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Morphology of Digestive Tract and Its Goblet Cells of Giurine Goby *Rhinogobius giurinus*

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Morphology, histology, and histochemical characteristics of the digestive tract of the guirine goby (*Rhinogobius giurinus*: Gobiidae), collected from the coast of Jeju Island, Korea, were investigated. The digestive tract of *R. giurinus*, which is a brackish water species, has a short (relative length of gut=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 the muscularis externa is thickest in the esophagus, which also contained the most mucus-secreting goblet cells ($P<0.05$). In *R. giurinus*, digestive function occurs in the anterior portion of the digestive tract, where mucus protects the tract from the actions of digestive enzymes and functions to activate digestion.

Key words: Digestive tract, Gobiidae, Goblet cell, RLG, *Rhinogobius giurinus*

Introduction

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

Gobies living in the tidal zone of the coastal area of Jeju Island include *Chasmichthys dolichognathus*, *Istigobius hoshinonis*, *Mugilogobius fontinalis*, and *Pterogobius virgo*. The giurine goby, *Rhinogobius giurinus*, is a gregarious brackish-water fish that lives in the estuary of the Cheonjiyeon waterfall, where it is being studied as part of an environmental project.

The digestive tracts of most teleosts consist of an oral cavity, pharynx, stomach, intestine, rectum, and anus. As the feeding habits of fish differ by species and the environment that they inhabit, the morphologies and structures of their digestive tracts also differ (Tanaka, 1969).

This study investigated the internal structures of the digestive tract, the characteristics of the epithelial tissues, and the distribution and characteristics of mucus-secreting goblet cells with respect to the habitat and ingesta of the giurine goby, *R. giurinus,* in order to provide basic information for understanding the digestive physiology and biology of the Gobiidae.

Materials and Methods

Specimens

Rhinogobius giurinus (n=20) were collected from July 2004 to September 2004 in the estuary below the Cheonjiyeon waterfall on Jeju Island, and 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 organs, from the esophagus to the anus, were removed from the body cavity. The length of the digestive tract (DL, 0.1 mm) and the relative lengths of the gut (RLG: digestive tract length/body length) were measured, and the stomach and intestinal contents were examined.

Histological observations

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

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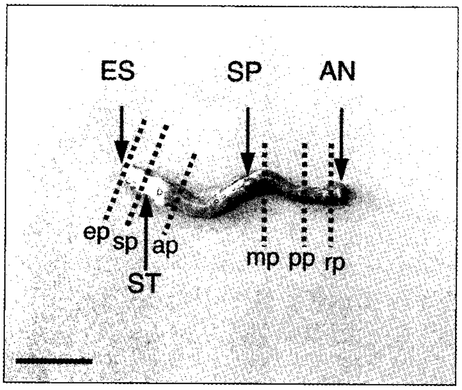

Fig. 1. External morphology of the digestive tract of *Rhinogobius giurinus*. AN: anus, ES: esophagus, SP: spleen, ST: stomach, ap: anterior intestine, ep: esophagus, mp: mid-intestine, pp: posterior intestine, rp: rectum, sp: stomach. Scale bar=10 mm.

hematoxylin and 0.5% eosin (HE) for histological observations and with Alcian blue (AB) at pH 2.5 and periodic acid-Schiff (PAS) to observe mucussecreting goblet cells.

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

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).

Results

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

Rhinogobius giurinus inhabits the soft, sandy bottom of the estuary below the Cheonjiyeon waterfall. The average lengths of the body and digestive tract were 8.4±0.2 and 3.5±0.2 cm, respectively, and the RLG was 0.42±0.02 (Table 1). The digestive tract is short. No clear border between the esophagus and stomach was observed, and the area between the stomach and anterior portion of the intestine had a slight depression. The digestive tract of this species is also narrow, and externally pyloric caeca were not observed (Fig. 1). The stomach contained mainly lugworms.

Histological characteristics

The muscularis externa of the esophagus of *R. giurinus* was well developed, while the lamina propria was less developed. The mucosal folds were regularly branched and distributed toward the lumen (Fig. 2-A). The average thickness of the muscularis externa and average length of the mucosal folds were 206.7±23.3 μm and 540.0±40.0 μm, respectively (Table 2).

