

## Histological Organization of Head Skin in the Female

*Devario regina* (Fowler, 1934),

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### บทคัดย่อ

การศึกษาผิวหนังส่วนหัวของปลาชิวใบไฝ (*Devario regina*) เพศเมีย (จำนวน 10 ตัว) ที่อาศัยในแม่น้ำตาปี ทางด้านโครงสร้างและองค์ประกอบของเซลล์ ด้วยเทคนิคการย้อมสี Periodic Acid Schiff (PAS) ผลการศึกษาพบว่าโครงสร้างผิวหนังส่วนหัวประกอบด้วย 5 ชั้น คือ คิวติเคิล (cuticle) หนังกำพร้า (epidermis) เบส เมน เมมเบรน (basement membrane) หนังแท้ (dermis) และ ไฮโพเดอมิส (hypodermis) โดยที่ชั้นหนังกำพร้า ถูกประกอบด้วยชั้นบุผิวหลาຍชั้น ที่สามารถแยกเป็น 3 ชั้นย่อย คือ ฐานเนื้อเยื่อ (stratum basal) ฟูซิฟอร์ม (fusiform) และคิวติคูลาร์ (cuticular layer) นอกจากนี้ในชั้นหนังกำพร้ายังพบเซลล์สร้างเมือก (mucous secreting cell) บริเวณด้านนอกของชั้นผิวหนังที่ทำปฏิกิริยาบวกกับสี PAS ชั้นผิวหนังแท้อยู่ใต้ชั้นหนังกำพร้าประกอบด้วย 2 ชั้นย่อย คือ stratum spongiosum และ stratum compactum ท้ายสุดคือ ชั้นบางของไฮโพเดอมิสซึ่งพบได้ในผิวหนังของปลาชนิดนี้ด้วย ถึงแม้ว่าข้อมูลครั้งนี้จัดเป็นการศึกษาเบื้องต้น แต่สามารถใช้เป็นข้อมูลพื้นฐานทางด้านมิญชวิทยาของปลาชิวใบไฝในอนาคตต่อไป

คำสำคัญ: ปลาชิวใบไฝ ปลา หัว มิญชวิทยา ผิวหนัง

### Abstract

Head skin histology in ornamental species, female *Devario regina* (n = 10) living in Tapee River, Thailand was investigated both characterization and cell compositions using Periodic Acid Schiff (PAS) staining method. As to our knowledge, the skin organization in this species was composed of five layers: cuticle, epidermis, basement membrane, dermis and hypodermis, respectively. The epidermis was lined by stratified epitheliums, which can be also classified into three sub-layers: stratum basal, fusiform

and cuticular layers. Among the epidermal layer, the mucous secreting cell was seen and located in the proximal region, which positively stained with Periodic Acid Schiff (PAS). The dermis was seen beneath the epidermis with composing of two sub-layers: stratum spongiosum and stratum compactum. Lastly, thin layer of hypodermis in skin histology was also observed in this species. Although, this information provides as preliminary study, it could be further applied to other studies of *D. regina*.

**Keywords:** *Devario regina*; Fish; Head; Histology; Skin

## Introduction

The integument or skin in the fish covers along the body, therefore the important role of integument was well-known as the first protective barrier against noxious agents from the environment and helps in others systems such as respiratory and excretory [1, 2]. Histological examination revealed that the integument is composed of six layers including cuticle, epidermis, basement membrane, dermis and hypodermis in some fishes, as suggested by Roberts [3]. In spite of its importance, previous investigations of this region particularly structure and cell compositions have been rarely found and out-of-date in some teleost fishes including *Morone saxatilis* [1] and *Gnathonemus petersii*, *Danio rerio* and *Pelvicachromis pulcher* [2]. The consequence of structural and histological details in all reports provided and applied to other studies for example histopathology and pathophysiology.

*Devario regina* is the ornamental fish in Thailand, which is required the several knowledge, especially histological characterization. Although, the skin histology and histochemistry in this species during juvenile stage has been reported [4], it is not histological observed the skin region in adult stage. In present study, we continuously attempted to

describe the histological structure and cell compositions of the head skin in the female *D. regina* during its adult stage, as revealed by PAS staining method.

## Materials and Methods

### Field sampling

Alive mature *D. regina* female with body length: ranging from 4-6 cm were used in this study. They were caught in fisheries reason (April 2012, n= 5 and November 2012, n= 5) from Tapee River, Chawang District, Nakhon Si Thammarat Province, Thailand (8°28'10" N, 99°29'45" E).

