average body weight of females is 28.17 ± 4.76 g and the average body length is 109.35 ± 5.59 mm. The average forefoot length of males is 15.46 ± 4.52 mm, and that of females is 17.61 ± 6.84 mm. The posterior foot is longer, about 1/5 of the body length, have 5 toes, the average posterior foot length of males is 22.63 ± 10.23 mm, and that of females is 22.02 ± 12.56 mm. The average nasal length of males is 6.10±1.40 mm, and that of females is 6.55 ± 0.20 mm. The average tail length of males is 17.57 ± 5.00 mm, and that of females is 17.34 ± 2.90 mm. There is no significant difference in above parameters between males and females ($P > 0.05$), which is showed in Table 1.

The parameter values of the digestive system are shown in Table 2 and Figure 4. The digestive tract of *Anourosorex squamipes* includes mouth, pharynx, esophagus, stomach, intestines, etc. The digestive gland is mainly the liver.

The average length of male esophagus is 52.04 ± 2.40 mm, female is 49.66 ± 6.93 mm. The stomach is the place where food is temporarily stored, which located at the upper left of the abdominal cavity, the shape is flexed, connected to the esophagus at the top, and the small intestine at the bottom. The average weight of male stomach is 1.60 ± 0.72 g, female is 0.87 ± 0.38 g. The overall weight of male stomach is larger than that of male and female, but there is no significant difference. The average total length of the male intestine is 31.73 ± 11.24 cm, the female is 33.50 ± 17.20 cm, but there is no significant difference in the length of male and female intestines. The male intestine weight is 2.40 ± 0.26 g, the female is 3.00 ± 0.64 g, and the *P* value is 0.099 (> 0.05). The liver is the largest digestive gland in the body of *Anourosorex squamipes*, which is located on the dorsal side of the sternum in the front of the abdomen. The live is divided into left lateral lobe, left medial lobe, right medial lobe and right lateral lobe. Their liver parenchyma is completely separated, and connected by only a small amount of connective tissue. The diaphragm surface of the liver is convex and fits with the diaphragm; the visceral surface is slightly concave, adjacent to the stomach, duodenum, kidney and other organs. The average weight of male liver is 2.10 ± 0.61 g, female is 1.63 ± 0.42 g, there is no significant difference of the liver weights between male and female ($P > 0.05$).

*Changes in villi height, crypt depth and muscle thickness of the small intestine*

The length of the villi of the ileum and duodenum of males in *Anourosorex squamipes* is larger than that of females. The thickness of the muscular layer and the depth of the crypts of the duodenum of males are generally larger than that of females, but they are different. Both are not significant ($P > 0.05$), but the depth of the ileum of males is generally greater than that of females, and the difference is extremely significant ($P < 0.01$), as showed in Figure 5 and Table 3.

**Table 1.** Physical sign measurement of *Anourosorex squamipes.*

| Gender | body weight* | body length** | forefoot** | Posterior foot** | nasal** | tail** |
|---|---|---|---|---|---|---|
| ♂ | 29.03 ± 2.44 | 110.64 ± 7.65 | 15.46 ± 4.52 | 22.63 ± 10.23 | 6.10 ± 1.40 | 17.57 ± 5.00 |
| ♀ | 28.17 ± 4.76 | 109.35 ± 5.59 | 17.61 ± 6.84 | 22.02 ± 12.56 | 6.55 ± 0.20 | 17.34 ± 2.90 |
| all | 28.60 ± 3.60 | 110.00 ± 6.62 | 16.53 ± 5.68 | 22.32 ± 11.39 | 6.32 ± 0.80 | 17.45 ± 3.95 |
| *P* value | 0.392 | 0.542 | 0.437 | 0.637 | 0.104 | 0.249 |

*Weight= g. **Length= mm.