A stomach was observed, but pyloric caeca were absent in the anterior intestine (Fig. 2-B). The average thickness of the muscularis externa and average length of the mucosal folds of the stomach were 122.5±8.2 μm and 608.3±40.6 μm, respectively (Table 2).

The mucosal folds of the anterior intestine were regularly branched and densely distributed toward the lumen (Fig. 2-C). The average thickness of the muscularis externa and average length of the mucosal folds were 45.8±3.8 μm and 481.7±24.6 μm, respectively (Table 2).

The mucosal folds of the mid intestine were mostly regularly branched and distributed toward the lumen (Fig. 2-D). The average thickness of the muscularis externa and average length of the mucosal folds were 49.2±2.9 μm and 390.0±14.7 μm, respectively (Table 2).

The mucosal folds of the posterior intestine were wider and longer than in other portions of the intestine and were distributed toward the lumen (Fig. 2-E). The average thickness of the muscularis externa and average length of the mucosal folds were 48.3±4.4 μm and 433.3±18.1 μm, 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. 2-F). The average thickness of the muscularis externa and average length of the mucosal folds were 43.3±25.4 μm and 286.7±16.2 μm, respectively (Table 2).

The muscularis externa of this fish consisted of two layers of smooth muscle cells. The cells in the inner layer were arranged circularly, and those in the outer layer were arranged longitudinally. The thickness decreased from anterior to posterior in the in-

Table 1. Body length, digestive tract length, and RLG of *Rhinogobius giurinus*. Values are the mean ± SE. RLG (relative length of gut = digestive tract length/body length).

	Rhinogobius giurinus
Sample number	20
BL (body length, cm)	8.4±0.2
DL (digestive tract length, cm)	3.5±0.2
RLG (relative length of gut)	0.42±0.02

