

A THESIS
FOR THE DEGREE OF MASTER OF SCIENCE

Histochemical Characteristics of the Digestive Tract
of the Gobiidae in Jeju Island, Korea

Sang-Woo Hur



Department of Marine biology
GRADUATE SCHOOL
CHEJU NATIONAL UNIVERSITY

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Sang-Woo Hur
(Supervised by professor Young-Don Lee)

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This thesis has been examined and approved.



Thesis director, Sang-Chul Chung, Prof. of Marine biology

Kyeong-Jun Lee, Prof. of Marine biology

Young-Don Lee, Prof. of Marine and Environmental Research Institute

Date

Department of Marine biology
GRADUATE SCHOOL
CHEJU NATIONAL UNIVERSITY

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국문초록

제주연안 조간대에 서식하는 망둑어과 어류인 바닥문절, *S. geneionema*, 별망둑, *C. gulosus* 과 기수지역인 천지연하구에 서식하는 망둑어과 어류인 검정망둑, *T. obscurus*, 갈문망둑, *R. giurinus* 소화관의 형태와 조직학적 및 조직화학적 특징을 조사하였다.

해산어인 바닥문절은 소화관의 길이가 짧고 (RLG 0.45), 단순한 형태를 나타냈으며, 무위어의 특징을 나타내었고, 유문수가 관찰되지 않았다. 소화관의 점막주름은 일반적인 가지화된 형태를 나타내었으며, 근육층의 경우 식도에서 가장 발달되었다. 점액을 분비하는 배상세포의 분포양상은 후장부에 가장 많이 분포하였으며, CCK-IR 세포의 경우 전장부에 가장 많이 분포하였으며, 적은 수의 CCK-IR 세포들이 전장부에서 직장까지 분포하는 특징을 나타내었다 ($P<0.05$).

해산어인 별망둑은 소화관의 길이가 짧고 (RLG 0.43), 위선이 잘 발달된 위를 가지고 있으며, 유문수는 가지고 있지 않았다. 소화관의 점막주름은 복잡한 수지상의 형태를 나타내었으며, 근육층의 경우 식도에서 가장 발달되었다. 점액을 분비하는 배상세포의 분포양상은 중장부에 가장 많이 분포하였으며, CCK-IR 세포의 경우 전장부에 가장 많이 분포하였고, 전장부와 중장부에서만 관찰되었다 ($P<0.05$).

기수어인 검정망둑은 소화관의 길이가 다소 길고 (RLG 0.66), 위의 위선과 점막고유관의 발달이 미약하였으며, 유문수가 관찰되지 않았다. 소화관의 점막주름은 일반적인 가지화된 형태를 나타내었으며, 근육층의 경우 식도에서 가장 발달되었다. 점액을 분비하는 배상세포의 분포양상은 중장부에 가장 많이 분포하였으며 ($P<0.05$), CCK-IR 세포의 경우 전장부와 중장부에서만 고르게 분포하는 경향을 나타내었다.

기수어인 갈문망둑은 소화관의 길이가 짧고 (RLG 0.42), 위선이 잘 발달된 위를 가지고 있으며, 유문수는 가지고 있지 않았다. 소화관의 점막주름은 일반적인 가지화된 형태를 나타내었으며, 근육층의 경우 식도에서 가장 발달되었다. 점액

을 분비하는 배상세포의 분포양상은 식도에서 가장 많이 분포하였으며, CCK-IR 세포의 경우 전장부에 가장 많이 분포하였고, 전장부와 중장부에서만 관찰되었다 ($P<0.05$).

해산어인 바닥문절과 별망둑은 주로 각각 후장부와 중장부에서 장을 보호하여 장 활성을 촉진시키고, 소화작용이 일어나는 것으로 생각되고, 기수어인 검정망둑과 갈문망둑은 주로 각각 중장부와 전장부에서 장을 보호하여 장 활성을 촉진시키고, 소화작용이 일어나는 것으로 생각되나 네 종의 섭취물에 대한 생물검정이 미비하여 차후에 먹이생태 측면에서 소화관 내용물 분석과 또한 염색성에 따른 배상세포의 특성에 관한 연구가 선행되어야 할 것으로 생각된다.



I . Introduction

Worldwide, there are 1,875 species in the family Gobiidae (Nelson, 1984), which is slightly smaller than that of the Cyprinidae. Gobies are found in both freshwater and seawater. In Korea, there are 22 genera and 46 species of the Gobiidae, most of which live in inland waters, including estuaries, rivers, and brackish inland seas (Kim, 1986).

Gobies living in the tidal zone of the coastal area of Jeju Island include *Pterogobius virgo*, *Istigobius hoshinonis*, *Chasmichthys dolichognathus*, and *Mugilogobius fontinalis*. The hairychin goby, *Sagamia geneionema*, lives in the arenaceous area of the tidal zone, while the gluttonous goby, *Chasmichthys gulosus*, inhabits tidal pools that are characterized by a continuous supply of freshwater and domestic sewage. The trident goby, *Tridentiger obscurus*, and the giurine goby, *Rhinogobius giurinus*, are gregarious brackish water fishes that live in the estuary of the Cheonjiyeon waterfall, where they are being studied as part of an environmental project.

The digestive tract of most teleosts consists of an oral cavity, pharynx, stomach, intestine, rectum, and anus. However, since the feeding habits of these fish differ by species and the environment that they inhabit, the morphologies and structures of their digestive tracts differ as well (Tanaka, 1969).

In this study, we investigated the internal structures of the digestive tracts, the characteristics of the epithelial tissues, and the distributions and characteristics of mucus-secreting goblet cells and CCK-producing cells with respect to the habitats and ingesta of individual species in order to provide a basis for understanding the digestive physiology and biology of the Gobiidae. Specifically, the subjects of this study were the hairychin goby, *S. geneionema*, the gluttonous goby, *C. gulosus*, the trident goby, *T. obscurus* and the giurine goby, *R. giurinus*. *S. geneionema* and *C. gulosus* inhabit in seawater, and *T. obscurus* and *R. giurinus* inhabit brackish waters.

II. Materials and methods

1. Specimens

S. geneionema and *C. gulosus* were collected from the Hamdeok-ri coastal area of Jeju Island, Korea. *T. obscurus* and *R. giurinus* were collected from an estuary at Cheonjiyeon waterfall on Jeju Island; these species are currently being studied as part of an environmental project conducted by the city of Seogwipo.

The collected specimens were transported to the Marine and Environmental Research Institute at Cheju National University, Korea. They were anaesthetized with 2-phenoxy-ethanol, and the body length (BL, 0.1 mm) was measured. The entire digestive tract, from the esophagus to the anus, was removed from the body cavity. The length of the digestive tract (DL, 0.1 mm) and the relative length of the gut (RLG: digestive tract length/body length) were measured, and the stomach and intestinal contents were examined.

2. Histological observations

Samples from six parts of the digestive tract (esophagus, stomach, anterior intestine portion, mid intestine portion, posterior intestine portion, rectum) were fixed in Bouin's solution, dehydrated in a graded series of ethanol, embedded in paraffin, and then cut in 5 μm cross and longitudinal sections.

Slides were stained with Hansen's hematoxylin and 0.5% eosin (HE) for histological observations, and were stained with Alcian blue (AB) at pH 2.5 and periodic-acid-Schiff (PAS) for observations of mucus-secreting goblet cells.

Microscopy of the length of the mucosal folds, thickness of the muscularis externa, and characteristics of the goblet cells from different regions of the digestive tract was carried out using a light microscope (Carl Zeiss, HBO 50) with Image scope 2.3 (Image Line, Inc.) software.

3. Immunohistochemistry

Six fish from each species were processed for immunohistochemistry. CCK-immunoreactive (IR) cells were visualized using the avidin-biotin complex (ABC) method (Hsu *et al.*, 1981). Microscope slides were coated with poly-L-lysine to promote tissue section adherence.

After the sections were deparaffinized and rehydrated, they were incubated in 0.5 mM periodic acid to block endogenous peroxides. After three rinses in 0.1 M phosphate-buffered saline (PBS; pH 7.2), nonspecific binding was blocked with 10% normal goat serum in PBS for 15 min. The solution was blotted off from the slides, primary CCK antiserum (1:8000, Sigma, Israel) was added, and the slides were incubated for 22 h at 4 °C in a moist chamber. After three rinses in PBS, the sections were incubated for 50 min at room temperature (around 23 °C) in anti-rabbit goat serum (IgG, Vector, USA) diluted 1:200 in PBS. After three more rinses in PBS, the sections were incubated for 1 h at room temperature (around 23 °C) with strept avidin labeled peroxidase diluted 1:100. After three rinses in PBS, the DAB substrate system (Vector) was added for peroxidase reactions. All samples were prepared on a clean bench and incubated in a moist chamber. After immunostaining, the sections were mounted in Canada balsam (Junsei, Japan), and CCK-IR cells were observed by light microscopy.

4. Statistical analysis

All data were subjected to a one-way ANOVA. Duncan's multiple comparisons test was conducted using SAS statistical software (SAS Institute, Cary, NC, USA). RLG of each species was subjected to a analysis of covariance and location test.



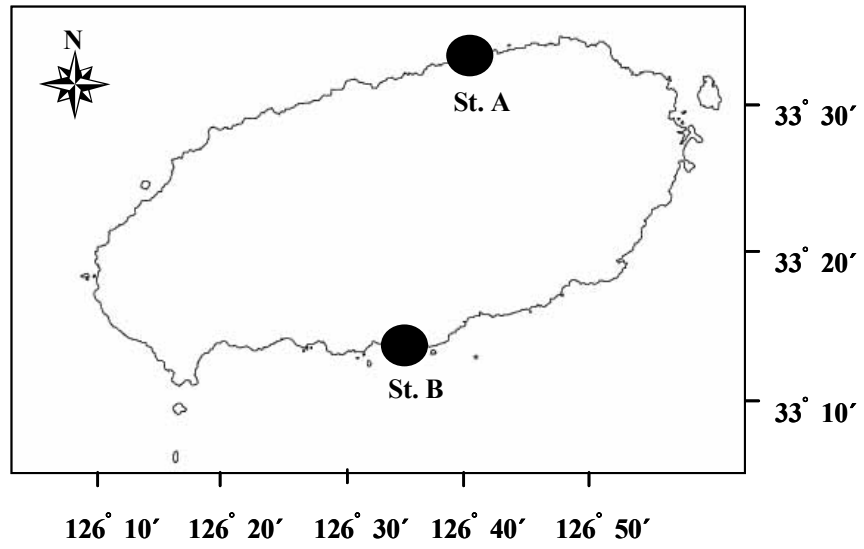


Fig. 1. Map showing the sampling area of the Gobiidae at the Jeju Island, Korea.
St. A: Hamdeok-ri, St. B: Seogwipo.

III. Results

1. Body length, digestive tract length, relative length of gut (RLG) and stomach contents

1) Hairychin goby (*S. geneionema*)

S. geneionema inhabits mainly the sandy bottom along the shores of shallow sea. The average length of body and the digestive tract of this fish were 6.7 ± 0.2 cm and 3.0 ± 0.1 cm, respectively, and the RLG was 0.45 (Table 1). The result of analysis of covariance was not significantly different compared to the other fish species investigated in this study. The result of location test was significantly different compared to *T. obscurus* (Table 1).

The digestive tract of this fish has a slightly curved shape, with a depression between the esophagus and the anterior intestine. pyloric caeca were not observed. The diameter of the rectum was larger than that of the other portions of the intestine (Fig. 2-A). The intestinal contents of the fish consisted of sand grains and small shrimps.

2) Gluttonous goby (*C. gulosus*)

C. gulosus mainly inhabits rock beds in tidal pools. Its average length of body and the digestive tract were 12.3 ± 0.2 cm and 5.3 ± 0.2 cm, respectively, and the RLG was 0.43 (Table 1). The result of analysis of covariance was not significantly different compared to the other fish species investigated in this study. The result of location test was significantly different compared to *T. obscurus* (Table 1). The digestive tract of this fish is short and gently curved, and the border between the esophagus and the stomach is not clear (Fig 2-B).

The digestive tract of this fish was very rough, and pyloric caeca were not observed. The

stomach contents of the fish consisted of small shrimps, crabs and a small amount of green algae.