### Preparation of head skin and histological analysis

All fish were euthanized by a rapid cooling shock [5] and immediately fixed in Davidson's fixative approximately 24 hrs at room temperature. After fixation, these head fish were cut and then processed under standard histological techniques [6]. The paraffin blocks were sectioned at 5-6  $\mu$ m thicknesses using the rotary microtome and stained with Periodic Acid Schiff (PAS) [7]. Stained tissues were analyzed for their histological structures and photographed by a Canon EOS 1100D digital camera.

## Results and Discussion

The histological sections and schematic diagram of the *D. regina* head showed that the skin was consisted of five layers; 1) cuticle, 2) epidermis, 3) basement membrane, 4) dermis and 5) hypodermis (Fig. 1 - Fig. 3), as previously agreed to Harder [8] and Dauod *et al.* [9] in *Mystus pelusius*. However, it differed from some studies because the head skin histology in *Gnathonemus petersi*, *Astronotus ocellatus* [10] and *Bagarius bagarius* [8] was constituted into two principle layers: epithelium and dermis.

The outermost layer of the head skin histology was the thin cuticle layer. Normally, this layer was removed in the routinely histological processes [1], as likely observed in our research. Variety of the functions of cuticle layer are well-known including protection the epidermal layer, the decrease of wounding, the prevent water penetration by osmosis and the reduce friction during swimming [8]. Under concentrated layer, the epidermis was lined by non-keratinizing stratified epithelium and can be basically classified into three sub-layers: stratum basal, fusiform and cuticular layers (Figs. 1B, 2B). The stratum basal was a thin layer and covered by the low simple cuboidal epithelium. This sub-layer was situated above a thin basement membrane (pinkish color to PAS reaction) (Fig. 1C). The oval basophilic nucleus of the epithelial cell was observed with surrounding by eosinophilic cytoplasm. Besides, our observation was seen the lymphocytes (1-2 cells) within the lymphocytic spaces (Fig. 2B). Mittal and Munshi [10] reported that the function of the lymphocytic space in freshwater fish was supply and up-take the

nutrient for the cell proliferation in the epithelium. In fusiform sub-layer, it was stratified layer and contained various cell types including mucous secreting (or goblet cell), sacciform (or sacciform granulated cell) and malpighian cells (or epidermal cell) (Figs. 1A-1C, 2A-2B). Several mucous secreting cells were observed. Under longitudinal sections, the oval shape of the mucous secreting cell was appeared in proximal region of the epidermis along the head skin. This cell was easily identified by magenta stain with PAS staining method. Similarly, the previous investigations have been reported in other fishes such as *Acipenser gueldenstaedii* and *Pangio kuhlii* [2]. It is noted that its roles used for drug reduction and predator evasion [2]. The oval shape of the sacciform cell was the largest cell types, which its size was larger than the mucous secreting cell. The function of the sacciform cell is unclear, however, it may produce a noxious substances to protect pathogenic microorganism [11] [12]. Another cell, irregular shape either cuboidal or irregular shapes of the malpighian cell layer was occurred in the proximal region; however, it changed into squamous shape in distal region. The function of this cell is unknown and is discussed with relating to filamentous, as indicating to produce the filament [11]. A cuticle layer or mucous coat in this layer was lined by the squamous epithelium, hence it was separately derived from the mucous [1]. Taste bud was still seen in some areas of the head skin (Fig. 2C). Its characteristic was a typically pear-shaped structure composing of several elongated taste cells. The role of taste bud is well-considered to be the chemoreceptor, which is acquiesced of the food [1].

Underlying the epidermis layer, the dermis was appeared. It could be divided into two sub-layers: stratum spongiosum (or outer papillary layer) and stratum compactum (Figs. 1A-1C, 2A-2B). The stratum spongiosum distinctly contained the loose connective tissue. This sub-layer was arranged parallel to the skin surface. Moreover, eosinophilic leukocytes, chromate- phores (or pigment cells) (Figs. 1C, 2A) and peripheral nerve were clearly seen. Of interest, the chromatophores in this species were only observed in stratum spongiosum layer. This result appeared most similar in mention to *Pangio kuhlii* and *Astronotus ocellatus* [2]. Conversely, *Astatotilapia lapiaburtoni* was detected the chromatophores both dermal and epidermal layers [2]. The stratum compactum was the large part of dermis that contained dense connective tissue. Lastly, the hypodermis was also basically observed between the stratum compactum and muscular elements. It was rarely seen in this study.