**Table 2.** Digestive system measurement of *Anourosorex squamipes.*

| Gender | esophagus (mm) | stomach (g) | length (cm) | weight (g) | liver (g) |
|---|---|---|---|---|---|
| ♂ | 52.04 ± 2.40 | 1.60 ± 0.72 | 31.73 ± 11.24 | 2.40 ± 0.26 | 2.10 ± 0.61 |
| ♀ | 49.66 ± 6.93 | 0.87 ± 0.38 | 33.50 ± 17.20 | 3.00 ± 0.64 | 1.63 ± 0.42 |
| all | 50.85 ± 4.67 | 1.24 ± 0.55 | 32.62 ± 14.22 | 2.70 ± 0.45 | 1.87 ± 0.52 |
| *P* value | 0.112 | 0.274 | 0.523 | 0.099 | 0.371 |

**Table 3.** Changes of villus height, cry*P* t de*P* th and basal layer thickness in the ileum, jejunum and duodenum of *Anourosorex squamipes*.

| | Gender | Villus height (μM) | Cry*P* t de*P* th (μM) | Basal layer thickness (μM) | Villus/cry*P* t (V/C) |
|---|---|---|---|---|---|
| Ileum | ♂ | 328.71 ± 40.94 | 101.56 ± 26.33 | 60.02 ± 15.00 | 3.42 ± 0.88 |
| | ♀ | 219.76 ± 23.92 | 67.94 ± 10.35 | 69.28 ± 18.02 | 3.29 ± 0.54 |
| | Average | 274.24 ± 32.43 | 84.75 ± 18.34 | 64.65 ± 16.51 | 3.36 ± 0.71 |
| | *P* value | 0.205 | 0.007 | 0.197 | 0.158 |
| Jejunum | ♂ | 269.88 ± 54.14 | 83.15 ± 12.98 | 60.24 ± 8.59 | 3.27 ± 0.59 |
| | ♀ | 232.68 ± 34.87 | 67.91 ± 9.64 | 61.20 ± 10.52 | 3.44 ± 0.36 |
| | Average | 251.28 ± 44.51 | 75.53 ± 22.62 | 60.72 ± 19.11 | 3.36 ± 0.48 |
| | *P* value | 0.050 | 0.239 | 0.463 | 0.142 |
| Duodenum | ♂ | 264.18 ± 29.19 | 126.97 ± 22.04 | 159.28 ± 27.11 | 2.13 ± 0.41 |
| | ♀ | 199.09 ± 31.38 | 73.15 ± 11.62 | 66.62 ± 13.71 | 2.75 ± 0.42 |
| | Average | 231.64 ± 30.29 | 100.06 ± 16.83 | 112.95 ± 40.82 | 2.44 ± 0.42 |
| | *P* value | 0.947 | 0.128 | 0.053 | 0.731 |