3) Trident goby (*T. obscurus*)

T. obscurus inhabits mainly rock beds and was collected from an estuary at the Cheonjiyeon waterfall. The average length of body and the digestive tract were 8.9 ± 0.4 cm and 5.8 ± 0.4 cm, respectively, and the RLG was 0.66 (Table 1). The result of analysis of covariance was not significantly different compared to the other fish species investigated in this study. The result of location test was significantly different compared to the other species studied (Table 1).

The digestive tract of this fish is longer than those of the other species studied and is gently curved. The diameter of the rectum was somewhat larger than that of other portions of the intestine, and pyloric caeca were not observed (Fig. 2-C). The stomach contents of the fish consisted of green algae and small lugworms.



4) Giurine goby (*R. giurinus*)

R. giurinus inhabits the soft, sandy bottom of the estuary at the Cheonjiyeon waterfall. The average length of body and the digestive tract were 8.4 ± 0.2 cm and 3.5 ± 0.2 cm, respectively, and the RLG was 0.42 (Table 1). The result of analysis of covariance was not significantly different compared to the other fish species investigated in this study. The result of location test was significantly different compared to *T. obscurus* (Table 1).

The digestive tract of this fish is shorter than that of the other species studied. A clear border between the esophagus and stomach was not seen, and the area between the stomach and the anterior portion of the intestine had a slight depression. The digestive tract of this fish is narrow, and pyloric caeca were not observed (Fig. 2-D). The stomach contents of the fish consisted mainly of lugworms.

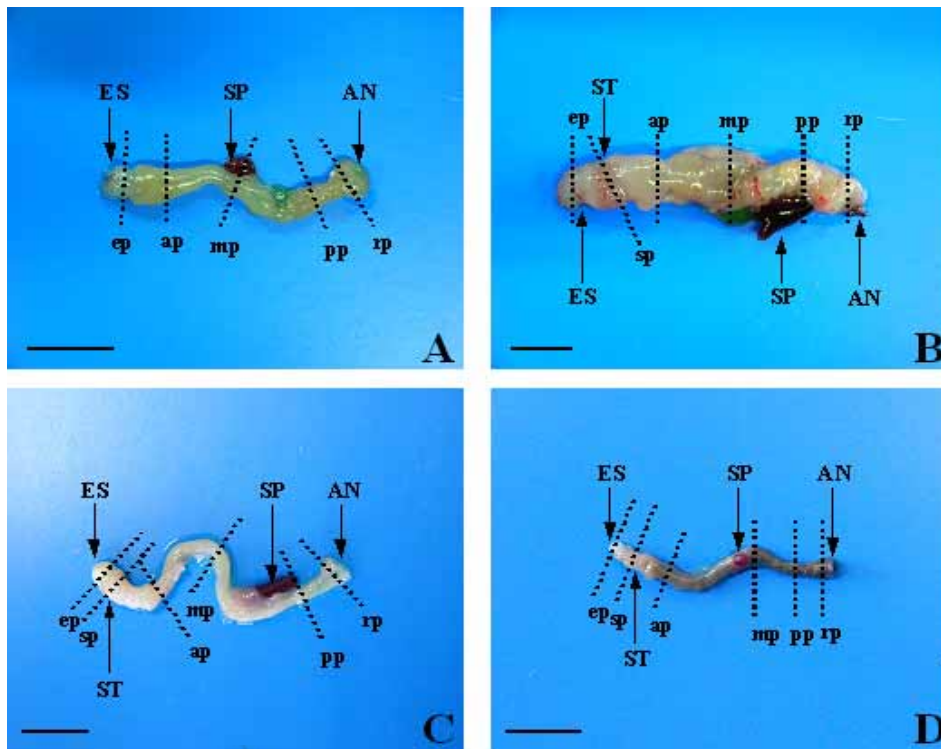


Fig. 2. External morphology of the digestive tract of the Gobiidae.

A: *S. geneionema*, B: *C. gulosus*, C: *T. obscurus*, D: *R. giurinus* AN: anus, ES: esophagus, SP: spleen, ST: stomach, ap: anterior intestine portion, ep: esophagus portion, mp: mid intestine portion, pp: posterior intestine portion, rp: rectum portion, sp: stomach portion. Scale bars indicate 10 mm.

Table 1. Body length, digestive tract length, RLG and relative growth equation of the Gobiidae

	<i>S. geneionema</i>	<i>C. gulosus</i>	<i>T. obscurus</i>	<i>R. giurinus</i>
Sample number	20	20	20	20
BL (body length, cm)	6.7±0.2	12.3±0.2	8.9±0.4	8.4±0.2
DL (digestive tract length, cm)	3.0±0.1	5.3±0.2	5.8±0.4	3.5±0.2
RLG (relative length of gut)	0.45±0.02	0.43±0.01	0.66±0.04	0.42±0.02
Relative growth equation	DL=0.925+0.3061BL	DL=0.5683+0.3803BL	DL=3.5921+0.2473BL	DL=0.5240+0.3550BL

Values are mean ± S.E.

RLG (relative length of gut: digestive tract length/body length).

2. Histological characteristics

1) Hairychin goby (*S. geneionema*)

The muscularis externa and lamina propria of the esophagus of *S. geneionema* were poorly developed. Mucosal folds were regularly distributed and extended toward the lumen of the esophagus (Fig. 3-A). The average thickness of the muscularis externa and length of the mucosal folds were $61.7 \pm 6.0 \mu\text{m}$ and $331.1 \pm 12.2 \mu\text{m}$, respectively (Table 2).

In this fish, a stomach and pyloric caeca were not observed. In the anterior intestine portion, the mucosal folds were regularly branched and extended toward the lumen (Fig. 3-B). They were densely distributed and paralleled the lamina propria. The average thickness of the muscularis externa and length of the mucosal folds were $36.7 \pm 3.1 \mu\text{m}$ and $410.6 \pm 14.3 \mu\text{m}$, respectively (Table 2).

The mucosal folds of the mid intestine portion were regularly branched and regularly distributed, extending toward the lumen (Fig. 3-C). The mean thickness of the muscularis externa and length of the mucosal folds were $36.7 \pm 4.3 \mu\text{m}$ and $328.9 \pm 14.3 \mu\text{m}$, respectively (Table 2).

In the posterior intestine portion, short and wide mucosal folds were regularly distributed, extending toward the lumen (Fig. 3-D). The average thickness of the muscularis externa and length of the mucosal folds were $52.2 \pm 11.6 \mu\text{m}$ and $298.9 \pm 20.5 \mu\text{m}$, respectively (Table 2).

The mucosal folds of the rectum were wider than those of the posterior intestine portion, regularly distributed, and extended toward the lumen (Fig. 3-E). The average thickness of the muscularis externa and the length of the mucosal folds were $31.7 \pm 2.8 \mu\text{m}$ and $169.4 \pm 6.4 \mu\text{m}$, respectively (Table 2). Both tissues were very poorly developed.

The muscularis externa of this fish was also made up of two layers of smooth muscle cells, which were circularly arranged in the inner layer and longitudinally arranged in the outer layer. The muscularis externa was thickest in the posterior portion of the intestine, and the

inner circular layer was more developed than the outer longitudinal layer.

The mucosal folds consisted of a single layer of columnar epithelium and were shorter and wider towards the posterior intestine portion.



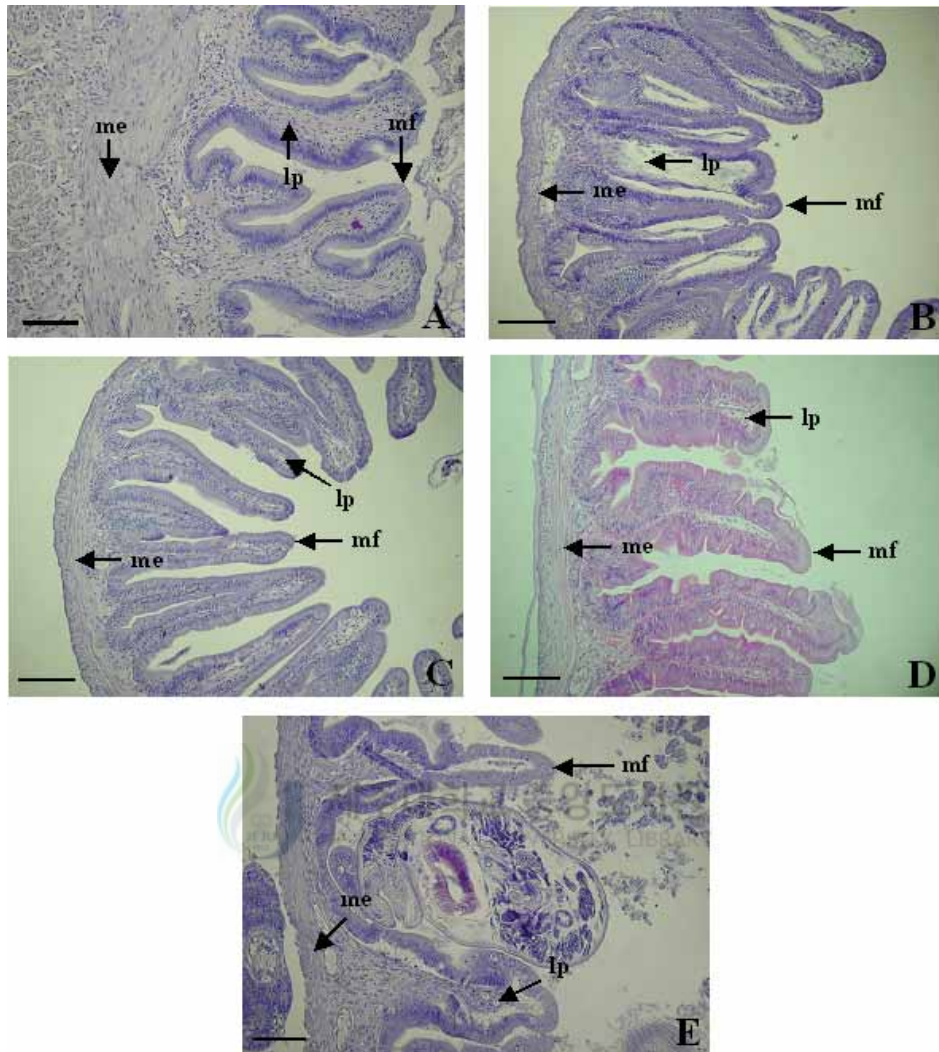


Fig. 3. Photomicrographs of cross section on the digestive tract of *S. geneionema*.
 A: esophagus, B: anterior intestine portion, C: mid intestine portion, D:
 posterior intestine portion, E: rectum, lp: lamina propria, me: muscularis
 externa, mf: mucosal fold. Scale bars indicate 50 μ m.

2) Gluttonous goby (*C. gulosus*)

The muscularis externa and lamina propria of the esophagus in this fish were well developed. The mucosal folds had a complex branched form and were regularly distributed, extending toward the lumen (Fig. 4-A). The average thickness of the muscularis externa and length of the mucosal folds were $395.6 \pm 31.9 \mu\text{m}$ and $852.2 \pm 55.4 \mu\text{m}$, respectively (Table 2).

In the gluttonous goby, a stomach was observed but pyloric caeca were not observed. The average thickness of the muscularis externa and the length of the mucosal folds were $233.3 \pm 42.9 \mu\text{m}$ and $1426.7 \pm 97.0 \mu\text{m}$, respectively (Table 2). The lamina propria was well developed and contained many gastric glands (Fig. 4-B).

The mucosal folds of the anterior intestine portion exhibited complex branching and were irregularly distributed, extending toward the lumen (Fig. 4-C). The average thickness of the muscularis externa and length of the mucosal folds were $138.9 \pm 17.2 \mu\text{m}$ and $628.9 \pm 42.4 \mu\text{m}$, respectively (Table 2).

In the mid intestine portion, the mucosal folds were complex and variously shaped (Fig. 4-D). The average thickness of the muscularis externa and length of the mucosal folds were $123.3 \pm 13.8 \mu\text{m}$ and $443.3 \pm 54.8 \mu\text{m}$, respectively (Table 2).

In the posterior intestine portion, the mucosal folds were slightly shorter but their arrangement was the same as in the mid intestine portion (Fig. 4-E). The average thickness of the muscularis externa and length of the mucosal folds were $158.9 \pm 11.1 \mu\text{m}$ and $288.9 \pm 27.4 \mu\text{m}$, respectively (Table 2).

