Oropharyngeal structure of the juvenile spotted Scat, *Scatophagus argus*
โครงสร้างออโรฟาริงค์ของปลาตะกรับ *Scatophagus argus* ระยะวัยรุ่น

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Abstract

A total of 20 juvenile-staged individuals of spotted Scat, *Scatophagus argus* with standard length of 5.06±0.32 cm was histologically examined for the oropharyngeal cavity by using standard paraffin method and histochemical analysis. Results showed an organizing of three tissue layers to make up for oropharyngeal structure including mucosa, submucosa and muscularis, respectively. Stratified epithelium of the mucosal layer with several cell types including mucous cells and taste buds occurred in all regions. With histochemical analysis, the mucous cell showed a strong positive reaction with Periodic acid-Shiff and alcian blue at pH 2.5, indicating the presence of the glycoprotein and mucopolysaccharide. Additionally, the oral cavity of *S. argus* also showed villiform teeth, which distributed in three areas (upper jaw, lower jaw and pharyngeal teeth). This is the first report about the

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histological observations of the oropharyngeal cavity in *S. argus* during juvenile stage, which could be applied to further studies about *S. argus*.

Keywords: Histology, Juvenile, Oral tissue, Spotted scat, Teeth

บทคัดย่อ

การศึกษานี้เป็นการศึกษาโครงสร้างออโรฟาริงค์ของปลาตะกรับ *Scatophagus argus* ระยะวัยรุ่น ที่ความยาวเหยียดเท่ากับ 5.06±0.32 cm จำนวน 20 ตัว ด้วยเทคนิคทางมิญชวิทยาและมิญชเคมี ผลการศึกษาทางมิญชวิทยา ออโรฟาริงค์มีโครงสร้างประกอบด้วย 3 ชั้น คือ มิวโคซา ซับมิวโคซา และมัสคูลาริส ตามลำดับ เยื่อบุผิวหลายชั้นของมิวโคซาที่มีเซลล์หลายชนิด ทั้งเซลล์สร้างสารเมือกและต่อมรับรส พบได้ตลอดแนวของออโรฟาริงค์ เมื่อพิจารณาด้วยเทคนิคมิญชเคมีพบว่า เซลล์สร้างสารเมือกมีปฏิกิริยาเชิงบวกกับพีเอเอชและอัลเซียนบลู พีเอช 2.5 ชี้ให้เห็นว่ามีองค์ประกอบเป็นสารกลุ่มไกลโคโปรตีนและมูโคโพลีเซคคาไรด์ นอกจากนี้ช่องปากของปลาตะกรับยังประกอบด้วยฟันชนิด villiform ที่มีการกระจาย 3 บริเวณ (บน ล่าง และฟาริงเคียลทีช) การศึกษาครั้งนี้จัดเป็นรายงานแรกของโครงสร้างออโรฟาริงค์ของปลาตะกรับในระยะวัยรุ่น ที่สามารถประยุกต์สำหรับการศึกษางานวิจัยที่เกี่ยวข้องกับปลาตะกรับในอนาคตต่อไป

คำสำคัญ : มิญชวิทยา ระยะวัยรุ่น เนื้อเยื่อปาก ปลาตะกรับ ฟัน

Introduction

An oral cavity and pharynx is are typically structures locating at the beginning part of digestive tract (Ezeasor, 1982; Fishelson et al., 2004) which provides information about feeding habitat and physiology in fishes (Rodrigues and Menin, 2005; Xiong et al., 2011). The gross anatomy and histology of the oral cavity and pharynx in teleost fish is composed of various kinds of epithelium, abundant mucus-secreting cells, teeth, taste buds and tongues, which are responsible for the multifunctional region (Ezeasor, 1982; Fishelson et al., 2004). Especially, the tongue in the oral cavity is an important tactile organ and plays an important role in the transportation as well as the swallowing of food in fish (Abbate et al., 2012a; 2012b). The feature of this organ is explained not only the evolution of organism life style but also the adaptation to different environmental conditions and feeding habitats (Iwasaki, 2002).

Scatophagus argus which belonges to the family Scatophagidae, is a dominant fish that play an important role in food chain of Pranburi Estuary, Thailand. The study on the morphology and histology of esophagus throughout the intestine in *S. argus* was previously identified (Senarat et al., unpublished), however, the oral and pharynx has never been examined. Therefore, the objective of this study was to investigate the oropharyngeal structure of the juvenile spotted Scat, *S. argus* in order to provide a basic knowledge on histological character to be used in further study.