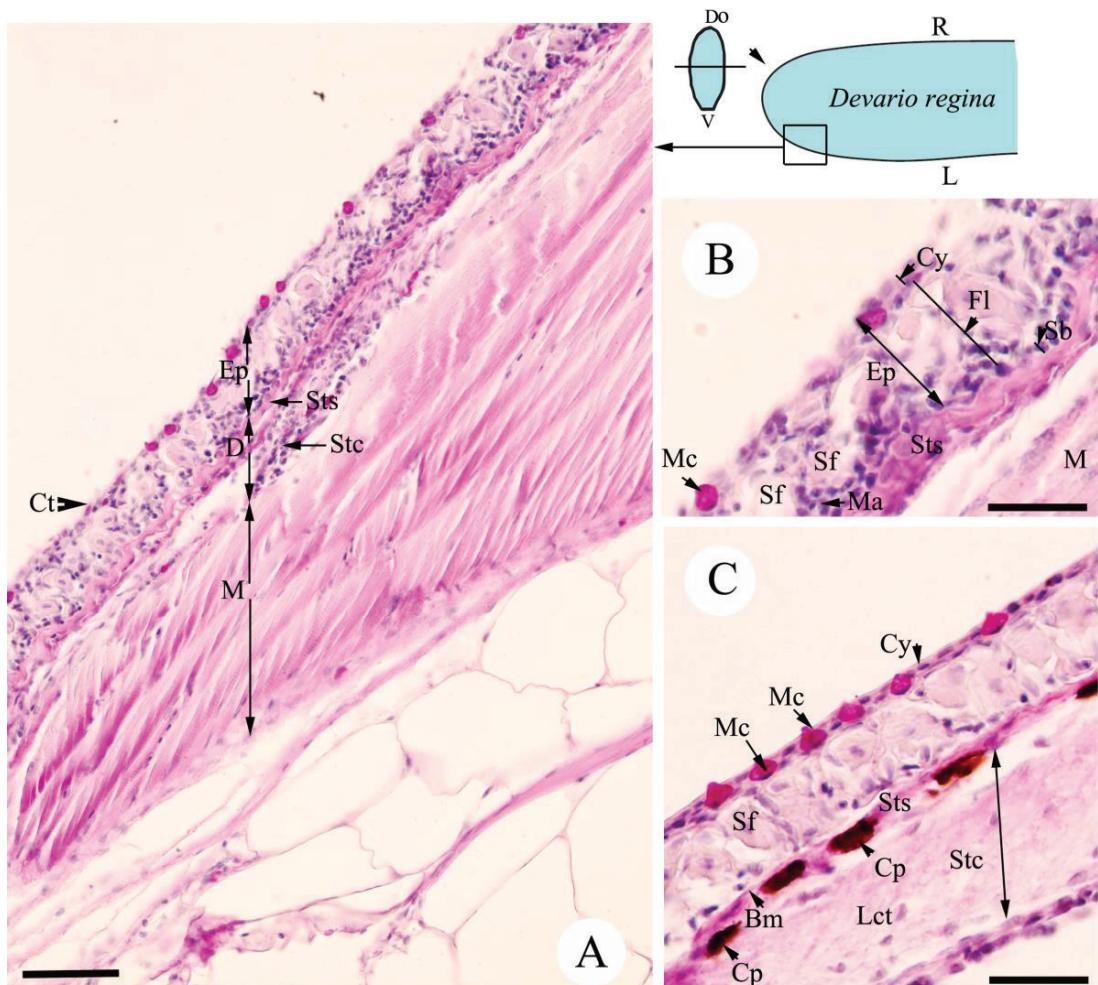
### Conclusion

Based on PAS analysis, we concluded that the head skin of *D. regina* was histologically composed of five layers: cuticle, epidermis, basement membrane, dermis and hypodermis,