The muscularis externa of the rectum was poorly developed but the mucosal folds were more complex and developed enough so that they filled the lumen (Fig. 4-F). The average thickness of the muscularis externa and length of the mucosal folds were $141.1 \pm 25.4 \mu\text{m}$ and $627.8 \pm 34.5 \mu\text{m}$, respectively (Table 2).

The muscularis externa of this fish was also made up of two layers of smooth muscle cells, which were circularly arranged in the inner layer and longitudinally arranged in the outer layer. The muscularis externa was widest in the posterior intestine portion, and the inner

circular layer was more developed than the outer longitudinal layer.

The mucosal folds consisted of a single layer of columnar epithelium and were shorter and wider from the anterior intestine portion to the rectum.



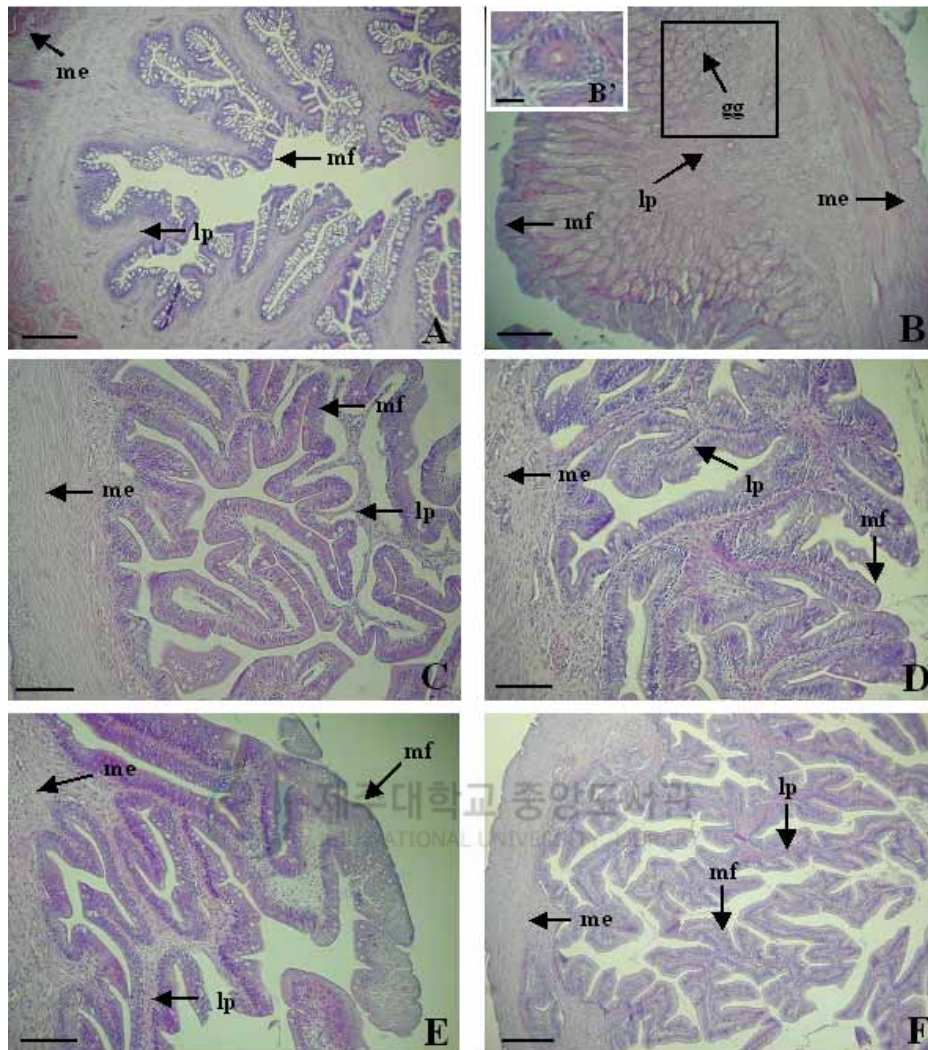


Fig. 4. Photomicrographs of cross section on the digestive tract of *C. gulosus*.

A: esophagus, B: stomach, C: anterior intestine portion, D: mid intestine portion, E: posterior intestine portion, F: rectum, gg: gastric gland, lp: lamina propria, me: muscularis externa, mf: mucosal fold. Scale bars indicate 50 μm . Magnified gastric gland in (B) was shown in (B'). B': Scale bar indicates 20 μm .

3) Trident goby (*T. obscurus*)

The mucosal folds and lamina propria of the esophagus in *T. obscurus* were well developed. The mucosal folds were regularly branched and extended towards the lumen (Fig. 5-A). The average thickness of the muscularis externa and length of the mucosal folds were $150.0 \pm 19.3 \mu\text{m}$ and $613.3 \pm 39.6 \mu\text{m}$, respectively (Table 2). The muscularis externa was poorly developed.

In the anterior intestine portion, a stomach was observed but pyloric caeca were not observed (Fig. 5-B). The average thickness of the muscularis externa and the length of the mucosal folds were $150.0 \pm 14.9 \mu\text{m}$ and $802.7 \pm 33.7 \mu\text{m}$, respectively (Table 2). The lamina propria was poorly developed and contained gastric glands (Fig. 5-B).

The mucosal folds of the anterior intestine portion were mostly regularly branched, but their arrangement was extremely diverse (Fig. 5-C). The average thickness of the muscularis externa and length of the mucosal folds were $57.3 \pm 2.5 \mu\text{m}$ and $612.0 \pm 43.0 \mu\text{m}$, respectively (Table 2).

The mucosal folds of the mid intestine portion were mostly regularly branched, densely distributed, and paralleled the lamina propria (Fig. 5-D). The average thickness of the muscularis externa and length of the mucosal folds were $75.8 \pm 9.6 \mu\text{m}$ and $486.7 \pm 31.3 \mu\text{m}$, respectively (Table 2).

The muscularis externa was thicker and the mucosal folds were longer in the posterior intestine portion, but the arrangement of the mucosal folds was the same as in the mid intestine portion, although their forms were diverse (Fig. 5-E). The average thickness of the muscularis externa and length of the mucosal folds were $85.0 \pm 8.7 \mu\text{m}$ and $536.7 \pm 28.8 \mu\text{m}$, respectively (Table 2).

The muscularis externa of the rectum was thicker and the mucosal folds were shorter than in the posterior intestine portion and the lamina propria was wider (Fig. 5-F). The average thickness of the muscularis externa and length of the mucosal folds were $87.8 \pm 14.6 \mu\text{m}$ and $491.1 \pm 24.1 \mu\text{m}$, respectively (Table 2).

The muscularis externa of this fish was made up of two layers of smooth muscle cells. Those of the inner layer were circularly arranged and those of the outer layer were longitudinally arranged. The thickness decreased from the anterior to the posterior intestine portion. The inner circular layer was more developed than the outer longitudinal layer.

The mucosal folds consisted of a single layer of columnar epithelium. While their arrangement was the same as in other portions of the intestine, they were relatively shorter in the mid intestine portion and longer in the posterior intestine portion.



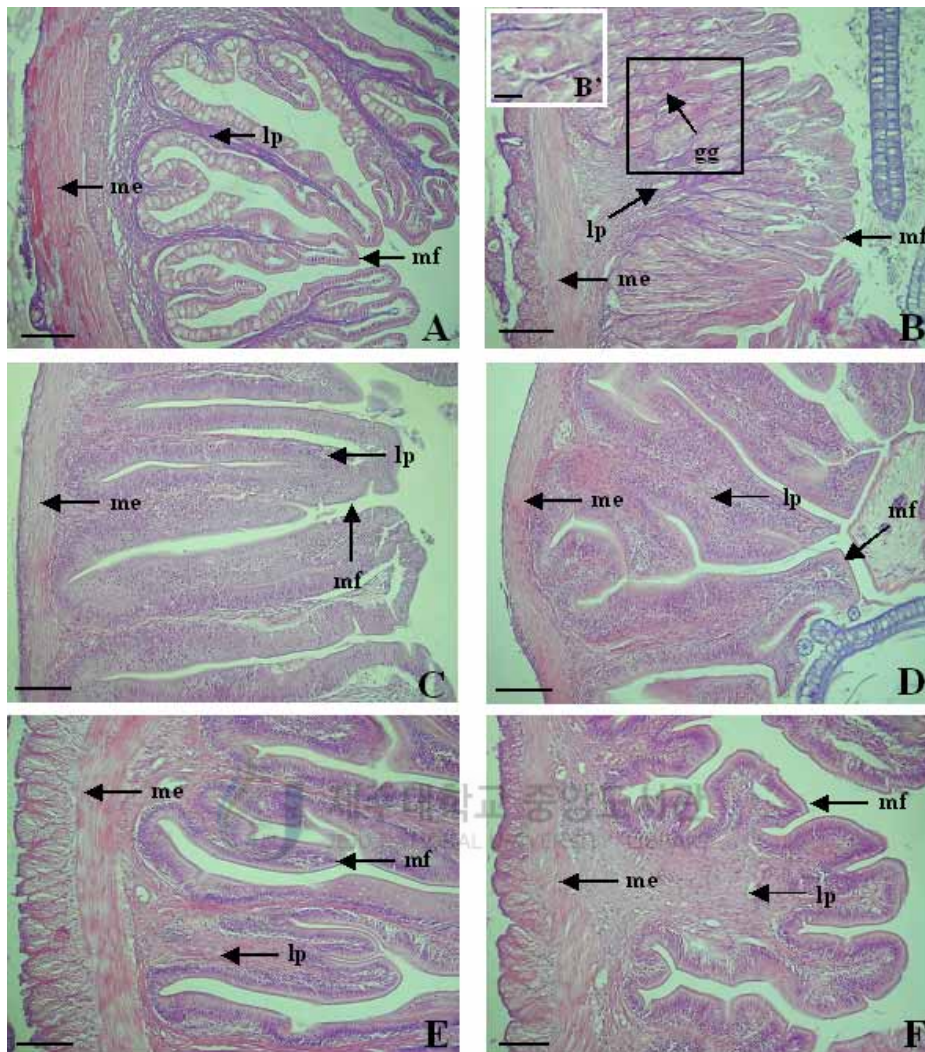


Fig. 5. Photomicrographs of cross section on the digestive tract of *T. obscurus*. A: esophagus, B: stomach, C: anterior intestine portion, D: mid intestine portion, E: posterior intestine portion, F: rectum, gg: gastric gland, lp: lamina propria, me: muscularis externa, mf: mucosal fold. Scale bars indicate 50 μm . Magnified gastric gland in (B) was shown in (B'). B': Scale bar indicates 20 μm .

4) Giurine goby (*R. giurinus*)

The muscularis externa of the esophagus of *R. giurinus* was more developed than that of *T. obscurus*, but the lamina propria was less developed. The mucosal folds were regularly branched and distributed toward the lumen (Fig. 6-A). The average thickness of the muscularis externa and length of the mucosal folds were $206.7 \pm 23.3 \mu\text{m}$ and $540.0 \pm 40.0 \mu\text{m}$, respectively (Table 2).

A stomach was observed but pyloric caeca were absent in the anterior intestine portion (Fig. 6-B). The average thickness of the muscularis externa and length of the mucosal folds were $122.5 \pm 8.2 \mu\text{m}$ and $608.8 \pm 40.6 \mu\text{m}$, respectively (Table 2).

The mucosal folds of the anterior intestine portion were regularly branched and densely distributed toward the lumen (Fig. 6-C). The average thickness of the muscularis externa and length of the mucosal folds were $45.8 \pm 3.8 \mu\text{m}$ and $481.7 \pm 4.6 \mu\text{m}$, respectively (Table 2).

The mucosal folds of the mid intestine portion were mostly regularly branched and distributed toward the lumen (Fig. 6-D). The average thickness of the muscularis externa and length of the mucosal folds were $49.2 \pm 2.9 \mu\text{m}$ and $390.0 \pm 14.7 \mu\text{m}$, respectively (Table 2).

The mucosal folds of the posterior intestine portion were wider and longer than in other portions of the intestine, and were distributed toward the lumen (Fig. 6-E). The average thickness of the muscularis externa and length of the mucosal folds were $48.3 \pm 4.4 \mu\text{m}$ and $433.3 \pm 18.1 \mu\text{m}$, respectively (Table 2).