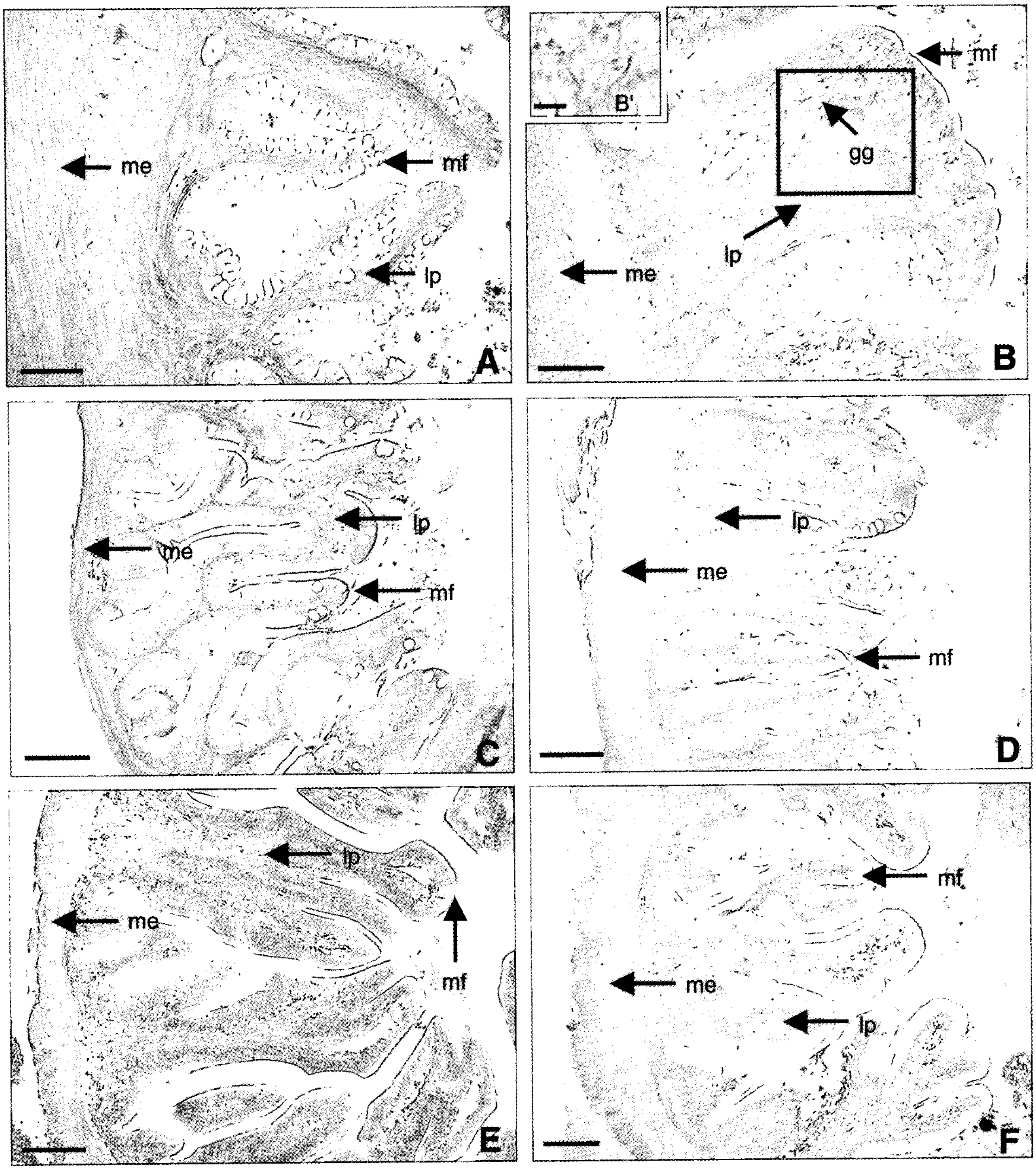

Fig. 2. Photomicrographs of cross sections of the digestive tract of *Rhinogobius giurinus*. A, esophagus; B, stomach; C, anterior intestine; D, mid-intestine; E, posterior intestine; F, rectum; gg, gastric gland; lp, lamina propria; me, muscularis externa; mf, mucosal fold. Scale bar = 50 μm. A magnification of the gastric gland in (B) is shown in B'. B', Scale bars = 20 μm.

Table 2. Histological features of the digestive tract of *Rhinogobius giurinus*.

	Muscularis externa thickness (μm)	Mucosal fold length (μm)
Esophagus	206.7±23.3	540.0±40.0
Stomach	122.5± 8.2	608.3±40.6
Anterior intestine portion	45.8± 3.8	481.7±24.6
Mid intestine portion	49.2± 2.9	390.0±14.7
Posterior intestine portion	48.3± 4.4	433.3±18.1
Rectum	43.3±25.4	286.7±16.2

Values are the mean ± SE.

