



## Histology study of Siamese freshwater batfish (*Oreoglanis siamensis*)

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### ABSTRACT

Siamese freshwater batfish (*Oreoglanis siamensis*) is considered as endangered species due to the limited habitat such as highland running water sources of Inthanon mountain, Chiang Mai province, Thailand. Moreover, this fish is threatened by the environmental changes and human hunting for food source. Therefore, this fish is listed in the wildlife protection of Thailand. Based on those reason this fish must be enhanced for conservation. Unfortunately, the information of this fish is little such as the feature of habitat, food item, reproduction, and diseases. Basically, histological study makes us know the information of animal cell and tissue for baseline of further study. Hence, this study aims to provide the information of Siamese freshwater batfish histology. Thirty fish samples were collected from Huay Khang Chang, Inthanon mountain using scoop net in October 2017. The fish organ samples were fixed in 10% neutral buffer formalin, dehydrated throughout of alcohol series by automatic tissue processing machine, embedded in paraffin block, 5 micrometer thickness, and stained by Hematoxylin & Eosin. The sections were observed under the microscope (Drawell, Smart series) and recorded by the camera (Zeiss, Scope.A1 and Zeiss, Axiocam 305 color). The histology of nasal barbel, gills, skin, brain, heart, liver, kidney, intestine, and stomach of Siamese freshwater fish is documented for the first time in Thailand.

## INTRODUCTION

Siamese freshwater batfish (*Oreoglanis siamensis*) is sisorid catfish from Inthanon mountain and considered as endangered species in Thailand. According to the study of this fish species biology, it lives in lotic water habitat with water temperature during 18-20°C at 1600 meter above sea level and contain a sand, gravel and rocks that effect on natural selection to be alive [1] Based on these conditions, this species is limited in some upper streams of the mountain. The characteristics of this species are strongly depressed head and body, inferior mouth, greatly enlarged paired fins, which modified to an adhesive apparatus, notch on posterior margin of lower lip are presence, nasal barbel reaching midway between its base and anterior orbital margin, and emarginate shaped caudal fin [2]. However, the biological data of this species is scanty, especially the histology study which is an important information for further histopathological study. Therefore, this study aims to demonstrate the histology information of Siamese freshwater batfish collected in Thailand for the first time.

## MATERIALS AND METHODS

Thirty adult fish samples were collected from Huay Khang Chang, Inthanon mountain, Chiang Mai province, Thailand (18°30'20"N,

98°29'56"E at 1,470 m above sea levels) in October 2017 by scoop nets [3]. The fish samples were measured and weighed. After that, the fish organs, including barbel, gills, skin, brain, heart, liver, kidney, stomach and intestine were fixed in 10% neutral buffered formalin for 24 hours. The organ tissues were dehydrated with ethanol series, cleared with xylene, embedded in paraffin, cut at 5 µm thickness by microtome and stained with hematoxylin and eosin (H&E) [4]. All stained slides were observed under the light compound microscope (Drawell, Smart series) and recorded by camera (Zeiss, Scope.A1 and Zeiss, Axiocam 305 color). The experiment was performed according to the protocol of Laboratory animal center Chiang Mai University (2561/AQ-0002).

## RESULTS

The section of fish nasal barbel was composed of taste bud barbel enclose to dermis layer, several goblet cells, melanophore pigments, numerous alarm substance cells. In addition, the connective tissue and a cartilage rod were central area of barbel were found (Figure 1).

Gills were flaps of tissue packed full of capillaries that located beside the skull, enclose to nostril and mouth cavity and under the operculum. Gill filament were covered by squamous epithelium with

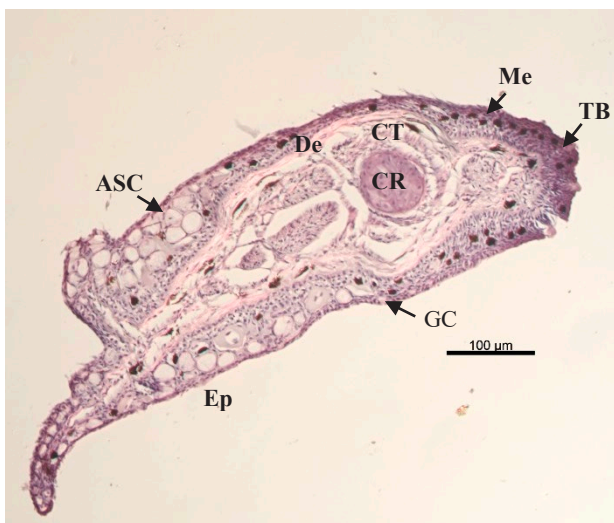
goblet or mucous cells presented especially at the apex of the filament. Gill lamellae includes an envelope of squamous epithelium and few goblet cells, usually thickness layer and contains the pillar cells inside. The efferent arteriole, which take the blood away from gills, was found at the apex of gill filament (Figure 2).