The lamina propria of the rectum was wider than in other portions of the intestine and the epithelium was well developed (Fig. 6-F). The average thickness of the muscularis externa and length of the mucosal folds were $43.3 \pm 25.4 \mu\text{m}$ and $286.7 \pm 16.2 \mu\text{m}$, respectively (Table 2).

As in the other species, the muscularis externa of this fish consisted of two layers of smooth muscle cells; those in the inner layer were circularly arranged while those in the outer layer ran longitudinally. The thickness was similar in the anterior and posterior intestine portion. The inner circular layer was more developed than the outer longitudinal

layer.

The mucosal folds consisted of a single layer of columnar epithelium. While their pattern of distribution did not change, they were shorter in the mid intestine portion and longer in the posterior intestine portion.



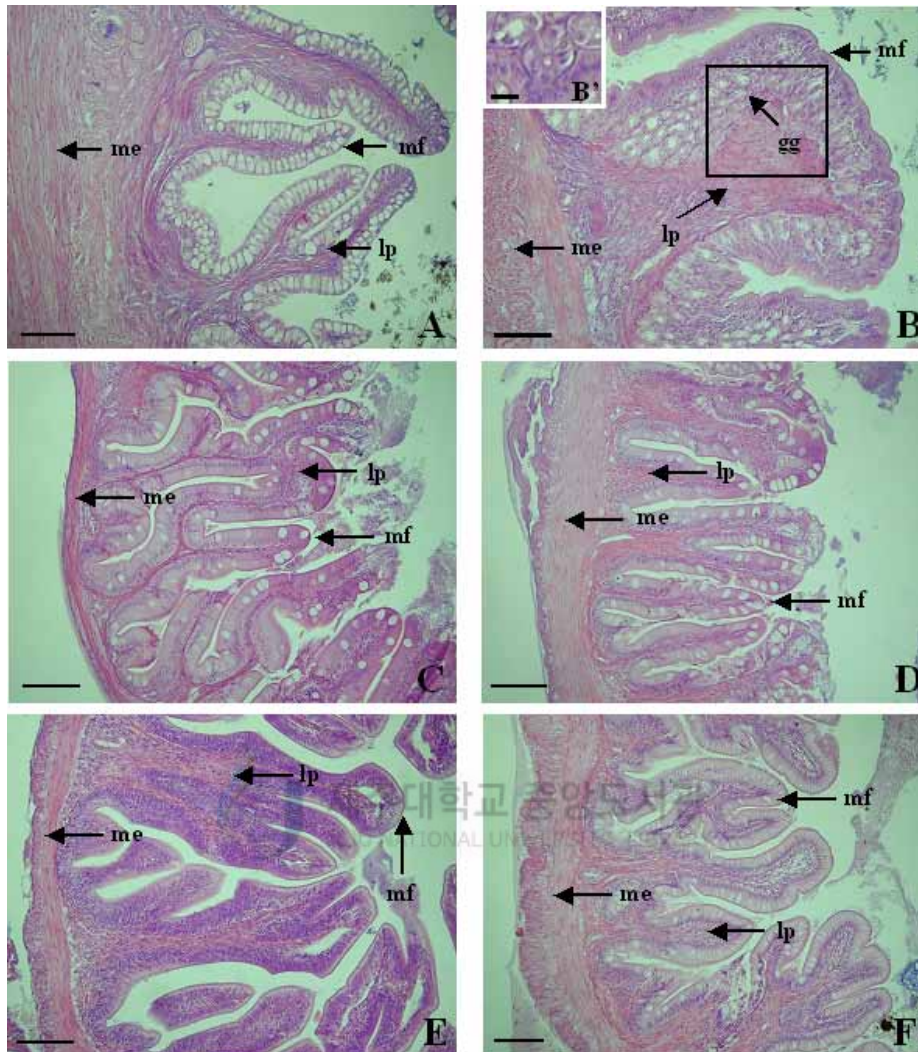


Fig. 6. Photomicrographs of cross section on the digestive tract of *R. giurinus*.

A: esophagus, B: stomach, C: anterior intestine portion, D: mid intestine portion, E: posterior intestine portion, F: rectum, gg: gastric gland, lp: lamina propria, me: muscularis externa, mf: mucosal fold. Scale bars indicate 50 μm . Magnified gastric gland in (B) was shown in (B'). B': Scale bar indicates 20 μm .

Table 2. Histological features of the digestive tract of the Gobiidae

	Muscularis externa thickness (μm)				Mucosal fold length (μm)			
	<i>S. geneionema</i>	<i>C. gulosus</i>	<i>T. obscurus</i>	<i>R. giurinus</i>	<i>S. geneionema</i>	<i>C. gulosus</i>	<i>T. obscurus</i>	<i>R. giurinus</i>
Esophagus	61.7 \pm 6.0	395.6 \pm 31.9	150.0 \pm 19.3	206.7 \pm 23.3	331.1 \pm 12.2	852.2 \pm 55.4	613.3 \pm 39.6	540.0 \pm 40.0
Stomach	.	233.3 \pm 42.9	150.0 \pm 14.9	122.5 \pm 8.2	.	1426.7 \pm 97.0	802.7 \pm 33.7	608.3 \pm 40.6
Anterior intestine portion	36.7 \pm 3.1	138.9 \pm 17.2	57.3 \pm 2.5	45.8 \pm 3.8	410.6 \pm 14.3	628.9 \pm 42.4	612.0 \pm 43.0	481.7 \pm 24.6
Mid intestine portion	36.7 \pm 4.3	123.3 \pm 13.8	75.8 \pm 9.6	49.2 \pm 2.9	328.9 \pm 14.3	443.3 \pm 54.8	486.7 \pm 31.3	390.0 \pm 14.7
Posterior intestine portion	52.2 \pm 11.6	158.9 \pm 11.1	85.0 \pm 8.7	48.3 \pm 4.4	298.9 \pm 20.5	288.9 \pm 27.4	536.7 \pm 28.8	433.3 \pm 18.1
Rectum	31.7 \pm 2.8	141.1 \pm 25.4	87.8 \pm 14.6	43.3 \pm 25.4	169.4 \pm 6.4	627.8 \pm 34.5	491.1 \pm 24.1	286.7 \pm 16.2

Values are mean \pm S.E.

“ . ” indicates stomachless.

3. Distribution and characteristics of Goblet cells

According to the location of the digestive tract and situation in that regions, different regional distributions and relative frequencies of goblet cells were observed. These differences are shown in Table 3 and Fig. 11.

1) Hairychin goby (*S. geneionema*)

The goblet cells of this fish were distributed throughout the entire digestive tract. Mucus-secreting goblet cells were detected in the esophagus (42 ± 26), anterior intestine portion (197 ± 28), mid intestine portion (271 ± 48), posterior intestine portion (339 ± 26) and rectum (250 ± 8) (Table 3). The greater abundance in the posterior intestine portion than in other portions of the digestive tract was significant ($P < 0.05$).

The goblet cells in the esophagus were mainly distributed within the epithelium and were spherical and oval (Fig. 7-A). Toward the posterior intestine portion, these cells were densely distributed and mainly oval (Fig. 7).

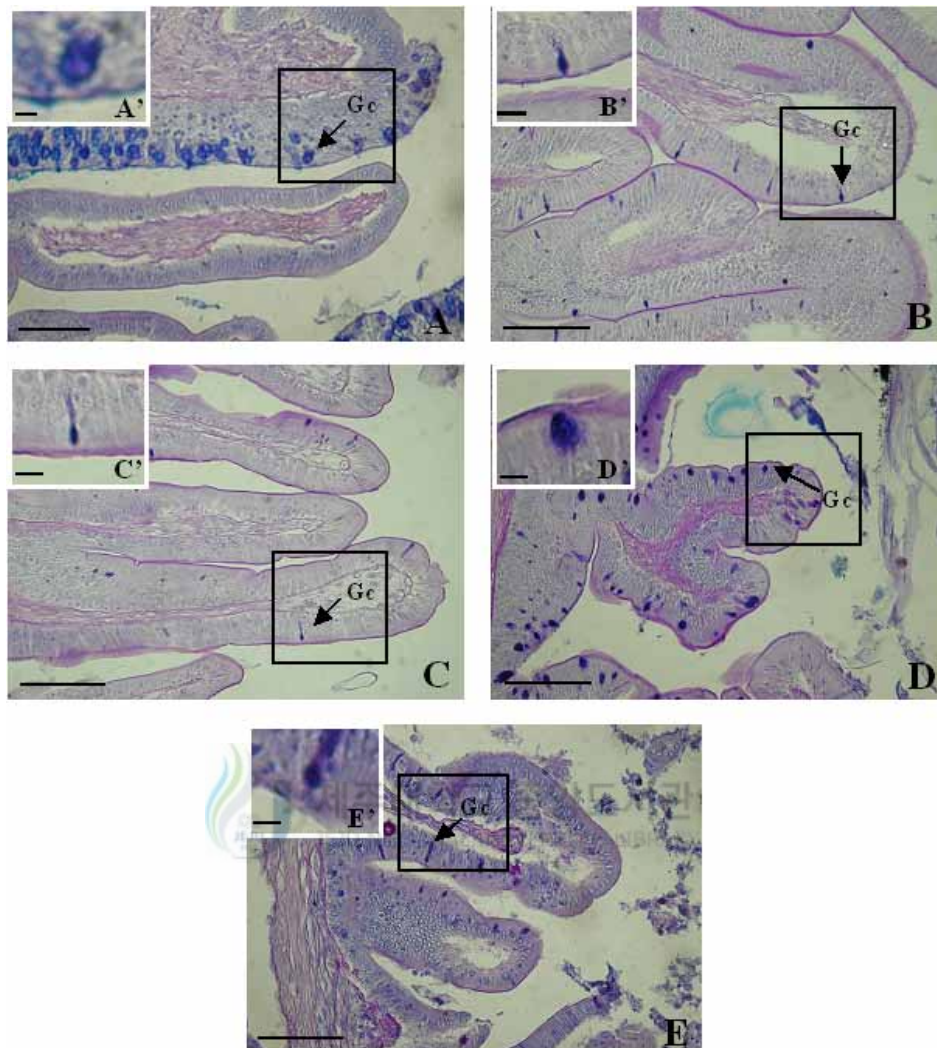


Fig. 7. Photomicrographs of goblet cells in the digestive tract of *S. geneionema*.
 A: esophagus, B: anterior intestine portion, C: mid intestine portion, D:
 posterior intestine portion, E: rectum, Gc: goblet cell. Scale bars indicate
 100 μm . Magnified goblet cells in (A-E) were shown in (A'-E'). Scale bars
 indicate 20 μm .

2) Gluttonous goby (*C. gulosus*)

Goblet cells were not detected in the stomach of *C. gulosus*. Mucus-secreting goblet cells were detected in the esophagus (1292±95), anterior intestine portion (873±126), mid intestine portion (1290±129), posterior intestine portion (784±49), and rectum (446±51) (Table 3). Thus, these cells were significantly more abundant in the esophagus and mid intestine portion ($P<0.05$) and decreased toward the rectum.

The goblet cells in the esophagus were mostly located in the epithelia of the mucosal folds, and were large and spherical (Fig. 8-A). In the other portions of the digestive tract, goblet cells were irregularly distributed from the upper to the lower parts of the mucosal folds (Fig. 8).