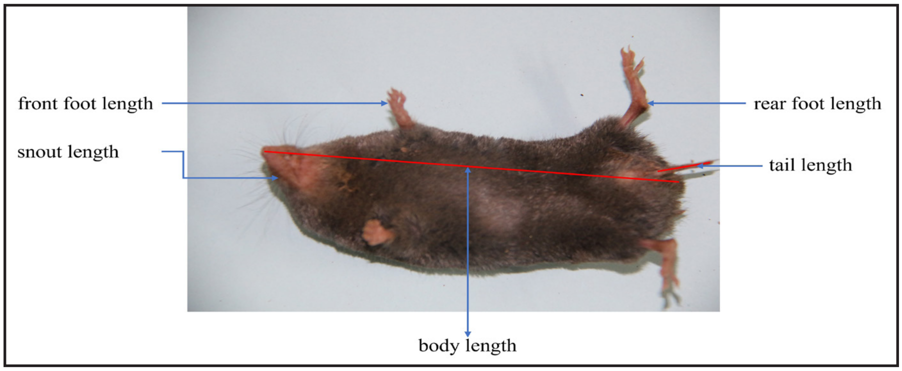


**Figure 1.** Determination of the appearance parameters of *Anourosorex squamipes*.

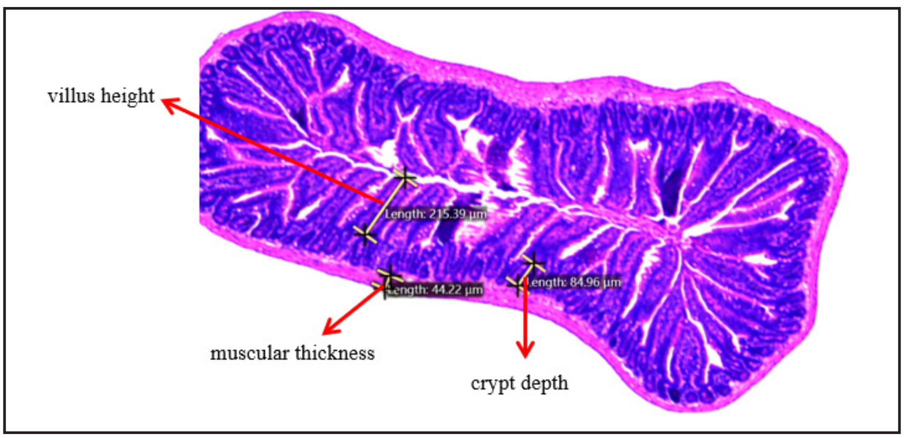


**Figure 2.** Determination of the villus height, crypt depth and muscular thickness of the small intestine of *Anourosorex squamipes*.

