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Micromorphological Observation of the Anterior Gut of Sulawesi Medaka Fish (*Oryzias celebensis*)

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A B S T R A C T

The use of medaka fish as a candidate animal model has been started which has similarities with the Zebra fish that was developed as an animal model. Sulawesi medaka fish (*Oryzias celebensis*) is a type of medaka fish that are endemic in the region of South Sulawesi. This research aims to observe the histology of anterior gut of Sulawesi medaka fish. Histological study on the anterior gut of Sulawesi medaka fish using buccal cavity and oesophagus organs. Histological observation showed that the mouth and buccal cavity are shared by the respiratory and digestive systems. Also in Sulawesi medaka fish we found the lining of the buccal cavity consists of mucoid epithelium on a thick basement membrane with numerous goblet cells. In general the structure of the anterior gut system in Sulawesi medaka fish similar with Zebra fish as well as other Teleostei fish.

Introduction

Fish medaka (*Oryzias sp*) is a fish that has several advantages as an experimental animal that is easy to nurseries, the reproductive cycle is short (maturation initial 2 months), embryos are transparent, the generation time is short, the genome size is small and has had a construction of transgenic (Naruse *et al.*, 1985; Denny *et al.*, 1998; Ishikawa, 2000). Some biological information to the molecular systematics of medaka fish, especially the Japanese medaka fish (*Oryzias latipes*) is also widely found (Andriani and Hassan, 2013; Takehana *et al.*, 2015). Needs medaka fish as an animal model has made researchers using medaka fish as animals test to create a wide variety of strains of medaka fish Oryzias latipes.

But in Indonesia as a distribution center of medaka fish species, information about particular medaka fish endemic to Sulawesi Island as Oryzias celebensis still very poor and have not been domesticated as well as to be further explored. Medaka fish as well as

Zebra fish have started to be used as experimental animals. Some mutant medaka fish species have also been made for screening diabetes drugs, cancer, especially skin cancer and other degenerative diseases.

Medaka (*Oryzias sp*) including a group of small fish on the taxonomic belonging to the family Adrianichthyidae (Kottelat *et al.*,1993; Nelson, 2006). The distribution of these fish covering a wide area from India to Japan and South along the islands of the Indo - Australian in the Wallacea line, namely Timor and Sulawesi (Moss and Wilson, 1998; Parenti, 2008). The discovery of the new species of medaka fish species is also increasing (Parenti and Soeroto, 2004, Parenti *et al.*, 2013)). From exploration results the researchers showed that Sulawesi has a particularly high endemism of the fish family Adrianichthyidae.

The high endemism is not only supported by the restrictions zoogeography as the island and the line of Wallacea Weber, but also because this is not a group of fish traded so the chances for migration very limited. These facts underlying the statements by some researchers that medaka fish can unravel the mystery of the evolution of the marine fauna that exist in Sulawesi

As development of Sulawesi medaka fish (*Oryzias celebensis*) as an animal model and in an effort to use Sulawesi medaka fish in the field of histology and pathology, research has been done of the anterior gut system in Sulawesi medaka fish. The results of this study are expected to be the gateway for the development of medaka fish as an animal model.

This research aims to develop medaka fish as an animal model through histological observation, especially on the observation of the anterior gut of Sulawesi medaka fish.

Materials and Methods

The samples of Sulawesi medaka fish about six adult fishes with average of size and weight. Sulawesi medaka fish were obtained from Pattunuang river, Karst, Rammang-rammang, Maros, South Sulawesi. The fishes and then kept in aquariums for temporary before sampling. Fish samples and then stored in 10% neutral buffered formalin. The Sulawesi medaka fish sizes are small and the observations made by one part of the body of the fish. The fishes are processed in the process of further histotechnique (Kiernan, 1990). Samples were dehydrated with graded series alcohol and clearing using xylol before embedding with paraffin. The tissues were then cut using microtome with 4 µm thickness, then samples were stained using Mayer's Hematoxylin eosin (HE) and others histochemical staining such as Alcian Blue staining (AB) and Periodic Acid staining (PAS) (Kiernan, 1990). Observations were made under a microscope which has been connected to the camera microscope (Olympus 22X with advanced Optilab) and also analyzed with image J programme.

Results and Discussions

A comprehensive study of the histology of the anterior gut of Sulawesi medaka fish had not been established y*et al.*,though few studies were conducted on the anatomy and histology of zebrafish (Menke *et al.*, 2011). Nevertheless, this research is very important to know about feeding behavior and habitat of Sulawesi medaka fish, thus providing fundamental information for further anatomical and physiological studies. Sections were observed anterior gut such as buccal cavity and oesophagus (Figure 1). The mouth and buccal cavity are shared by the respiratory and digestive systems according to previous study (Takashima and Hibiya, 1995; Roberts and Ellis 2001).

Fig.1 Buccal cavity. Consisted of mucoid epithelium on a thick membrane with numerous Goblet cells (arrows). A: 10x10, B, C: 40x10. A, B: HE staining, C: AB staining