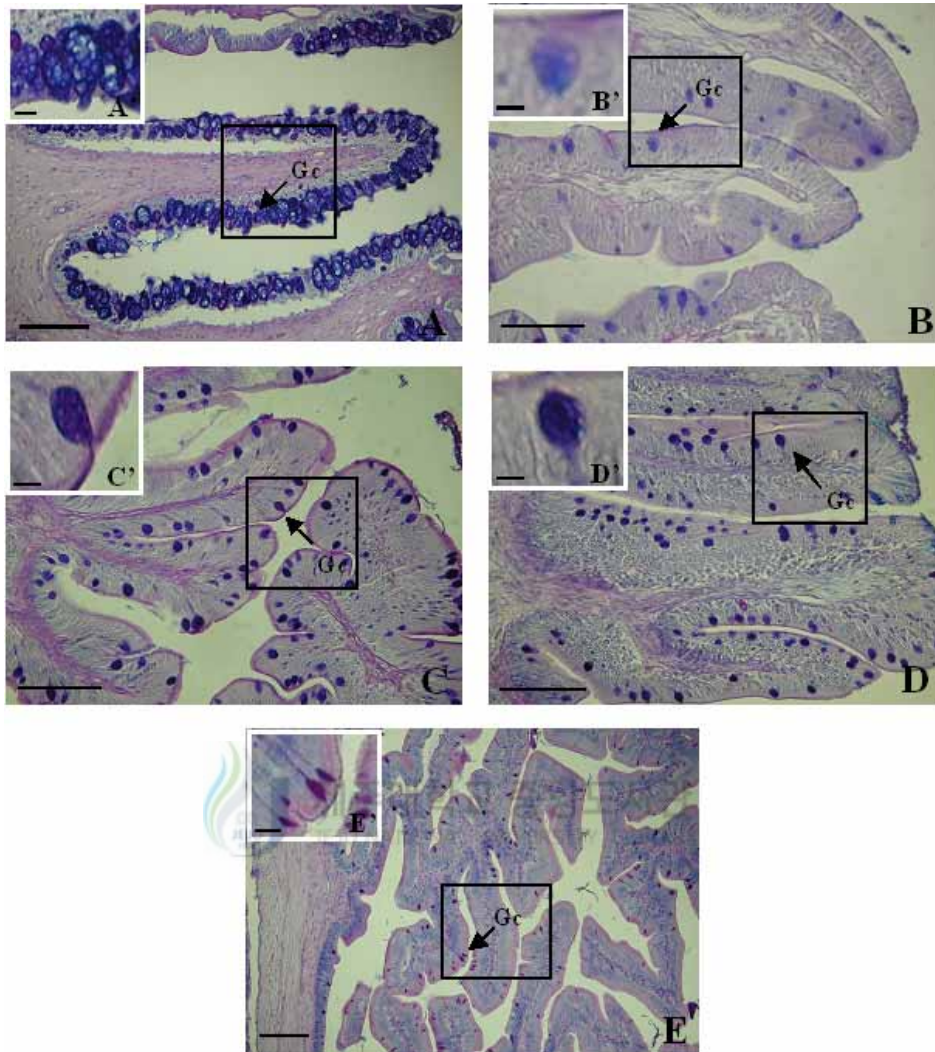


Fig. 8. Photomicrographs of goblet cells in the digestive tract of *C. gulosus*.

A: esophagus, B: anterior intestine portion, C: mid intestine portion, D: posterior intestine portion, E: rectum, Gc: goblet cell. Scale bars indicate 100 μm . Magnified goblet cells in (A-E) were shown in (A'-E'). Scale bars indicate 20 μm .

3) Trident goby (*T. obscurus*)

Goblet cells were not detected in the stomach of the trident goby. Mucus-secreting goblet cells were detected in the esophagus (994±154), anterior intestine portion (310±57), mid intestine portion (1402±131), posterior intestine portion (405±135), and rectum (368±41) (Table 3). They were significantly more abundant in the mid intestine portion ($P<0.05$) and decreased in number toward the rectum.

The goblet cells in the esophagus were densely distributed within the epithelium of the mucosal folds and were mainly large and oval (Fig. 9-A). In the other portions of the digestive tract, the cells were irregularly scattered from the upper to the lower parts of the mucosal folds and were spherical and oval (Fig. 9).



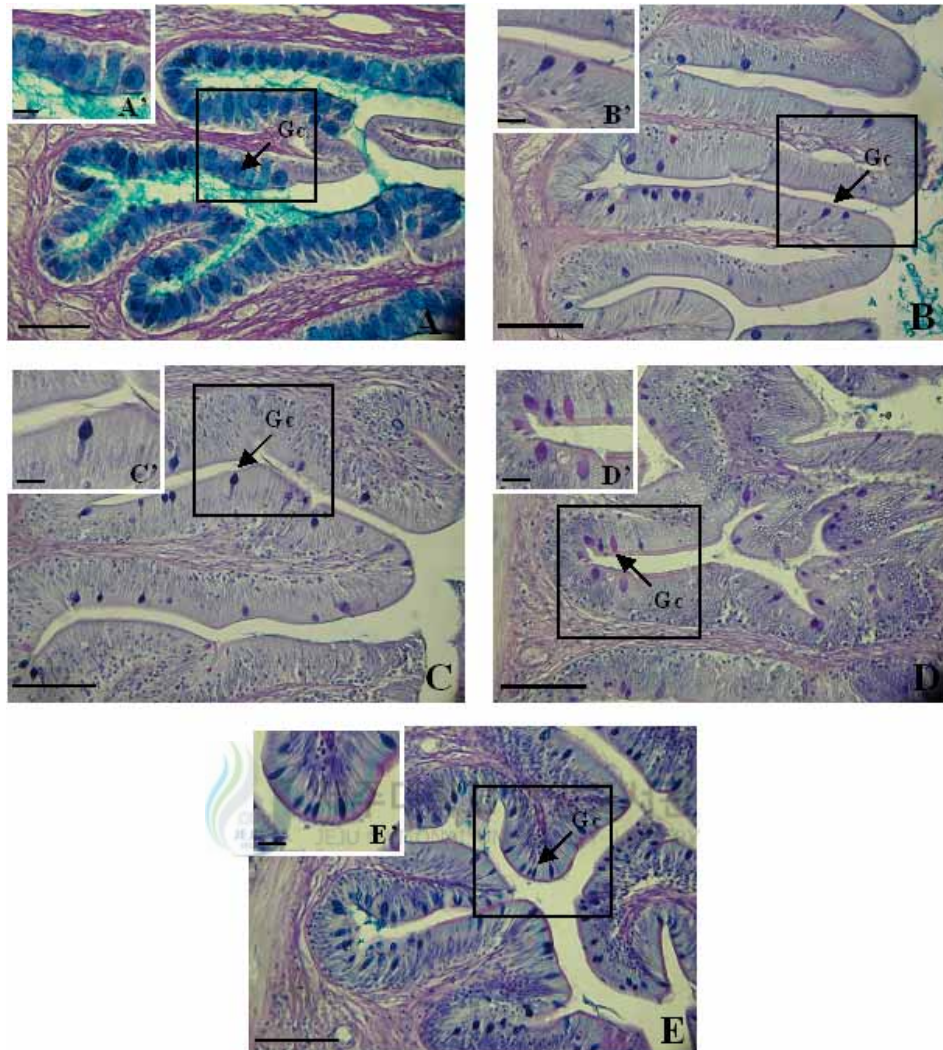


Fig. 9. Photomicrographs of goblet cells in the digestive tract of *T. obscurus*.

A: esophagus, B: anterior intestine portion, C: mid intestine portion, D: posterior intestine portion, E: rectum, Gc: goblet cell. Scale bars indicate 100 μm . Magnified goblet cells in (A-E) were shown in (A'-E'). Scale bars indicate 20 μm .

4) Giurine goby (*R. giurinus*)

Goblet cells were not detected in the stomach of the giurine goby. Mucus-secreting goblet cells were detected in the esophagus (920 ± 17), anterior intestine portion (641 ± 107), mid intestine portion (510 ± 42), posterior intestine portion (379 ± 101), and rectum (287 ± 55) (Table 3). They were significantly more abundant in the esophagus ($P < 0.05$). The number of goblet cells decreased toward the rectum.

The goblet cells in the esophagus were densely distributed and filled the lumen. They were large and spherical or oval (Fig. 10-A). From the anterior intestine portion to the mid intestine portion, the cells were mainly spherical and were distributed throughout the mucosal folds. From the posterior intestine portion to the rectum, mainly oval shaped goblet cells were observed; these were also distributed throughout the mucosal folds (Fig. 10).



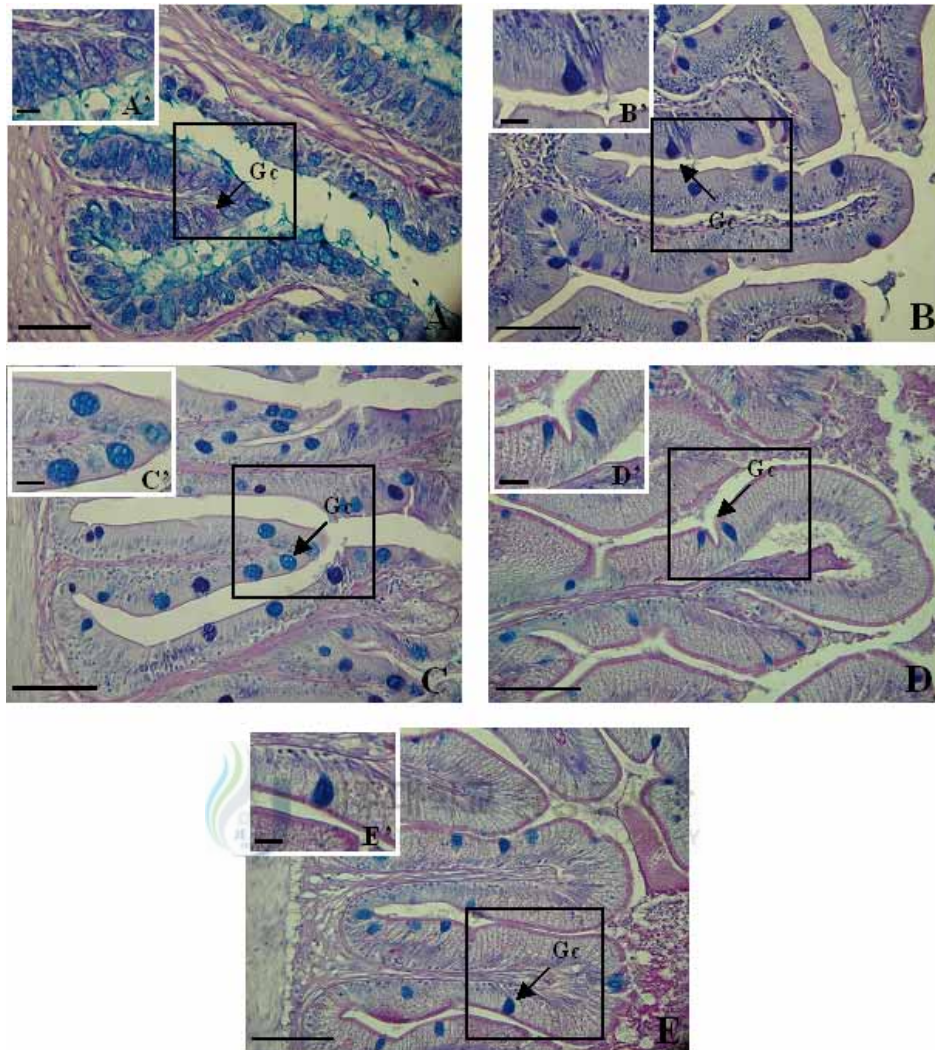


Fig. 10. Photomicrographs of goblet cells in the digestive tract of *R. giurinus*.

A: esophagus, B: anterior intestine portion, C: mid intestine portion, D: posterior intestine portion, E: rectum, Gc: goblet cell. Scale bars indicate 100 μm . Magnified goblet cells in (A-E) were shown in (A'-E'). Scale bars indicate 20 μm .

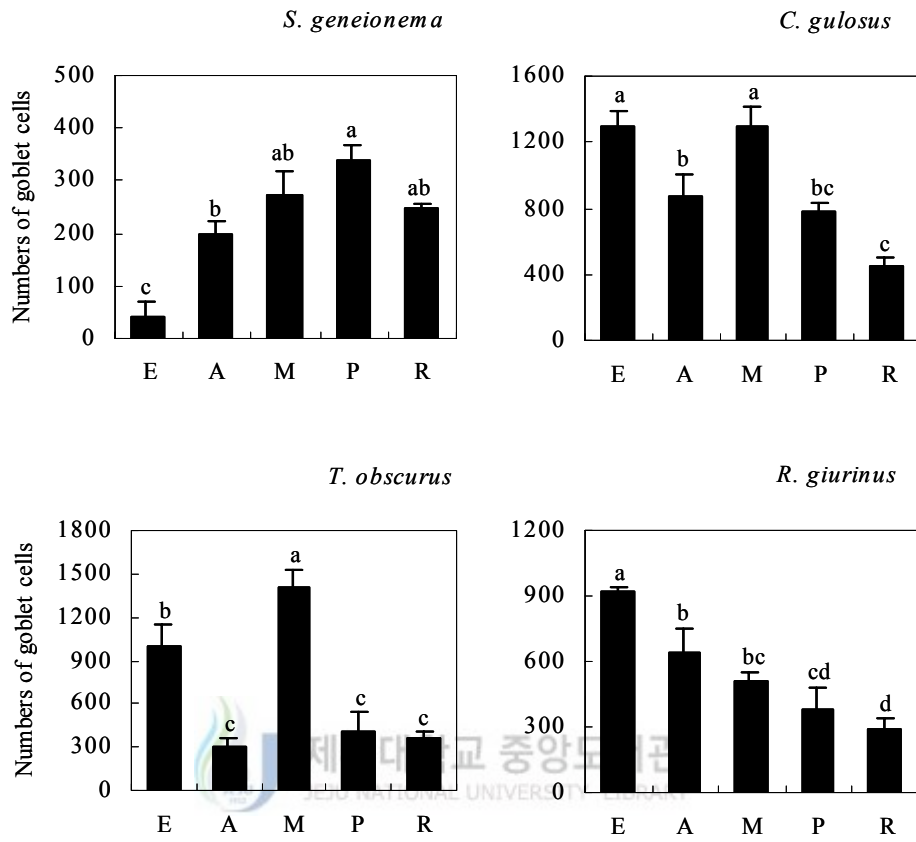


Fig. 11. Change of the goblet cell of the digestive tract of the Gobiidae.