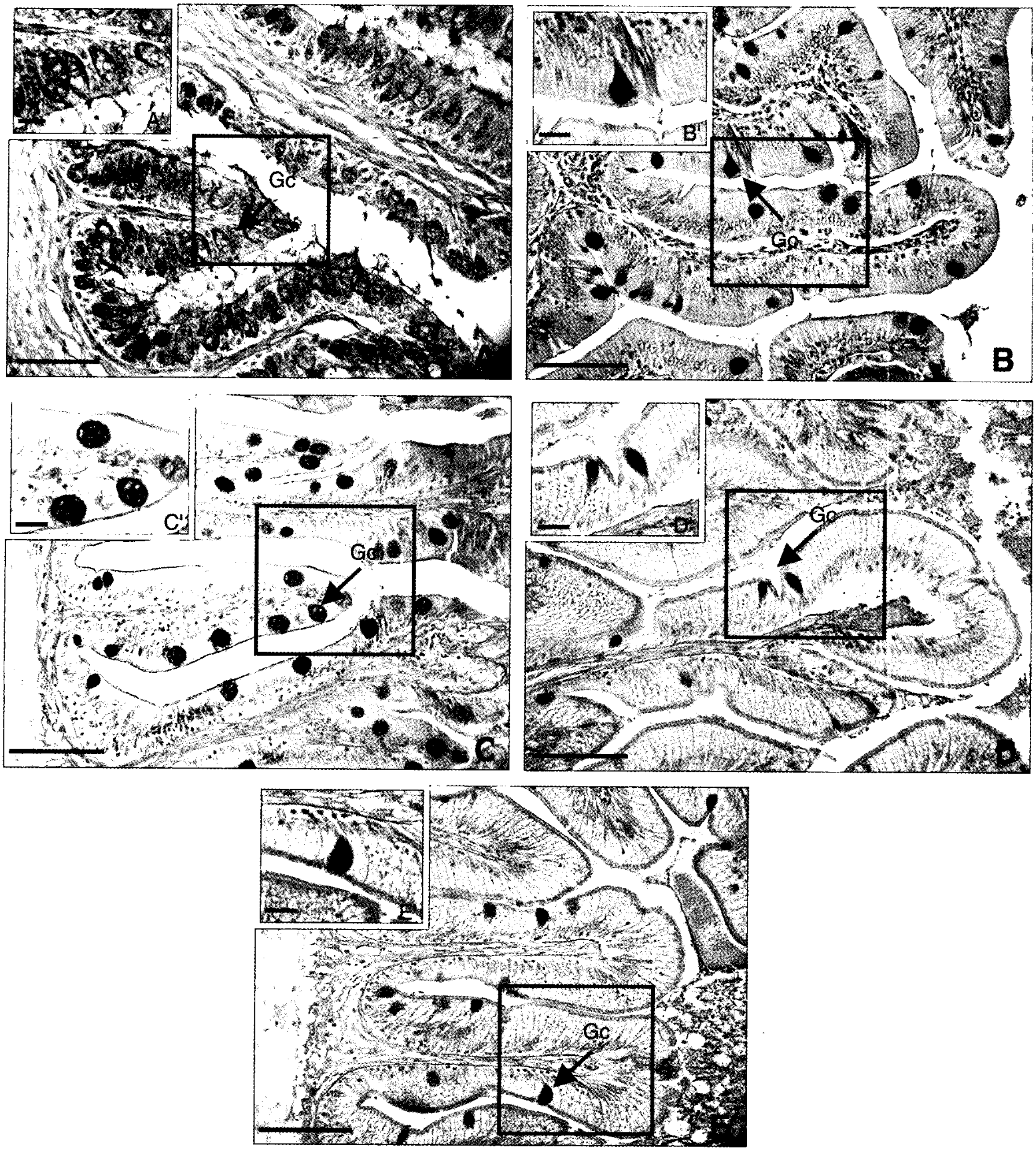

Fig. 3. Photomicrographs of goblet cells in the digestive tract of *Rhinogobius giurinus*. A, esophagus; B, anterior intestine; C, mid-intestine; D, posterior intestine; E, rectum; Gc, goblet cell. Scale bar = 100 μm. Magnifications of the goblet cells in (A-E) are shown in (A'-E'). Scale bar = 20 μm.

testine. The inner circular layer was more developed than the outer longitudinal layer. The mucosal folds consisted of a single layer of columnar epithelium. While the distribution pattern did not change, the cells were shorter in the mid-intestine and longer in the posterior intestine.

Distribution and characteristics of goblet cells

The distribution and relative frequency of goblet cells differed according to the location in the digestive tract. These differences are shown in Table 3 and Fig. 4. Goblet cells were not detected in the stomach of the giurine goby. Mucus-secreting goblet cells were detected in the esophagus (920±17), anterior (641±107), mid-intestine (510±42), posterior intestine (379±101), and rectum (287±55) (Table 3).

Table 3. Numbers of goblet cells in the digestive tract of *Rhinogobius giurinus*

	Numbers of goblet cells/tissue section
	Rhinogobius giurinus
Esophagus	920±17[a]
Stomach	None
Anterior intestine portion	641±107[b]
Mid intestine portion	510±42[bc]
posterior intestine portion	397±101[cd]
Rectum	287±55[d]

Values are the mean ± SE. Superscripts with different letters in the same column indicate significantly different values ($P < 0.05$).