Fish skin was composed of three layers including epidermis, dermis and hypodermis. The middle layer of epidermis was composed of large alarm substance cells, which usually round or oval shape, having the distinct nuclei in the central location. Although, this layer was covered by squamous epithelium, invisible goblet cell, usually melanin pigment also found in this layer. Dermis were located between

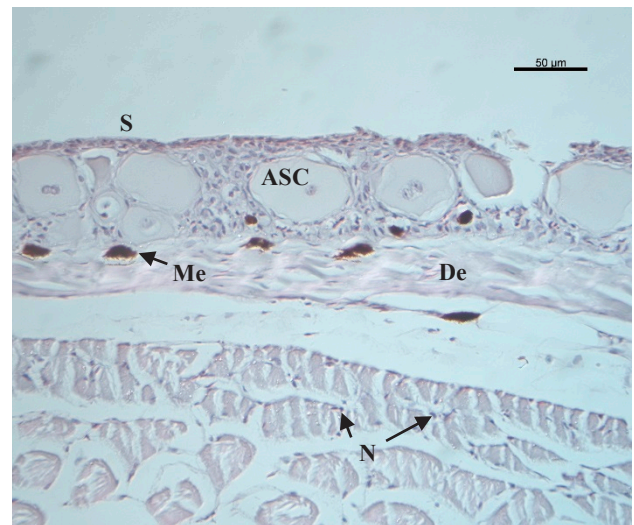
epidermis and muscle. In addition, muscle fibre bundles with numerous peripheral nuclei were observed. The hypodermis contained the loose connective tissue (Figure 3).

Central nervous system (CNS) contained neurons and non-neurons such as neuroglia, endothelia, ependymal cells and pericytes of blood vessel walls. The telencephalon (cerebrum) included numerous nerve cells that located in olfactory lobes (Figure 4).

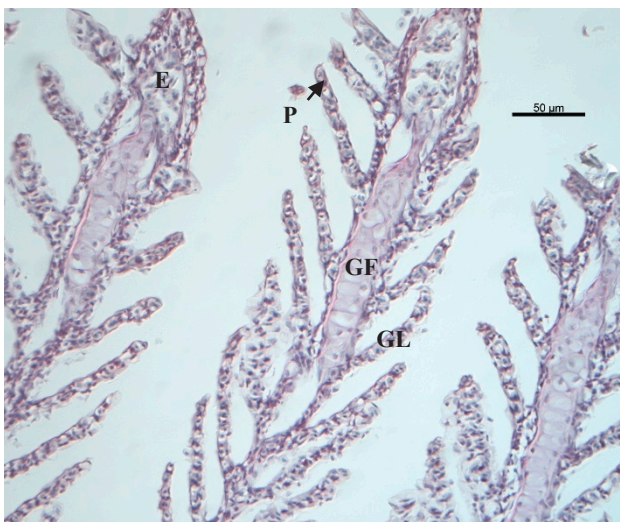
Heart of this fish was located in the chamber immediately behind gill arch and between operculum. The heart was covered by outer layer or epicardium and the inner layer, myocardium, which comprised of loose cardiac muscle (Figure 5).



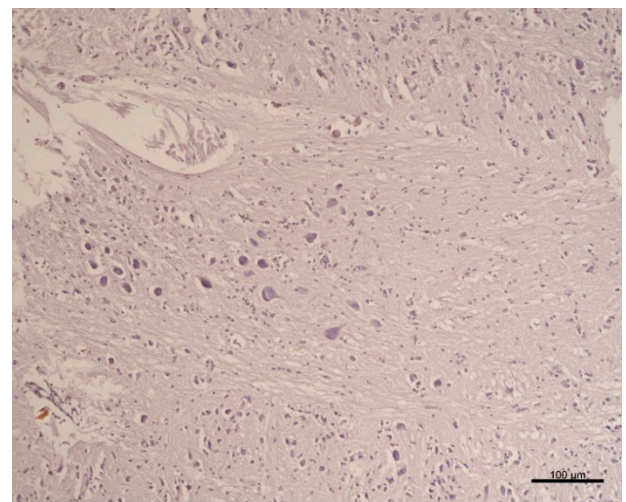
**Figure 1.** The nasal barbel of mature Siamese fresh water batfish. The major histological components visible are epidermis (EP); dermis (De); taste bud (TB); melanophore (Me); alarm substance cell (ASC); goblet cell (GC); connective tissue (CT); cartilage rod (CR) 20x.