A: anterior intestine portion, E: esophagus portion, M: mid intestine portion, P: posterior intestine portion, R: rectum portion. Vertical bars denote standard error of means. Different superscript on the bars are significantly different ($P < 0.05$).

Table 3. Number of goblet cells in the digestive tract of the Gobiidae

	Numbers of goblet cells/tissue section			
	<i>S. geneionema</i>	<i>C. gulosus</i>	<i>T. obscurus</i>	<i>R. giurinus</i>
Esophagus	42±26 ^c	1292±95 ^a	994±154 ^b	920±17 ^a
Stomach	.	None	None	None
Anterior intestine portion	197±28 ^b	873±126 ^b	310±57 ^e	641±107 ^b
Mid intestine portion	271±48 ^{ab}	1290±129 ^a	1402±131 ^a	510±42 ^{bc}
posterior intestine portion	339±26 ^a	784±49 ^{bc}	405±135 ^c	397±101 ^{cd}
Rectum	250±8 ^{ab}	446±51 ^e	368±41 ^e	287±55 ^d

Values are mean ± S.E. in same column superscripted with different letters are significantly different ($P<0.05$).
 “. . .” indicates stomachless.

4. Distribution and characteristics of CCK-IR cells

1) Hairychin goby (*S. geneionema*)

CCK-IR cells were not detected in the esophagus, but were found with various frequencies in the anterior intestine portion and extending to the rectum. The numbers of CCK-IR cells were recorded from anterior intestine portion (124 ± 27), mid intestine portion (30 ± 8), posterior intestine portion (8 ± 1) and rectum (4 ± 1) (Table 4). Thus, the highest frequency was observed in the anterior intestine portion, with decreasing frequencies toward the rectum ($P < 0.05$).

Spindle-shaped and a few spherical cells were dispersed among the epithelial cells. CCK-IR cells extended from the epithelium to the intestinal lumen, with their thin apexes pointing toward the lumen (Fig. 12).



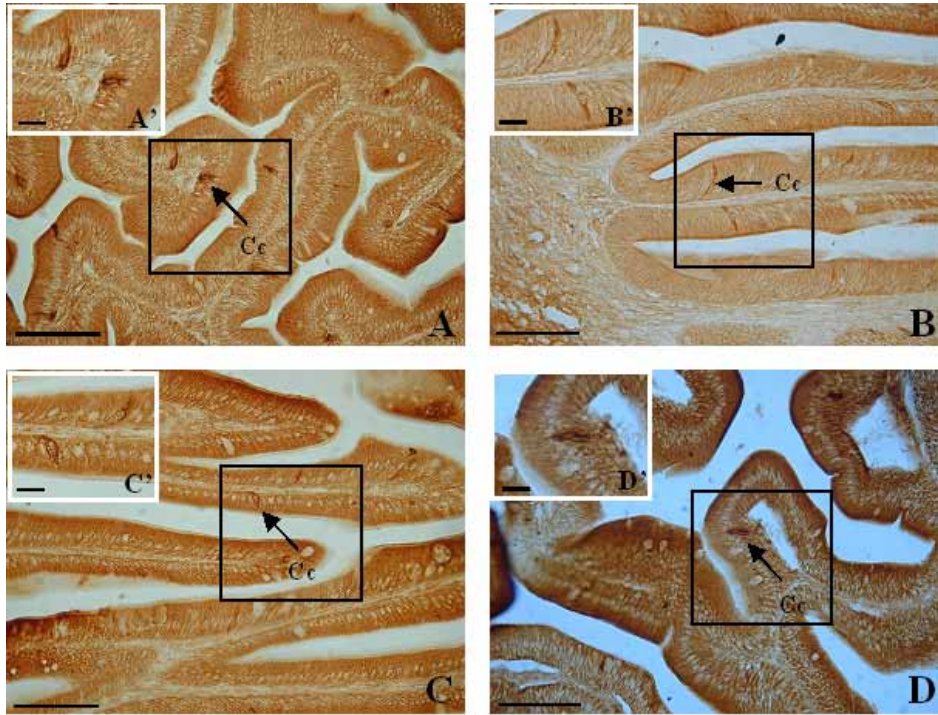


Fig. 12. Photomicrographs of CCK-IR cells in the digestive tract of *S. geneionema*.

A: anterior intestine portion, B: mid intestine portion, C: posterior intestine portion, D: rectum, Cc: CCK-IR cell. Scale bars indicate 50 μm . Magnified goblet cells in (A-D) were shown in (A'-D'). Scale bars indicate 20 μm .

2) Gluttonous goby (*C. gulosus*)

CCK-IR cells were observed from the anterior to the mid intestine portion. The numbers of CCK-IR cells were recorded from anterior intestine portion (175 ± 5), mid intestine portion (38 ± 26) (Table 4). Thus, from the anterior intestine portion to the mid intestine portion, the frequency of CCK-IR cells rapidly decreased ($P < 0.05$).

Spindle-shaped cells and a few spherical and oval cells were dispersed among the epithelial cells of the mucosal folds. CCK-IR cells extended from the epithelium to the lumen of the intestine, with their thin apices pointing toward the lumen (Fig. 13).

3) Tridint goby (*T. obscurus*)

CCK-IR cells were observed in the anterior and mid intestine portion. The numbers of CCK-IR cells were recorded from anterior intestine portion (26 ± 15), mid intestine portion (23 ± 10) (Table 4). Thus, CCK-IR cells were scattered throughout the anterior and mid intestine portion.

Spindle shaped cells and a few spherical cells were dispersed among the epithelial cells of the mucosal folds (Fig. 14), but the frequencies were less than in *S. geneionema* and *C. gulosus*.

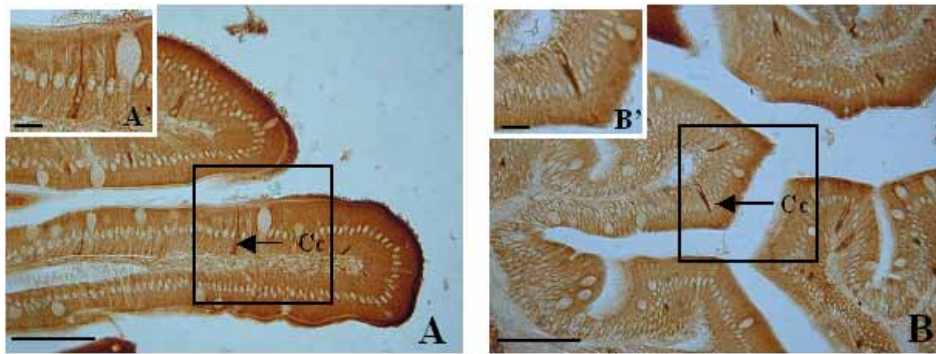


Fig. 13. Photomicrographs of CCK-IR cells in the digestive tract of *C. gulosus*.

A: anterior intestine portion, B: mid intestine portion, Cc: CCK-IR cell.
 Scale bars indicate 50 μm . Magnified CCK-IR cells in (A-B) were shown
 in (A'-B'). Scale bars indicate 20 μm .

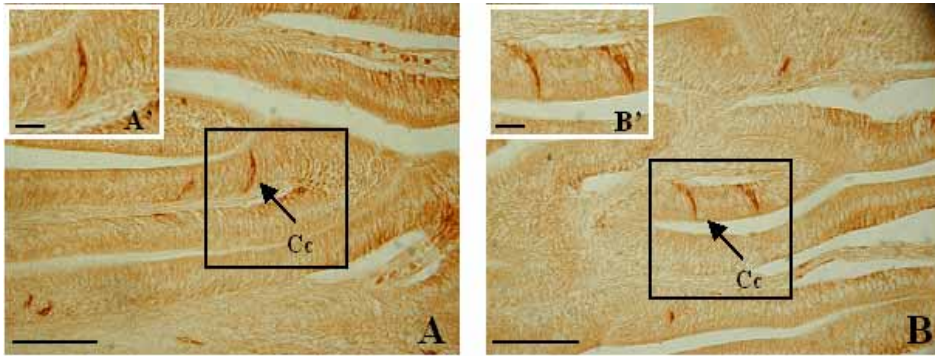


Fig. 14. Photomicrographs of CCK-IR cells in the digestive tract of *T. obscurus*.

A: anterior intestine portion, B: mid intestine portion, Cc: CCK-IR cell.
Scale bars indicate 50 μm . Magnified CCK-IR cells in (A-B) were shown
in (A'-B'). Scale bars indicate 20 μm .

4) Giurine goby (*R. giurinus*)

CCK-IR cells were observed only in the anterior and mid intestine portion. The numbers of CCK-IR cells were recorded from anterior intestine portion (83 ± 23), mid intestine portion (10 ± 4) (Table 4). Thus, compared to the anterior intestine portion, the frequency of CCK-IR cells in the mid intestine portion was greatly decreased ($P < 0.05$).

Spindle cells were dispersed among the epithelial cells of the mucosal folds (Fig. 15). CCK-IR cells extended from the epithelium to the lumen but were less abundant than in *S. geneionema* and *C. gulosus*.





Fig. 15. Photomicrographs of CCK-IR cells in the digestive tract of *R. giurinus*.

A: anterior intestine portion, B: mid intestine portion, Cc: CCK-IR cell.
Scale bars indicate 50 μm . Magnified CCK-IR cells in (A-B) were shown
(A'-B'). Scale bars indicate 20 μm .

Table 4. Number of CCK-IR cells in the digestive tract of the Gobiidae

	Numbers of CCK-IR cells/tissue section			
	<i>S. geneionema</i>	<i>C. gulosus</i>	<i>T. obscurus</i>	<i>R. giurinus</i>
Esophagus	None	None	None	None
Stomach	.	None	None	None
Anterior intestine portion	124±27 ^a	175±5 ^a	26±15	83±23 ^a
Mid intestine portion	30±8 ^b	38±26 ^b	23±10	10±4 ^b
posterior intestine portion	8±1 ^b	None	None	None
Rectum	4±1 ^b	None	None	None

Values are mean ± S.E. in same column superscripted with different letters are significantly different ($P<0.05$).
 “. ” indicates stomachless.

Table 5. Morphological and histochemical features of the digestive tract of the Gobiidae

	Stomach		Intestine		
	Degree of development	Mucosal fold	Form	Goblet cell	CCK-IR cell
<i>S. geneionema</i>	stomachless		regular branch	posterior intestine	whole intestine
<i>C. gulosus</i>	well developed lamina propria and gastric gland		complex branch	mid intestine	anterior and mid intestine
<i>T. obscurus</i>	well developed muscularis externa		regular branch	mid intestine	anterior and mid intestine
<i>R. giurinus</i>	well developed lamina propria and gastric gland		regular branch	anterior intestine	anterior and mid intestine

IV. Discussion

In the fish digestive system, the length of the digestive tract is closely related to the feeding habits of a species. Carnivorous fish have a short digestive tract. For example, in *Anguilla japonica*, RLG is 0.46 and *Gobio gobio* is 0.68-0.85. The RLG of carnivores is less than 1. In omnivorous fishes, the digestive tract is longer, e.g., *Rutilus rutilus*, RLG is 1.0-1.09 and *Cyprinus carpio* is 1.85. The RLG of omnivorous fish is between 1 and 2. In contrast, the digestive tract of herbivorous fish is much longer. *Sarotherodon mossambicus*, RLG is 6.29 and *Chanos chanos* is 8.5. RLG of herbivorous fish ranges from 2 to more than 5 (Ferraris *et al.*, 1987; Hsu and Wu, 1979; Takeuchi, 1991).