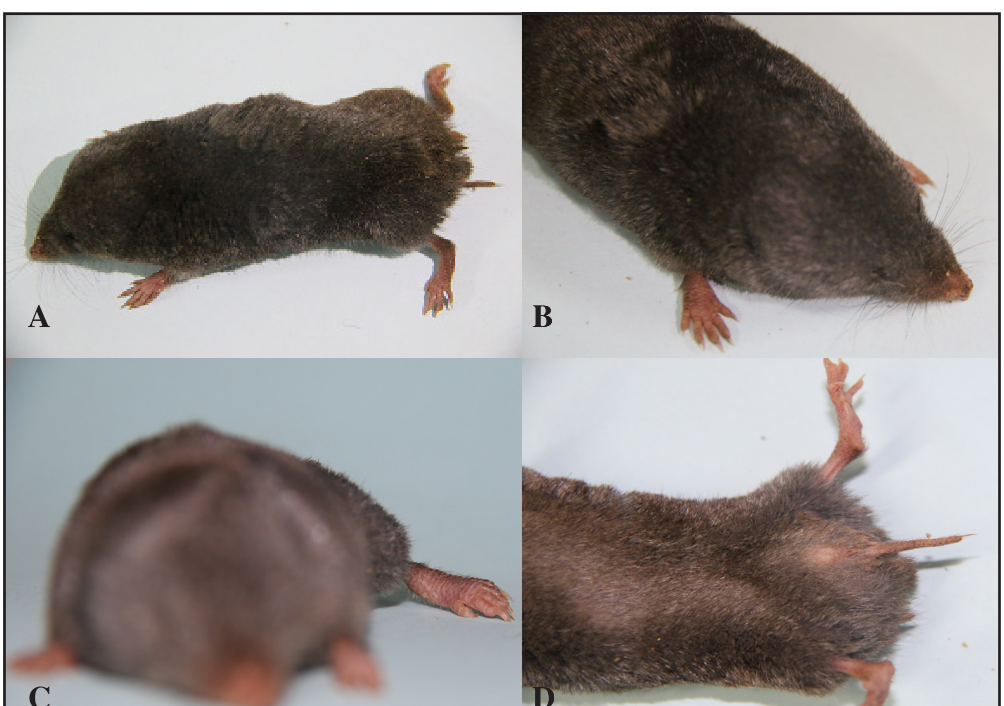


**Figure 3.** Appearance features of *Anourosorex squamipes*: A- Overall shape; B- Forefoot; C- Hind feet & D- Tail.

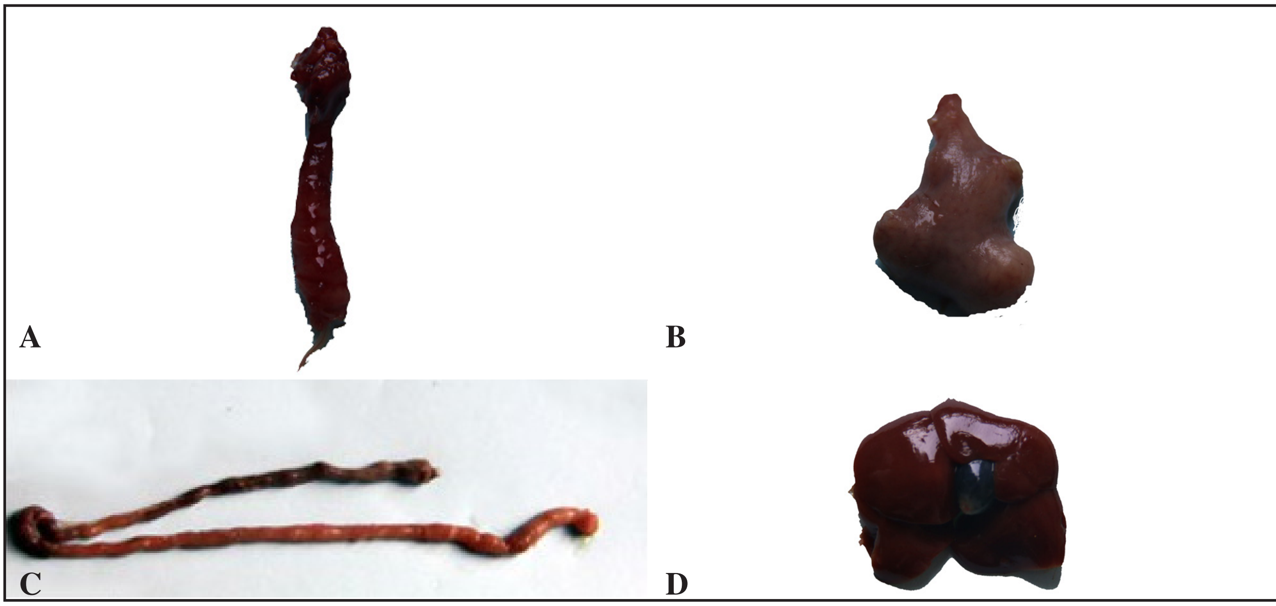


**Figure 4.** Characteristics of digestive system in *Anourosorex squamipes*: A- Esophagus; B- Stomach; C- Intestines & D- Liver.