**Figure 3.** Muscular system of mature Siamese freshwater batfish. The major histological components are squamous epithelium (S); alarm substance cells (ASC); melanin pigment (Me); dermis (De); peripheral nuclei (N). 40x.

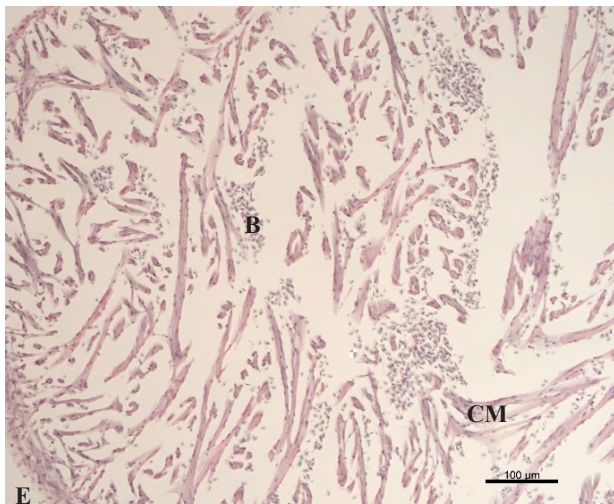


**Figure 2.** Gill filament of mature Siamese fresh water batfish. The major histological components are efferent arteriole (E); pillar cells (P); gill filament (GF); gill lamellae (GL). 40x.

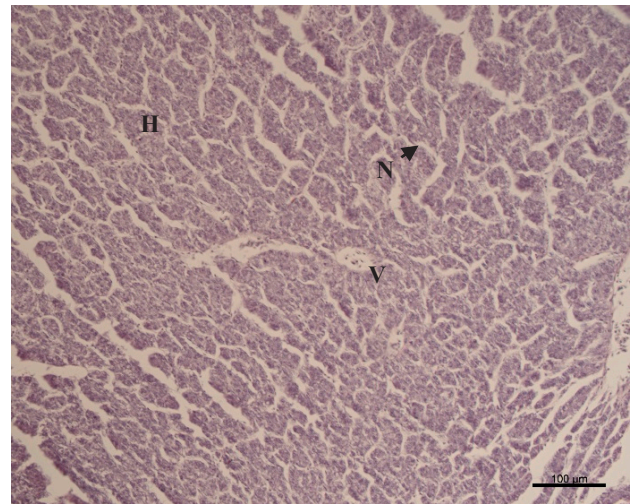


**Figure 4.** The telencephalon or cerebrum of mature Siamese fresh water batfish showed numerous nerve cells. 20x.





**Figure 5.** The heart of mature Siamese freshwater batfish. The major histological components are epicardium (E); cardiac muscle (CM); blood cells (B). 20x



**Figure 6.** The liver of mature Siamese freshwater batfish. The major histological components are hepatocytes (H); hepatic portal vein (V); peripheral nuclei (N). 20x.

Fish liver was located on dorsal side of the body cavity with gastrointestinal tract. The hepatocytes showed peripheral nuclei and hepatic portal vein was present. All blood from the hepatic portal vein through the blood sinusoid and drains into the central vein (Figure 6).

The kidney of this fish was located close to the vertebral column. The renal corpuscle contains a glomerulus that surrounded by Bowman's capsule. The renal tubule connected to the renal corpuscle (Figure 7).

The stomach of this fish was Y-shaped and consisted of mucosa layer with the stratified columnar epithelial. The mucus cell was covered with cilia. The numerous gastric glands were inserted in lamina propria, deep pink stained, and some connective tissues were found (Figure 8).

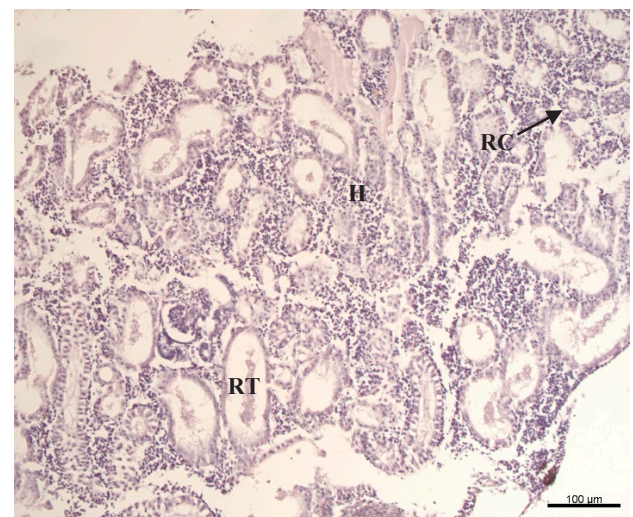
The mucosa layer of intestine was composed of high squamous columnar epithelial whereas the lamina propria consisted of connective tissue. The submucosa had some lymphocytes and erythrocytes (Figure 9).