In this study, the ingesta of *S. geneionema*, a stomachless species, consisted mostly of sand grains and small shrimps; its RLG was 0.45, which is typical of carnivorous fish. The stomach contents of *C. gulosus* were mostly small shrimps, crabs, and a small amount of green algae. Its RLG was 0.43 consistent with it being a carnivorous fish. The RLG of *T. obscurus*, which inhabits brackish water, was 0.66 and was thus also in the range of RLG of carnivorous fish. However, examination of its stomach contents, which consisted of green algae and lugworms, suggested that this species is omnivorous. The RLG of *R. giurinus* was 0.42, consistent with it being carnivorous. The stomach contents of this species consisted mainly of lugworms. Despite these findings, information regarding the feeding habits of these four species remains incomplete, and further investigations into their modes of food ingestion and the contents of their stomachs and intestines are needed.

In teleosts, digestive activity in the stomach can be classified as being either physical or chemical. Physical action depends on the degree of development of the muscularis externa and lamina propria of the stomach, while chemical action depends on the development of the gastric glands (Lee *et al.*, 1998; Lee and Chin, 1999). In fish whose lamina propria is much less well developed than the muscularis externa, large quantities of smooth food can be digested. In contrast, in fish whose muscularis externa is much less well developed than the

lamina propria, the food must be hard and can be digested only in small amounts (Lee and Chin, 1999). The muscularis externa of the stomach of *C. gulosus* is only slightly developed while the lamina propria is well developed and includes abundant gastric glands, which carry out chemical digestion. In addition, when the stomach was cut open and its contents examined, shrimps and crabs were found. The structure of the stomach of *C. gulosus* allows this fish to digest hard food in small amounts. In contrast, the gastric glands and lamina propria of *T. obscurus* are less well-developed than the muscularis externa, and the stomach of this species contained green algae and lugworms. Unlike in *C. gulosus*, the structure of the stomach of *T. obscurus* allows the digestion of large amounts of smooth food. In *R. giurinus*, the muscularis externa and lamina propria are somewhat developed whereas the gastric glands are more developed than those of *T. obscurus*. The stomach contents of *R. giurinus* consisted of lugworms, and its structure resembled that of *C. gulosus*, indicating that *R. giurinus* is able to digest hard food in small quantities.

The digestive tracts of different fish species vary in morphology and structure. Analyses of the digestive systems of several species of fish have been conducted, including mummichog, *Fundulus heteroclitus* (Babkin and Bowie, 1928); flounder, *Platichthys flesus* (Jenkins *et al.*, 1992); and Nile tilapia, *Oreochromis niloticus* (Morrison and Wright, 1999). Few studies have investigated the families Atherinidae, Blenniidae, Cobitidae, Cyprinidae, Gobiidae, Mugilidae, and Scaridae. In addition, the digestion and absorption properties of the digestive tracts of several stomachless fish belonging to the family Labridae have been studied (Al-Hussaini, 1947, 1949; Barrington, 1942; Chao, 1973; Ishida, 1936; Szarski, 1965).

The digestive tracts of most teleosts consist of an oral cavity, pharynx, stomach, intestine, rectum, and anus. Some Cypinidae and Gobiidae lack a stomach and pyloric caeca (Tanaka, 1969). We histologically examined *S. geneionema*, which has neither a stomach nor pyloric caeca, and *C. gulosus*, *T. obscurus*, and *R. giurinus*, which also do not have pyloric caeca. The muscularis externa of *S. geneionema* is thinner and the mucosal folds are shorter compared to the other species of fish investigated in this study.

The goblet cells in the fish digestive tract produce a lubricant for the mucosal surface,

which prevents the mucous membrane from being damaged by physical or chemical action and protects it from the actions of digestive enzymes (Allen *et al.*, 1986). Goblet cells in the digestive tracts of several species of fish have been studied with respect to mucus secretion: *Konosirus punctatus*, *Carassius carassius*, *Parasilurus asotus*, *Thamnaconus modestus*, and *P. majors* (Jo *et al.*, 1984). The histochemical characteristics of intestinal mucus-secreting cells have been analyzed in *Acanthopagrus schlegelii*, *Erosa erosa*, and *Sebastes inermis* (Byeon and Jo, 1985), as well as in *Pleuronichthys cornutus*, *Paralichthys olivaceus*, *Acanthogobius hasta*, *Zoarcetes gillii*, and *Lagocephalus wheeleri* (Choi, 1996). According to these studies, the shapes and sizes of the goblet cells in the digestive tract depend on the species of fish and the intestinal locations of the cells. The amounts of mucin also depend on the species of fish and the particular region of the digestive tract, with the number of goblet cells generally increasing posteriorly (Reifel and Travill, 1979). Lee and Chin (1995) reported that the number of goblet cells of *D. temmincki*, a stomachless fish, increased toward the posterior portion of the intestine and the rectum. Similarly in *S. geneionema*, which is also a stomachless fish, the numbers of AB-PAS positive goblet cells increased from the anterior toward the posterior portion. In terms of the shape of these cells, the front and middle basal parts are slim and the long back part is oval and rounded. In *C. gulosus*, the goblet cells, which are AB-PAS positive, are most abundant and most densely arranged in the mid intestine, and their shape ranges from largely oval to round. Like *C. gulosus*, the goblet cells of *T. obscurus* are AB-PAS positive and are distributed mostly in the mid intestine; the number of cells decreases toward the rectum. The cells are small and oval. In contrast to the goblet cells of *S. geneionema*, those of *R. giurinus* are distributed mostly in the vicinity of the esophagus and decrease in number toward the rectum. The cells are AB-PAS positive and are rounded and oval. Thus, it appears that the distribution of goblet cells and their various forms depends on the type of food consumed and the species of fish; however, further and more detailed studies regarding the physiological mechanisms are needed.

In *S. geneionema* and *C. gulosus*, which live in seawater, their digestive action occurs in

the posterior intestine portion and the mid intestine portion to protected and functions to activate digestion. In contrast, in *T. obscurus* and *R. giurinus*, which are brackish water fish, their digestive action occurs in the mid intestine portion and the anterior portion to protected and functions to activate digestion. Further studies of the modes of food ingestion by these fish, the contents of their digestive tracts, and the staining characteristics of the goblet cells need to be carried out.

In fish, several intestinal peptide hormones have been identified, mainly by means of immunohistochemical techniques (Fritsch *et al.*, 1978; Noalillac-Depeyre and Hollande, 1981; Holmgren *et al.*, 1982; El-Salhy, 1984a; El-Salhy, 1984b; Hansen *et al.*, 1987; Garcia-Hernandez *et al.*, 1994; Reinecke *et al.*, 1997; Kurokawa *et al.*, 2000). Hormonal factors seem to play important roles in the intestine of vertebrates, including fish (Lebenthal and Lebenthal, 1999). Cholecystokinin (CCK) is a major hormone controlling digestion and participates in the regulation of food intake and satiety (Peyon *et al.*, 1997), as well as having stimulatory effects on pancreatic secretion (Bell, 1979; Lhosted *et al.*, 1994; Biederbick and Elsasser, 1998). CCK is produced by endocrine cells scattered among the epithelial cells lining the intestine and, upon exposure to the appropriate food signals in the intestinal lumen, is released into the circulation (Liddle, 1997). In teleosts, CCK has been shown to retard gastric emptying (Olsson *et al.*, 1999), regulate the secretion of pancreatic enzymes, stimulate gall bladder contractions *in vitro* and *in vivo*, and regulate intestinal peristalsis (Aldman and Holmgren, 1987; Einarsson *et al.*, 1997; Rajjo *et al.*, 1988; Andrew and Young, 1988; Aldman *et al.*, 1992). Most studies, however, have focused on the timing of CCK expression and its distribution pattern in other species of fish, e.g., olive flounder (*P. olivaceus*), Atlantic halibut (*Hippoglossus hippoglossus*), and bluefin tuna (*Thunnus thynnus*), whose CCK-IR cells are found only in the anterior midgut, or in the early life stages of teleosts (Kurogawa *et al.*, 2000; Kamisaka *et al.*, 2001; Kamisaka *et al.*, 2002). In ayu, *Plecoglossus altivelis*, and Atlantic cod, *Gadus morhua*, CCK-IR cells are distributed as far as the posterior intestine (JÖsson *et al.*, 1987; Kamisaka *et al.*, 2003). In our study, CCK-IR cells in the digestive tract of the Gobiidae were found to differ remarkably in their

regional distributions and relative frequencies. In *C. gulosus*, *T. obscurus*, and *R. giurinus*, these cells were only observed in the anterior and mid intestine portion. This distribution pattern is quite similar to that of olive flounder, *P. olivaceus*, Atlantic halibut, *H. hippoglossus*, and bluefin tuna, *T. thynnus*. In contrast, in the stomachless teleost *S. geneionema*, CCK-IR cells were observed throughout the digestive tract. This is also the case in ayu, *P. altivelis* (Kamisaka *et al.*, 2003). Furthermore, in *S. geneionema*, the highest frequency of CCK-IR cells was observed in the anterior intestine, with a small number of cells distributed as far as the rectum. This result suggests that food digested by acid in the stomach moves on and is then delayed in the anterior and mid intestine, thereby ensuring effective stimulation of the CCK-producing cells. Also, the distribution pattern of CCK-IR cells closely resembles that of goblet cells. In stomachless teleosts, ingested food easily passes through the digestive tract until it reaches the posterior intestine portion. The distribution pattern of CCK-IR cells in *S. geneionema*, for example, seems to be well adapted to promoting optimal control of the digestive process. Our study suggests that CCK-IR cells are distributed effectively to make up for the effects of the lack of a stomach on the digestive process.



V. Summary

The morphology, histology, and histochemical characteristics of the digestive tract of four species of the Gobiidae, the hairychin goby (*Sagamia geneionema*), gluttonous goby (*Chasmichthys gulosus*), trident goby (*Tridentiger obscurus*), and guirine goby (*Rhinogobius giurinus*), collected from the coastal area of Jeju Island, Korea, were investigated.

S. geneionema, which is a seawater species, has a short (RLG = 0.45), simple digestive tract, and lacks both a stomach and pyloric caeca. The mucosal folds are regularly branched and the muscularis externa is thickest in the esophagus. The numbers of mucus-secreting goblet cells is highest in the posterior intestine portion ($P < 0.05$), while CCK-IR cells are scattered throughout the intestine.

C. gulosus is also a seawater species. It has a short (RLG = 0.43), simple gut and stomach, but pyloric caeca are not observed. In the stomach, the lamina propria is well developed and contains many gastric glands. The mucosal folds have a complex branched form, and the muscularis externa is thickest in the esophagus. Mucus-secreting goblet cells are most abundant in the mid intestine portion ($P < 0.05$), whereas CCK-IR cells are observed only in the anterior and mid intestine portion.

The digestive tract of *T. obscurus*, which is a brackish water species, is longer (RLG = 0.66) than that of the other species studied. This fish has a simple stomach but pyloric caeca are absent. The mucosal folds are regularly branched and the muscularis externa is thickest in the esophagus. Mucus-secreting goblet cells were most abundant in the mid intestine portion ($P < 0.05$). CCK-IR cells are found in the anterior and mid intestine portion.

R. giurinus, which is also a brackish water species, has a short (RLG = 0.42), simple, and narrow gut. The gastric glands are well developed in the stomach, but pyloric caeca are absent. The mucosal folds are regularly branched, and, as in the other species, the muscularis externa is thickest in the esophagus, which also contained the largest number of mucus-secreting goblet cells ($P < 0.05$). CCK-IR cells are present only in the anterior and mid

intestine portion.

In *S. geneionema* and *C. gulosus*, which live in seawater, their digestive action occurs in the posterior intestine portion and the mid intestine portion to protected and functions to activate digestion. In contrast, in *T. obscurus* and *R. giurinus*, which are brackish water fish, their digestive action occurs in the mid intestine portion and the anterior portion to protected and functions to activate digestion. Further studies of the modes of food ingestion by these fish, the contents of their digestive tracts, and the staining characteristics of the goblet cells need to be carried out.