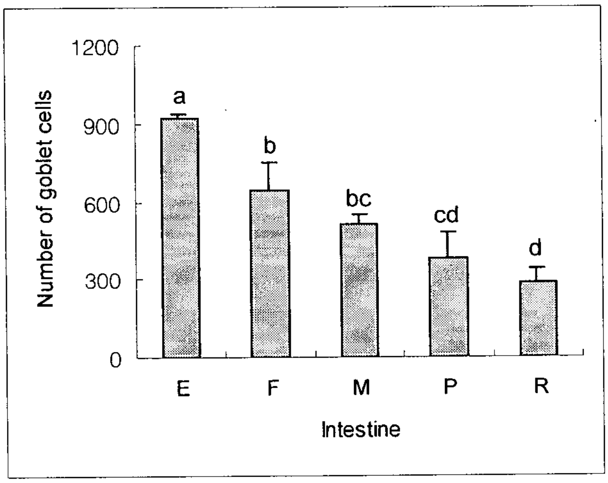

Fig. 4. Change in the distribution of goblet cells in the digestive tract of *Rhinogobius giurinus*. A: anterior intestine, E: esophagus, M: mid-intestine, P: posterior intestine, R: rectum. Vertical bars denote the standard error of the means. Different superscripts on the bars indicate significant differences ($P < 0.05$).

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. 3-A). From the anterior to mid-intestine, the cells were primarily spherical and were distributed throughout the mucosal folds. From the posterior intestine to the rectum, mainly oval shaped goblet cells were observed; these were also distributed throughout the mucosal folds (Fig. 3).

Discussion

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

In this study, the RLG of *R. giurinus* was 0.42, which is consistent with its being carnivorous. The stomachs of this species contained mainly lugworms. Nevertheless, information on the feeding habits of this species is incomplete, and further investigations of its mode of food ingestion and stomach and intestinal contents are needed.

In teleosts, the digestive activity in the stomach can be classified as 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 with lamina propria that are much less well developed than the muscularis externa, large quantities of smooth food can be digested. By contrast, in fish with muscularis externa that are 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). In *R. giurinus*, the muscularis externa and lamina propria are somewhat developed, whereas the gastric glands are well developed. The stomachs of *R. giurinus* contained lugworms, 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. The digestive systems of several species of fish have been analyzed, including the mummichog, *Fundulus heteroclitus* (Babkin and Bowie, 1928), flounder, *Platichthys flesus* (Jenkins *et al.*, 1992), and Nile tilapia, *Oreochromis niloticus* (Morrison and Wright, 1999). In addition, the digestion and absorption properties of the digestive tracts of several stomachless fish belonging to the family Labridae have been studied (Ishida, 1936; Barrington, 1942; Al-Hussaini, 1947, 1949; Szarski, 1965; Chao, 1973). Few studies have investigated the families Atherinidae, Blenniidae, Cobitidae, Cyprinidae, Gobiidae, Mugilidae, and Scaridae.

The digestive tract of most teleosts consists of an oral cavity, pharynx, stomach, intestine, rectum, and anus. Some Cyprinidae and Gobiidae lack a stomach and pyloric caeca (Tanaka, 1969). We examined *R. giurinus*, which histologically lacks pyloric caeca.

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, including *Konosirus punctatus*, *Carassius carassius*, *Parasilurus asotus*, *Thamnaconus modestus*, and *Pagrus major*, have been studied with respect to mucus secretion (Jo *et al.*, 1984). The histochemical characteristics of intestinal mucus-secreting cells have been analyzed in *Acanthopagrus schlegelii*, *Erosa erosa*, *Sebastes inermis* (Byeon and Cho, 1985), *Pleuronichthys cornutus*, *Paralichthys olivaceus*, *Acanthogobius hasta*, *Zoarces 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 amount of mucin also depends on the fish species and the particular region of the digestive tract, with the number of goblet cells generally increasing posteriorly (Reifel and Travill, 1979). The goblet cells of *R. giurinus* are distributed mostly in the esophagus and decrease in number toward the rectum. The cells are AB-PAS positive and are rounded and oval. Therefore, 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, more detailed studies of the physiological mechanisms are needed.

In *R. giurinus*, which are brackish water fish, digestive action occurs in the anterior portion of the digestive tract, where mucus protects the tract from the actions of digestive enzymes and functions to activate digestion. Further studies of the modes of food ingestion, the contents of digestive tracts, and the staining characteristics of the goblet cells of this species need to be carried out.

Acknowledgments

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