## DISCUSSIONS

The histology of Siamese freshwater batfish showed the same characteristics as carnivorous scaleless fishes. The stomach of this fish was abundant of gastric glands similar *Clarias gariepinus* in fundic regions [5], *Claris batrachus* [6]. By comparison, the lamina propria of this fish also larger than some carnivorous fishes such as *Channa striata* and *C. batrachus*, but the muscularis layer of this fish was thicker than submucosa. The small vessels were observed between muscularis and serosa. Blood vessels were larger than other catfish such as *Clarias batrachus* [7].

Pillar cells was observed inside the gill lamellae which function to sustain the gill branch and make the small pore to transfer red blood cells in respiratory systems [7]. It was the same in general fishes in Silurid fish, such as *C. lazera* presented the secondary lamellae and interlamellar epithelium with pillar cells in normal tissue [8], whereas the armoured catfish (*Hypostomus plecostomus*) demonstrated the pillar cells in second gill lamellae [9].

Numerous alarm substance cells in dermis layer were found in the skin of this fish indicate that this fish was susceptible for the stimulator for example the water factor changes [10]. Also, *Plotosus lineatus* was showed a lot of alarm substance cells in dermis [11]. The nasal barbel



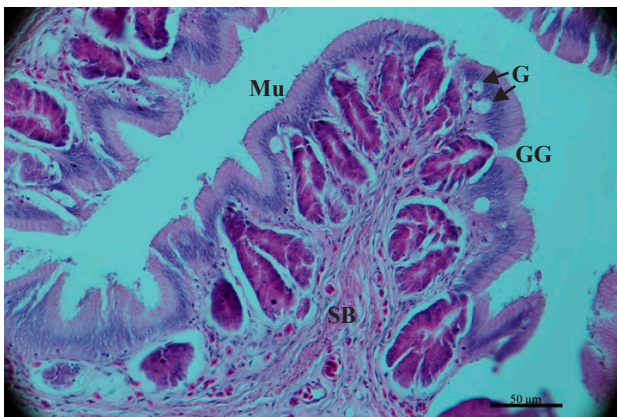
**Figure 7.** The kidney of mature Siamese freshwater batfish. The major histological components are renal tubule (RT); renal corpuscle (RC); haemopoietic tissue (H). 20x.

of this fish showed taste bud on tip of barbel, this organ was found in the same of other catfish such as *Ictalurus punctatus* [12].

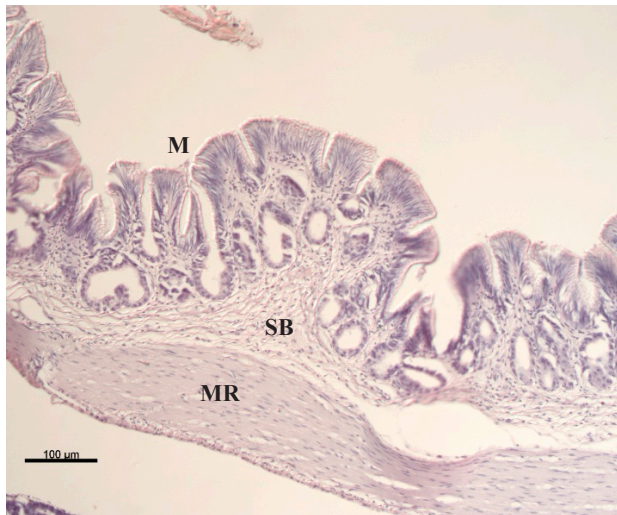
Liver of this fish including hepatocytes that control metabolism and macromolecules analysis for living activities. Vein was inserted between hepatocytes that receive red blood cell from heart and transferred to blood sinusoids by branching vein [7]. Kidney contained the renal corpuscle included the numerous of glomerulus for ammonia and waste elimination by the circulatory systems. For the kidney structure was also same in others fish [11,12]

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**Figure 8.** The Stomach of mature Siamese freshwater batfish. The major histological components are mucus cell (Mu); goblet cell (G); gastric glands (GG); submucosa (SB). 40x.



**Figure 9.** The intestine of mature Siamese freshwater batfish. The major histological components are mucosa layer (M); submucosa (SB); muscularis (MR). 20x.

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