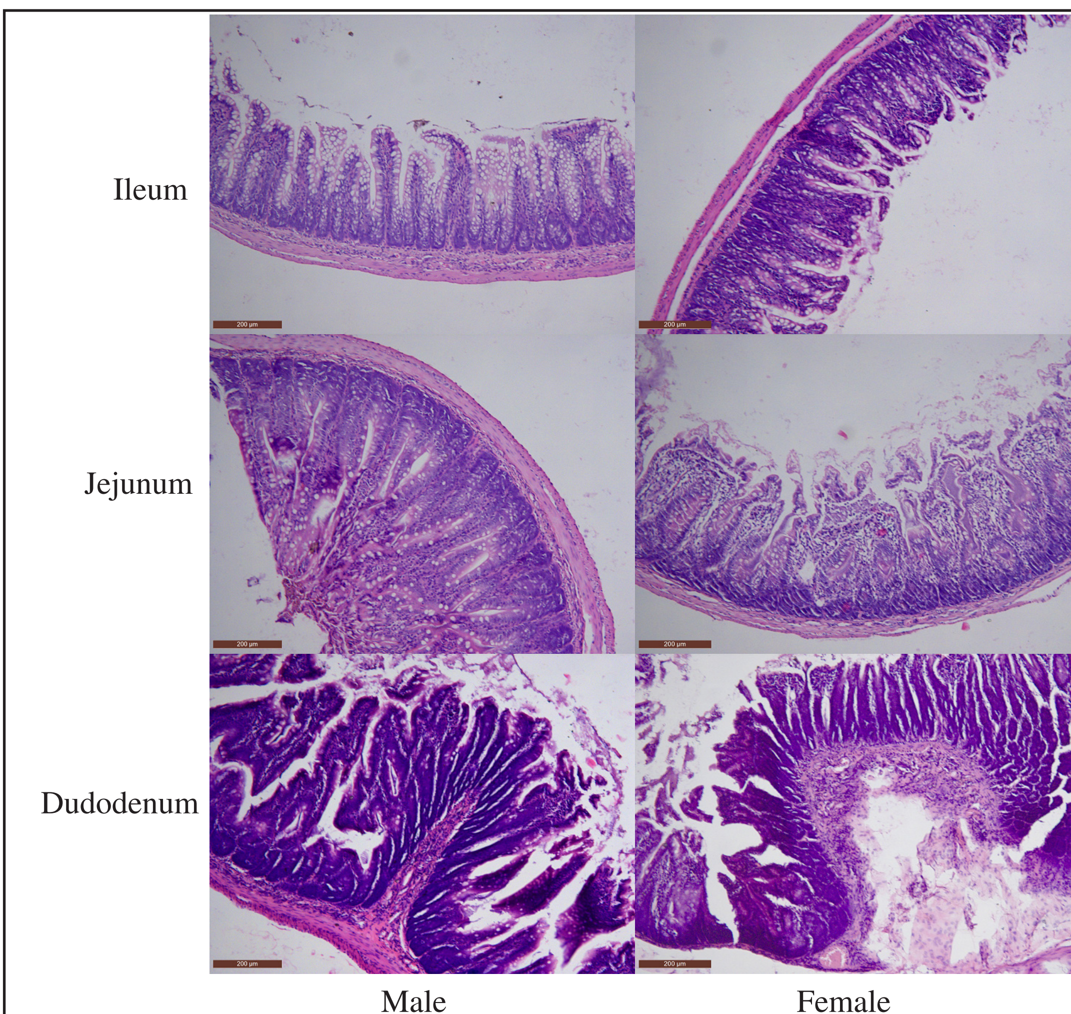


**Figure 5.** Small intestinal morphology of male and female *Anourosorex squamipes*.

## DISCUSSION

Animal morphological characteristics are closely related to their life habits, unique organs and lifestyles ensure that they thrive on earth. In this study, the appearance and digestive tract histomorphology of *Anourosorex squamipes* were measured and analyzed, aim to investigate the characteristics of appearance and digestive system, compare the differences between male and female and analyze the possible reasons for the differences.

Unique characters are found by observing the appearance of *Anourosorex squamipes*, the snout is blunt and short, the eyes are degenerated, and the tail is very short and covered with scales. The blunt and short snout may be related to its feeding habits, this structure is good for its digging and gathering. The degeneration of the eyes led to its visual degeneration, which may be related to the living environment of *Anourosorex squamipes*. The long-term nocturnal life of *Anourosorex squamipes* in underground with minimal exposure to sunlight, and combined with the humid underground environment, led to their ocular deterioration [50]. The tail of *Anourosorex squamipes* is covered with scales and is extremely short, which can reduce the friction between tail and soil during the movement, this could protect the tail [17]. Its forefoot is short and blunt, and slightly sturdy, which provides the necessary congenital conditions for *Anourosorex squamipes* to dig.

This study found that there are certain differences in the appearance parameters of male and female *Anourosorex squamipes*, but are not significant. The average weight of *Anourosorex squamipes* is 28.60 ± 3.60 g, average body length is 110.00 ± 6.62 mm, average forefoot length is 16.53 ± 5.68, and average hindfoot length is 22.32 ± 11.39 mm. There is no significant difference in appearance between males and females, and male *Anourosorex squamipes* are almost indistinguishable from females in terms of body weight, body length, hind foot length and tail length. This may be due to the fact that the food abundance of the *Anourosorex squamipes*' habitat, and the amount of activity during feeding, but these 2 factors are not significantly different between males and females [54].

Studies have shown that *Anourosorex squamipes*, which is dominated by carnivorous animals, has a shorter intestine than that of animals with plant and insect food [36,49,53]. The length of the intestine is only 32.62 ± 14.22 cm, which is about 2.9 times the body length of 110.00 ± 6.62 mm. In this study, we calculated that the intestinal weight/body weight ratio, as well as the intestinal length/body length ratio, were lower in *Anourosorex squamipes* than in rodents such as *Apodemus agrarius* [38,49], indicating that the feeding habits of *Anourosorex squamipes* is mainly carnivorous, which is consistent with the findings of Jiang *et al*. [18] that 65% of the gastric contents of *Anourosorex squamipes* were carnivorous, 27% were phytophagous, and 8% were insect based. In this experiment, the esophagus, stomach, intestine, liver and other parameters of *Anourosorex squamipes* have little difference between males and females, indicating that *Anourosorex squamipes* don't have obvious distinctions between males and females in food intake.