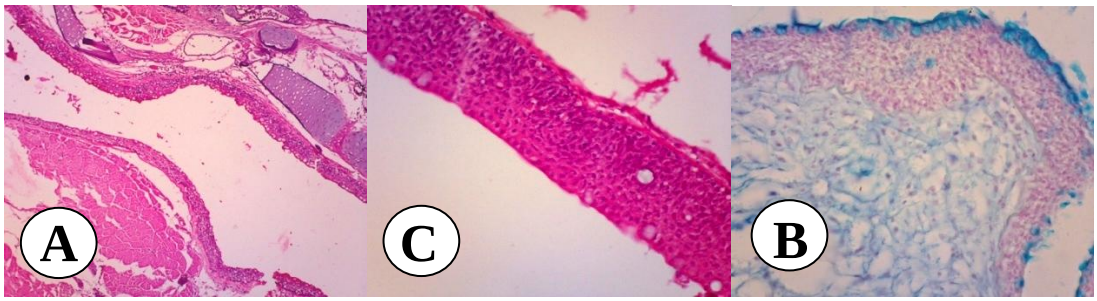


Fig.2 Buccal cavity (a) and oesophagus (b). A: 10x10, B: 40x10, HE staining

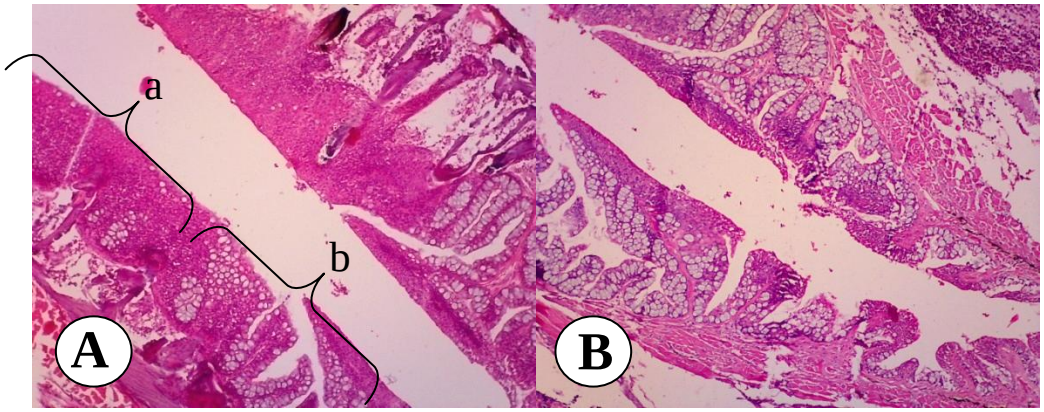
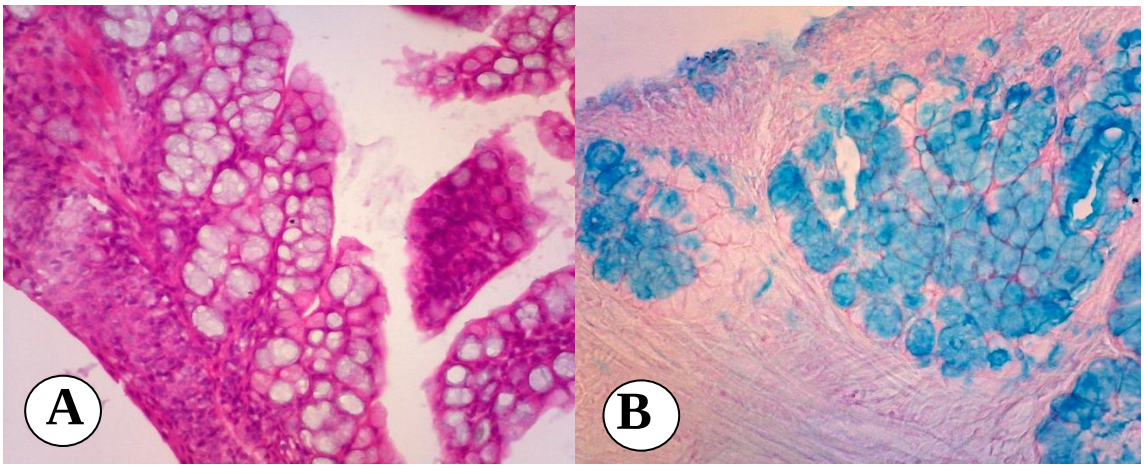


Fig.3 Oesophagus of Sulawesi medaka fish consisted of epithelial cells and Goblet cells on the mucosal. A, B: 40x10. A: HE staining, B: AB staining



The digestive function is confined to selection, seizure, and orientation of food before transfer to the intestines. In the zebrafish, the mouth and the perioral regions have many taste buds. Also in Sulawesi medaka fish we found the lining of the buccal cavity consists of mucoid epithelium on a thick basement membrane with numerous

goblet cells (Figure 1). In Sulawesi medaka fish as well as zebrafish the buccal cavity leads into the esophagus (Figure 2), which encompasses blind diverticula (esophageal sacs), a pharyngeal pad, and teeth where food can be ground (Roberts and Ellis, 2001) and continued to intestine.

Oesophagus of the Sulawesi medaka fish consisted of epithelial cells and Goblet cells on the mucosal areas (Figure 3). The number of Goblet cells gradually decreased from anterior to posterior region of eosophagus (Figure 2).

The Goblet cells, that abundant at anterior oesophagus and decreased in posterior region of oesophagus, produce mucoid substance and lubricates esophageal surface so that food bolus easily swallowed. The findings were similar to the oesophagus of other fishes, which demonstrated numerous mucous cells and then reacted positively to Alcian blue and PAS stains (Al Abdulhadi, 2013). Furthermore, the oesophageal mucus is important in immunological mechanisms against viral and bacterial infection and also osmoregulatory function.

The results of this observation indicate that the anterior digestive system of bungo fish and zebrafish have similarities with other teleost fish.

In general the structure of the anterior gut system in Sulawesi medaka fish similar with zebrafish as well as other Teleostei fish. The results of this study can be used as a basis in the development of studies of the digestive system in fish in particular and diseases that exist in the digestive system.

However, more studies should be carried out for deeper understanding of the digestion process and nutrient absorption of those fish as well as a fish animal model.

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