VI. References

- Aldman, G. and S. Holmgren. 1987. Control of gall bladder motility in the rainbow trout, *Salmo gairdneri*. Fish Physiol. Biochem., 4: 143-155.
- Aldman, G., D. Grove and S. Holmgren. 1992. Duodenal acidification and intra-arterial injection of CCK8 increase gallbladder motility in the rainbow trout, *Oncorhynchus mykiss*. Gen. Comp. Endocrinol., 86: 20-25.
- Al-Hussaini, A.H. 1947. The feeding habits and the morphology of the alimentary tract of some teleost living in the neighbourhood of the Marine Biological Station, Ghardaqa, Red Sea. Publ. Mar. Biol. Stn. Al-Ghardaqa, 5: 1-61.
- Al-Hussaini, A.H. 1949. On the functional morphology of the alimentary tract of some fish in the relation to differences in their feeding habits: anatomy and histology. Q. J. Microsc. Sci., 90: 109-139.
- Allen, A., D.A. Hutton, A.J. Leonard, J.P. Pearson and L.A. Sellers. 1986. The role of mucus in the protection of the gastroduodenal mucosa. Scand J. Gastroenterol, 21(suppl. 125): 71-77.
- Andrew, P.O.L.R. and J.Z. Young. 1988. The effect of peptides on the motility of the stomach intestine, intestine and rectum in the skate (*Raja*). Comp. Biochem. Physiol., C 89: 343-348.
- Babkin, B.P. and D.J. Bowie. 1928. The digestive system and its function in *Fundulus heteroclitus*. Biol. Bull., 54: 254-277.
- Barrington, E.J.W. 1942. Gastric digestion in the lower vertebrates. Biol. Rev., 17: 1-27.
- Biederbick, A. and H.P. Elsässer. 1998. Diurnal pattern of rat pancreatic acinar cell replication. Cell Tiss. Res., 291: 277-283.
- Bell, F.R. 1979. The relevance of the new knowledge of gastrointestinal hormones to veterinary science. Vet. Sci. Commun., 2: 305-314.

- Byeon, K.A. and U.B. Cho. 1985. Histochemical properties on mucosubstances of the intestine in *Sparus swinhonis* (Günther), *Erosa erosa* (Langsdorf) and *Sebastes inermis* (Cuvier et Valenciennes). Journal of Science, Pusan National University, 40: 251-269. (in Korean)
- Chao, L.N. 1973. Digestive system and feeding habits of the cunner, *Tautoglabrus adspersus*, a stomachless fish. Fish. Bull., 71: 565-586.
- Choi, J.S. 1996. Histochemical studies of mucosubstances in the intestine of *Pleuronichthys cornutus*, *Paralichthys olivaceus*, *Acanthogobius hasta*, *Zoarces gillii* and *Lagocephalus wheeleri*. MS. Thesis, Pusan National University, pp. 5-24. (in Korean)
- Einarsson, S., P.S. Davies and C. Talbot. 1997. Effect of exogenous cholecystokinin on the discharge of the gallbladder and the secretion of trypsin and chymotrypsin from the pancreas of the Atlantic salmon, *Salmo salar* L. Comp. Biochem. Physiol., 117C: 63-67
- El-Salhy, M. 1984a. Occurrence of polypeptide YY(PYY) and pancreatic polypeptide (PP) in the gastrointestinal tract of the bony fish. Biom Res., 5: 441-444.
- El-Salhy, M. 1984b. Immunocytochemical investigation of the gastroenteropancreatic (GEP) neurohormonal peptides in the pancreas and gastrointestinal tract of the dogfish, *Squalus acanthias*. Histochemistry. 80: 192-205.
- Ferraris, R.P., J.D. Tan and M.C. De La Cruz. 1987. Development of the digestive tract of milk fish *Chanos chanos* (Forsskaal): Histology and histochemistry. Aquaculture, 61: 241-257.
- Fritsch H.A.R., S.V. Noorden and A.G.E. Pearse. 1978. Localization of somatostatin and gastrin-like immunoreactivity in the gastrointestinal of *Ciona intestinalis* L. Cell Tiss. Res., 186: 181-185.
- Garcia-Hernandez, M.P., M.T. Lozano and B. Agulleiro. 1994. Ontogeny of some endocrine cells of the digestive tract in sea bass (*Dicentrarchus labrax*): An immunocytochemical study. Cell Tiss. Res., 277: 373-383.

- Hansen, G.N., B.L. Hansen and P.N. Jorgensen. 1987. Insulin-, glucagon-, and somatostatin-like immunoreactivity in the endocrine pancreas of the lungfish, *Neoceratodus forsteri*. *Cell Tiss. Res.*, 248: 181-185.
- Holmgren, S., C. Vaillant and R. Dimanline. 1982. VIP-, substance P-, gastrin/CCK-, bombesin-, somatostatin- and glucagon-like immunoreactivities in the gut of the rainbow trout. *Cell Tiss. Res.*, 223: 141-153.
- Hsu, Y.L. and J.L. Wu. 1979. The relationship between feeding habits and digestive protease of some freshwater fishes. *Bull. Inst. Zool., Acad. Sin.*, 18: 45-53.
- Hsu, S.M., L. Raine and H. Fanger. 1981. Use of Avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. *J. Histochem.*, 29: 577-580.
- Ishida, J. 1936. Distribution of the digestive enzymes in the digestive system of the stomachless fishes. *Annot. Zool. Jap.*, 15: 263-284.
- Jenkins, P.G., A.L. Pulsford and J.E. Harris. 1992. Microscopy of the absorptive cells and gut associated lymphoid tissue of the flounder *Platichthys flesus*. *J. Mar. Biol. Ass. UK*, 72: 553-567.
- Jo, U.B., B.S. Kim., I.J. Chai., S.Y. Back and I.S. Shin. 1984. Histochemical properties of mucosubstances on the intestinal mucous cells in the teleosts. *Journal of Science, Pusan National University*, 37: 1-15. (in Korean)
- Jönsson, A.C., S. Holmgren and B. Holstein. 1987. Gastrin/CCK-like immunoreactivity in endocrine cells and nerves in the gastrointestinal tract of the cod, *Gadus morhua*, and the effect of peptides of the gastrin/CCK family on cod gastrointestinal smooth muscle. *Gen. Comp. Endocrinol.*, 66: 190-202.
- Kamisaka, Y., G.K. Totland, M. Tagawa, M. Tanaka and I. Rønnestad. 2001. Ontogeny of cholecystokinin (CCK)-immunoreactive cells in the digestive tract of Atlantic halibut, *Hippoglossus hippoglossus*, larvae. *Gen. Comp. Endocrinol.*, 123: 31-37.
- Kamisaka, Y., T. Masuma, M. Kurokawa, T. Suzuki, G.K. Totland, I. Rønnestad, M.

- Tagawa and M. Tanaka. 2002. Ontogeny of cholecystokinin-immunoreactive cells in the digestive tract of blue fin tuna, *Thunnus thynnus*, larvae. *Sarsia.*, 87: 258-262.
- Kamisaka. Y., Y. Fujii, S. Yamamoto, T. Kurokawa, I. Rønnestad, G.K. Totland, M. Tagawa and M. Tanaka. 2003. Distribution of cholecystokinin-immunoreactive cells in the digestive tract of the larval teleost, ayu, *Plecoglossus altivelis*. *Gen. Comp. Endocrinol.*, 134: 116-121.
- Kim, I.S., Y.J. Lee and Y.U. Kim. 1986. Synopsis of the Family Gobiidae (Pisces, Perciformes) from Korea. *Bull. Korean Fish. Soc.*, 19(4): 387-403. (in Korean)
- Kurokawa, T., T. Suzuki and T. Andoh. 2000. Development of cholecystokinin and pancreatic polypeptide endocrine system during the larval stage of Japanese flounder, *Paralichthys olivaceus*. *Gen. Comp. Endocrinol.*, 120: 8-16
- Lebenthal, A. and E. Lebenthal. 1999. The ontogeny of the small intestine epithelium. *J. Paren. Enter. Nutri.*, 23(5): 3-6.
- Lee, J.S., K.S. Jeong and S.H. Huh. 1998. Internal morphology and histochemistry of the digestive tract in the spotted sea bass, *Lateolarax* sp., *Bull Fish. Sci. Inst. Yosu Nat'l Univ.*, 7: 105-113. (in Korean)
- Lee, J.S. and P. Chin. 1995. Morphology and histochemical characteristics of the alimentary tract in surfperch, *Ditrema Temmincki*. *Korean J. Ichthyol.*, 7: 140-149. (in Korean)
- Lee, J.S. and P. Chin. 1999. Morphology and histology of the digestive tract of the black sea bream, *Acanthopagrus schlegeli*. *J. Korean Fish. Soc.*, 32(5): 642-648. (in Korean)
- Lhosted, E.F., M. Fiszlewicz, A.M. Gueugneau, T. Tranchant and T. Corring. 1994. Early adaptation of pancreas to a protein-enriched diet: Role of cholecystokinin and gastrin-releasing peptide. *Pancreas.*, 9: 624-632.
- Liddle, R.A. 1997. Cholecystokinin cells. *Annu. Rev. Physiol.*, 59: 221-242.
- Morrison, C.M. and J. R. Wright Jr. 1999. A study of the histology of the digestive

- tract of the Nile tilapia. *J. Fish Biol.*, 54: 597-606.
- Nelson, J.S. 1984. *Fishes of the World* (2nd). John Wiley & Sons. 523 pp.
- Noaillac-Depeyre, J. and E. Hollande. 1981. Evidence for somatostatin, gastrin and pancreatic polypeptide-like substance in the mucosa cells of the gut in fishes and without stomach. *Cell Tiss. Res.*, 216: 193-203.
- Olsson, C., G. Aldman, A. Larsson and S. Holmgren. 1999. Cholecystokinin affects gastric emptying and stomach motility in the rainbow trout, *Oncorhynchus mykiss*. *J. Exp. Biol.*, 202: 161-170.
- Peyon, P., X.W. Lin, B.A. Himick and R.E. Peter. 1997. Molecular cloning and expression of cDNA encoding brain precholecystokinin in goldfish. *Peptides.*, 19: 199-210.
- Rajjo, I.M., S.R. Vigna and J.W. Crim. 1988. Actions of cholecystokinin-related peptides on the gall bladder of bony fishes *in vitro*. *Comp. Biochem. Physiol.*, C90: 267-273
- Reifel, C.W. and A.A. Travill. 1979. Structure and carbohydrate histochemistry of the intestine in ten teleostean species. *J. Morph.*, 162: 343-360.
- Reinecke, M., C. Muller and H. Segner. 1997. An immunohistochemical analysis of the ontogeny, distribution and coexistence of 12 regulatory peptides and serotonin in endocrine cells and nerve fibers of the digestive tract of the turbot, *Scophthalmus maximus* (teleostei). *Anat. Embryol.*, 195(1): 87-101
- Szarski, H. 1965. Cause of the absence of a stomach in Cyprinidae. *Bull. Acad. Pol. Sci. Biol.*, 4: 155-156.
- Takeuchi, T. 1991. Digestion and nutrition. In: Itazawa, Y. and I. Hanyu (eds.), *Fish physiology*, Koseisha-Koseikaku, Tokyo, pp. 67-101. (in Japanese)
- Tanaka, M. 1969. Studies on the structure and function of the digestive system in larvae at the stage of first feeding. *Japan. J. Ichthyol.*, 16(2): 164-174.

감사의 글

지난 7년간 제주에서의 학부과정과 대학원과정을 보내면서 지금의 이 시점까지 너무나 부족한 저를 늘 일깨워 주시고 정성과 열정으로 정확하고 올바른 출발점으로 인도하여주신 이영돈 지도교수님의 은혜와 고마움에 진심으로 머리 숙여 감사드립니다.

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그리고 늘 곁에서 사랑과 믿음으로 묵묵히 바라보며 부족한 나를 이끌어준 사랑하는 유미와 친아들과 같이 보살펴주신 유미의 아버님, 어머님께도 진심으로 감사드립니다. 또한 늘 친형이상으로 많은 격려와 힘을 주신 강성종, 강성보 형님과 래피아텍의 강순구 부사장님, 고희진, 고범호 선배님께도 감사드립니다.

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모두 감사드리고 사랑합니다.

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