Materials and Methods

All healthy specimens of *S. argus* during the juvenile stage were used in the present study. Fish with standard length at 5.06±0.32 cm were caught as by catch during the fishing season (October to November 2015) from Paknam Pranburi Estuary (n = 20, 2 stations (N 12°24'15.8" / E 099°58'25.6" 2 and N 12°24'21.6" / E 099°58'37.1"). The fish was rapidly euthanized using cooling shock (Wilson et al., 2009). The heads were dissected out and fixed overnight in Davidson's fixative, at 4°C for approximately 24 hrs. After a routine histological technique, the paraffin sections with thickness of 6-7 μm were stained with Harris's hematoxylin and eosin (H&E), periodic acid-Schiff (PAS) and aniline blue (AB) pH 2.5. to identify the basic structure and the chemical details (Bancroft and Gamble, 2002; Presnell and Schreibman, 1997). Finally, the sections were observed and photographed by a Canon EOS 1100D digital camera.

Results and Discussion

The histological and histochemical examinations showed that the oral cavity, which is the anterior-most region of the digestive tract, was composed of three distinctive layers including mucosa, submucosa and muscularis (Figures 1A-1E, 2A-2B). The mucosal epithelium of the oral area was covered by stratified polygonal squamous epithelium without ciliated structure (Figures 1A-1B), as found in most of teleost fish (Albrecht et al., 2001) such as *Cyprinus carpio* and *Gnathonemus petersii* (Genten et al., 2008). Moreover, the villiform teeth were also microscopically found in both upper and lower jaws of this fish (Figure 1A). it is consistent with

previous observations in other fishes such as *P. maculatus* (Godinho, 1967) and *Trichomycterus brasiliensis* (Lütken, 1874) (Oliveiraribeiro and Menin, 1996). As noted above, a part of the villiform teeth could be divided into two stages based on histological features: immature and mature teeth (Figure 1A). Mature teeth usually composed of different components including (i) a dentine layer, (ii) enamel carp (enamel coating) and (ii) pulp core. A few immature teeth were seen among the oral epithelium (Figure 1A). This tooth included (i) scleroblast, (ii) odontoblaste producing the pre-dentine and (iii) enamel organ (Figure 1A).

The taste buds were lined up along the mucosal epithelium (anterior to posterior regions) of the oral cavity (Figure 1B). Each taste bud was a typical pear-shape and located in the epidermal hillock. It was also reacted positively with the PAS method (Figure 1C). High magnification revealed that the taste bud was composed of sensory cells. Each cell had an elongate-shaped nucleus and its basement was supported by the connective tissue (Figure 1C), as observed in the most of teleost fish (Genten et al., 2008). Moreover, the specialized sustentacular cells were located at the periphery of the taste cell (Figure 1C).

A few mucous cells were prismatic only at the posterior region of the oral cavity. It reacted positively to PAS reaction (Figure 1D). These reactions indicated the presence of the glycoprotein, as likely shown in the oral cavity in *Trachelyopterus striatulus* (Santos et al., 2015). It suggests that the function of the chemical secretions may be concerned with mechanical protection against bacterial invasion (Santos et al., 2015). The submucosal layer appeared with lining beneath the mucosa. It appeared to consist of loose connective tissue (Data not shown).

At the floor of the oral cavity, the tongue was exhibited by using PAS reaction. The tongue was composed of the following layers: mucosa, submucosa and cartilaginous structure (hyaline cartilage) (Figure 1E). The tongue structure of the mucosal thickening was covered by stratified epithelium, which continuously appeared from the oral epithelium. The taste bud was also scattered in the mucosal epithelium at the apex of the tongue and was supported by submucosa and cartilaginous structure. It was in line with the observation in other marine fish species, *Sparus aurata* (Linnaeus, 1758) (Abbate et al., 2012a).