Liver is the largest digestive gland in *Anourosorex squamipes*, which is reddish brown with 4 separated lobes, and connected only by a small amount of connective tissue, and it is adjacent to the stomach, duodenum, kidney, and other organs. Liver is an important metabolic organ with many physiological functions, and its weight can be used as one of the indicators of changes in physiological functions [2,19,33]. In this study, we found that the average liver weight of male *Anourosorex squamipes* was 2.10 ± 0.61g, and that of females was 1.63 ± 0.42 g, males were slightly heavier than females, but they were not significant ($P > 0.05$). Research found that most liver cells of *Anourosorex squamipes* have dual nuclei, which is presumed to be related to their active metabolism. In addition, liver is an important organ of heat production, the difference of liver weight indirectly reflects the difference of animal adaptive heat production ability [1,52]. In comparison with another study [32] we found that the average liver weight of *Anourosorex squamipes* was similar to that of skunks. Therefore, we think that the adaptive heat production capacity and adaptation to cold and humid environments of *Anourosorex squamipes* are similar to that of skunks. It is speculated that the stress resistance of male is slightly higher than that of female, because the liver weight of male *Anourosorex squamipes* is also heavier than that of female. Stomach is the place where animals temporarily store and preliminarily digest and absorb food. It is reported that there is a close relationship between stomach size and the amount of food that can be taken in at one time or the time for foraging [21,30,36]. The results of this study showed that the stomach capacity of males is larger than that of females, which can store more food. By comparing the intestinal length of females and males, it is found that the intestinal length of males is relatively smaller than females, indicating that the male's ability to digest and absorb food is slightly weaker than females, which is consistent to another animal [41]. However, the liver weight of male *Anourosorex squamipes* is larger than female, indicating that the physiological metabolic capacity of male is larger than female. It is speculated that this may be related to the difference in the amount of activity between males and females in daily life. Males are more active and require more energy, but their digestion and absorption abilities are weaker than females. Therefore, the male *Anourosorex squamipes* needs more food to provide energy to meet the daily physiological metabolism, and the larger stomach capacity provides the essential conditions for the male to store more food.

Small intestine is the main part of food digestion and nutrient absorption. The intestinal villus height (VH), crypt depth (CD), VH/CD value, intestinal wall thickness can reflect the histological morphology of intestinal mucosa and intestinal health, and be used as important indicators to measure the health of the body and the digestion and absorption function of the small intestine [5,15,26]. Crypts are the source of new villi cells exist between the villi of the small intestine, and the depth reflects the cell production rate. The shallower crypts indicate that the cell maturation rate is increased and the absorption function is enhanced [6,39], and the intestinal mucosa can be repaired quickly. The VH/CD ratio comprehensively reflects the functional status of the small intestine [14]. In this study, we found that the crypt depth of male *Anourosorex squamipes* is generally greater than that of females, especially in the ileal crypt depth (see Tables 3, 4 & 5), indicating that the female's cell maturation rate is greater than that of the male. It is speculated that the female has a greater demand for nutrient absorption and better digestion and absorption of food, because females need to give birth to offspring and require greater nutrition. At the same time, it was also found that the height of female intestinal villi is slightly smaller than that of males, we speculated that females' intestines are longer than males, the food stays in the intestines for longer, which is helpful for the digestion and absorption of food adequately, but it also takes longer to eat, the abrasion of intestinal villi is relatively stronger [25,32]. Comparing the intestinal weight of females and males, it is found that the intestinal weight of males is smaller than that of females, which confirms the previous point. Therefore, when we need to raise *Anourosorex squamipes* for a long time under experimental conditions, if the females give birth to offspring, we can add appropriate amounts of synbiotics, yeast extracts and other substances to the feed [28,48] to increase the height of intestinal villi, reduce the depth of crypts, enhance digestion and absorption, reduce the prevalence of enteritis, reduce the mortality.

## CONCLUSION

The appearance of *Anourosorex squamipes* is somewhat different from male and female in most digestive system parameters, but the difference is small. For the digestive system, it is found that the adaptability and food storage capacity of male *Anourosorex squamipes* is greater than that of females, and the differences in crypt depth may be due to the greater nutrient absorption demand of females for breeding offspring.

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***Ethical approval*.** The study was approved by The Animal Local Ethics Committee (Number: 2020-011), China West Normal University.

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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