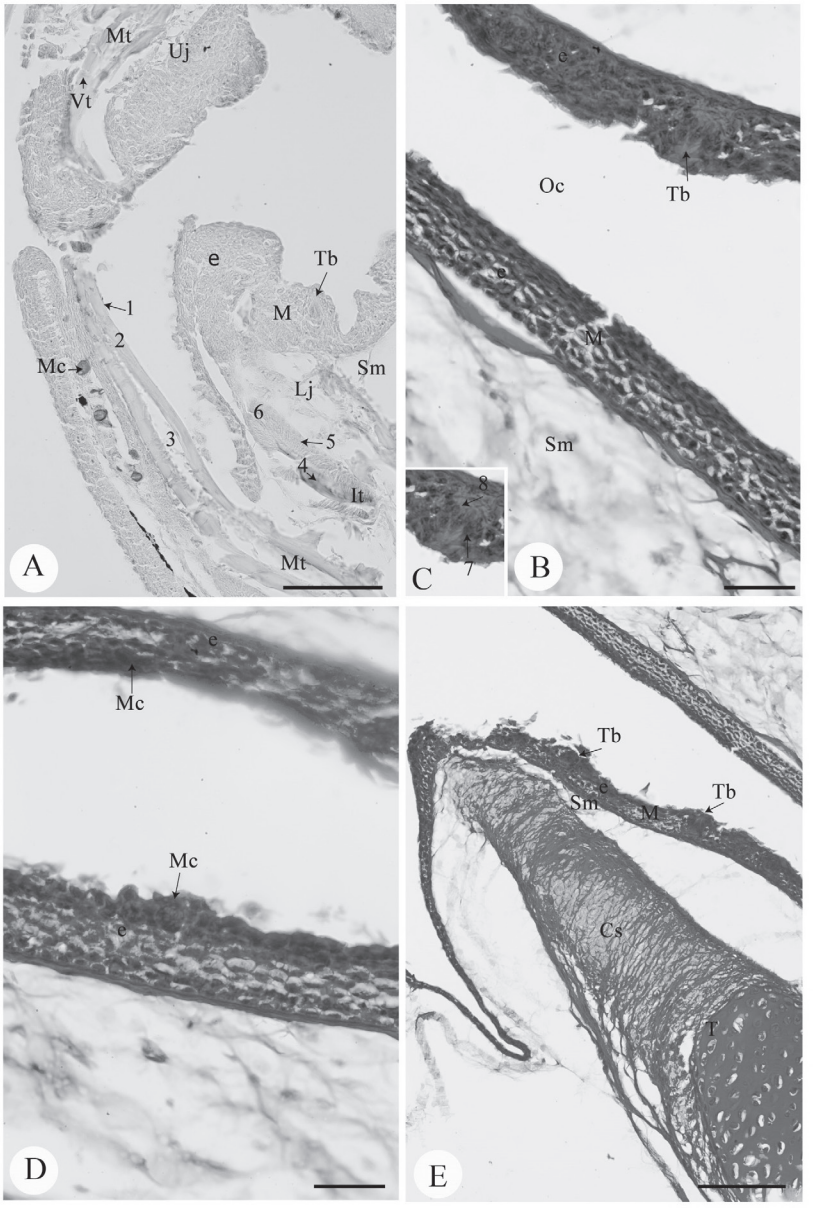

Figure 1. Histological structure of oral cavity (Oc) and tongue (T) in *Scatophagus argus* during juvenile stage. e = epithelial layer, Cs = Cartilaginous layer, It = immature tooth, Lj = lower jaw, M = mucosa, Mc = mucous cell, Mt = mature tooth, Oc = oral cavity, Sm = submucosa, T = tongue, Tb = taste bud, Uj = upper jaw, Vt = velifrom teeth, 1 = enamel carp, 2 = dentine, 3 = pulp core, 4 = dentine of immature tooth, 5 = odentoblast, 6 = enamel organ, 7 = taste cell, 8 = sustentacular cell. Note: Scale bar 300 μm (A); 100 μm (D-E); 50 cm (B)

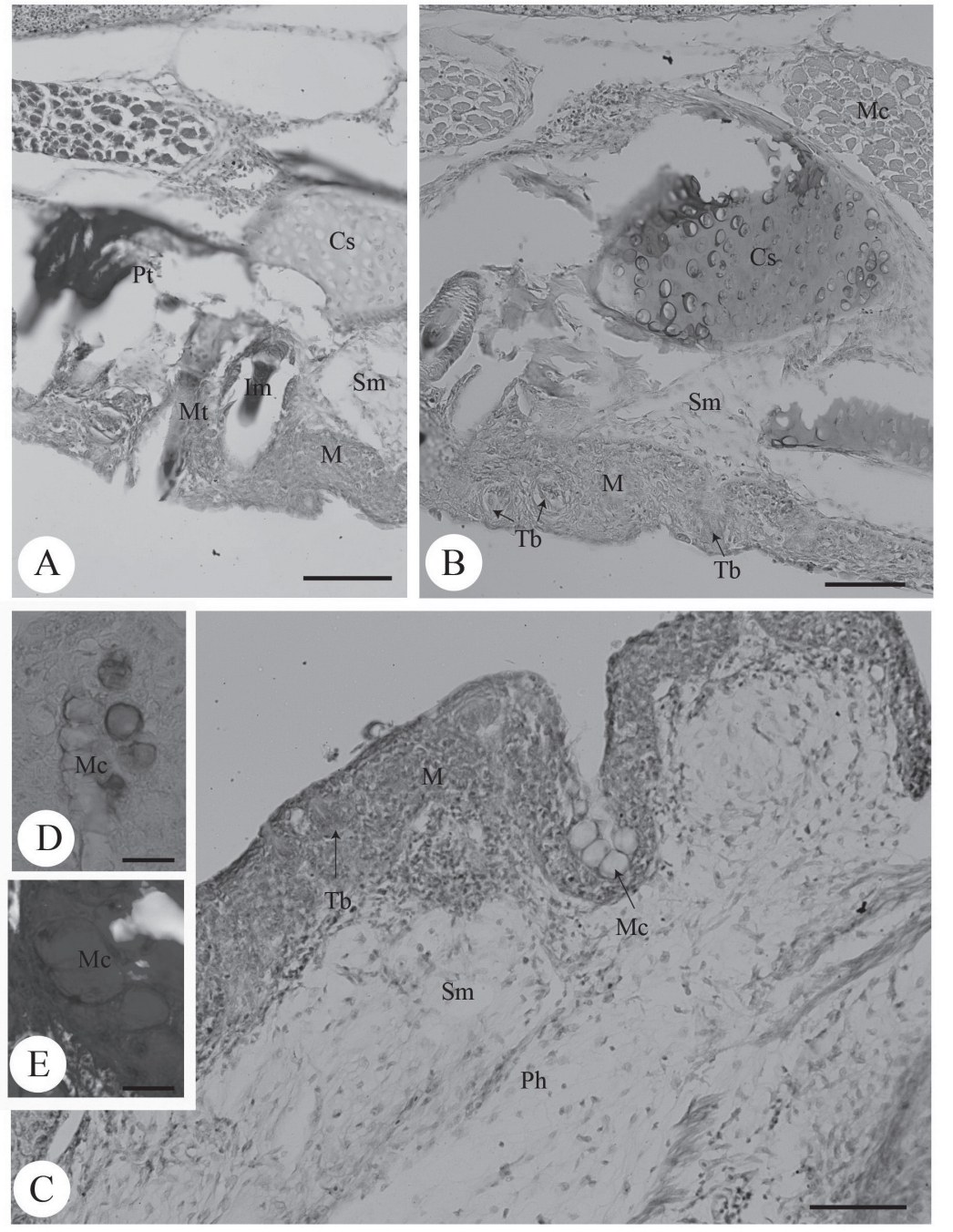

Figure 2. Histological structure of pharynx (Ph) in *Scatophagus argus* during juvenile stage. Cs = cartilaginous layer, Im = immature tooth, M = mucosa, Mc = mucous cell, Mt = mature tooth, Sm = submucosa, Pt = pharyngeal plate, Tb = taste bud, Note: Scale bar 100 μm (A-C); 10 μm (D-E)

Progressing posteriorly from the oral cavity, the pharyngeal teeth exclusively exhibited the formation of the elongated teeth, as also the pharyngeal villiform dental plate (Figure 2A-2B). These teeth may generally relate to food capturing and guiding, as suggested by Rodrigues et al. (2006) who studied pharyngeal teeth in *Leporinus macrocephalus*. The pharyngeal villiform dental plate was appeared in both immature and mature teeth (Figure 2A), which was similar to previous findings in several fish species (Genten et al., 2008) such as *Conorhynchos conirostris* (Rodrigues and Menin, 2005) and *S. trigonocephalus* (Schuingues et al., 2013).

The pharynx of *S. argus* continuously showed as a short-lengthed structure. It was lined with stratified epithelium, as closely structured with oral epithelium, submucosal and muscularis layers, which typically appeared to extend from the oral structure (Figure 2C). The mucous cells and the taste bud commonly occurred in the epithelial pharynx (Figure 1C). The mucous cell was an oval shape and reacted positively with PAS and AB, indicating production of the glycoprotein and mucopolysaccharide substances (Figure 2D-2E).

An important conclusion forming in this study was shown that the basic histological characterizations of the oral cavity and pharynx in the *S. argus* was composed of three layers: mucosa, submucosa and muscularis, All results from our observation will be applied to support the digestive physiology and ultrastructure.

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