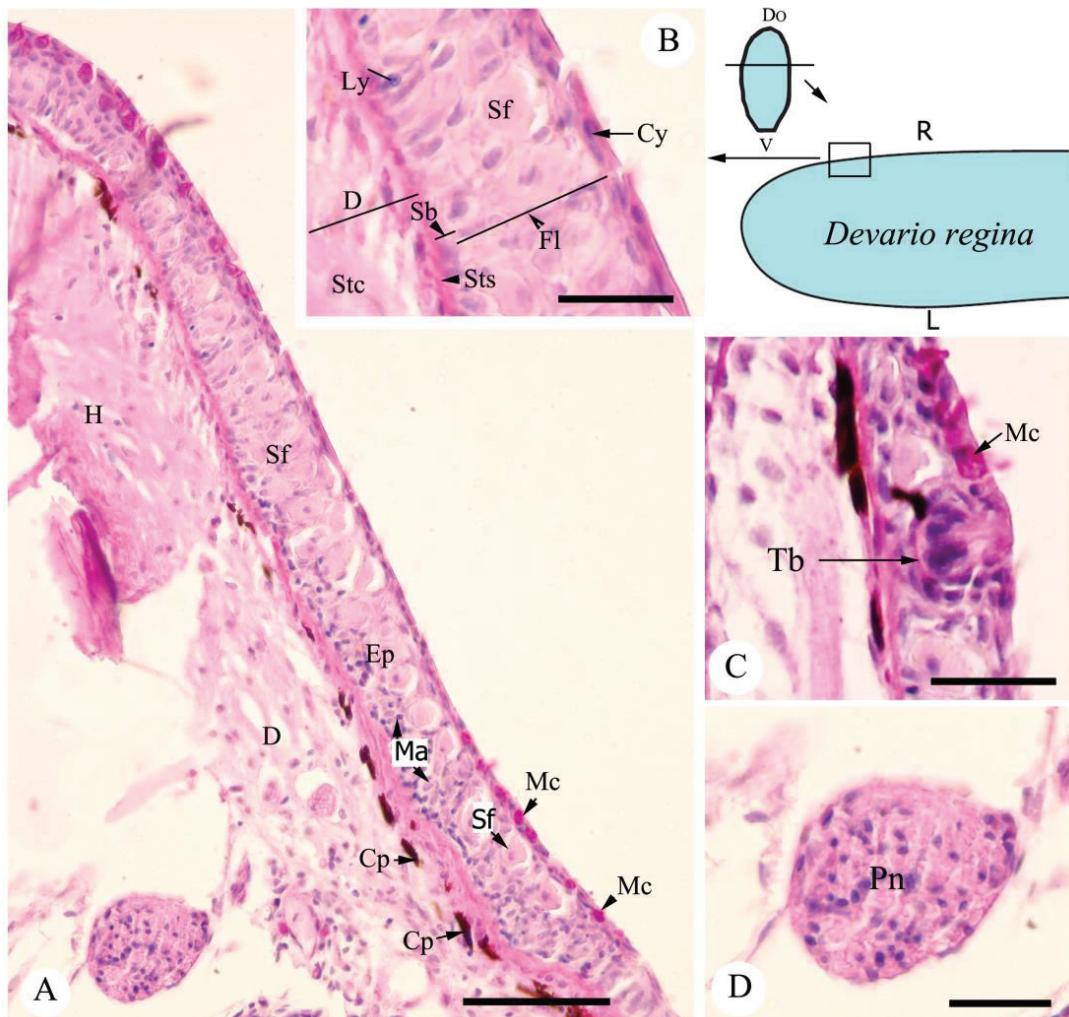
which is shown in both histological images (Figs. 1-2) and schematic diagram (Fig. 3). However, its hypodermis was noted that it was slightly observed. This result provides to increase the information in the basic histology.

### Acknowledgements

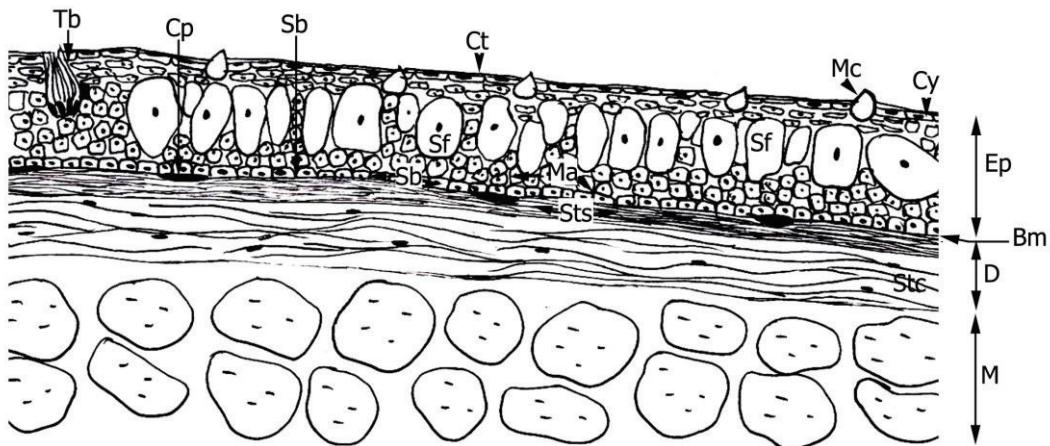
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**Figure 1** Section through the left part (L) of the *Devario regina* head. In this micrograph the skin can be histologically observed (A) and cell compositions (B-C). Note: Bm, basement membrane; Cp, chromatophore; Ct, cuticle; Cy, cuticular layers; D, dermis; Do, dorsal part; Ep, epidermis; Fl, fusiform layer; Lct, loose connective tissue; M, muscular element; Ma, malpighian cell; Mc, mucous secreting cell; R, right head; Sb, stratum basal; Sf, sacciform cell; Stc, stratum compactum; Sts, stratum spongiosum; v, ventral part. Scale bar, A = 100  $\mu$ m and B-C = 50  $\mu$ m; Periodic Acid Schiff (PAS)



**Figure 2** Section through the right part (R) of the head (H), *Devario regina*. In this micrograph the skin can be histologically observed (A) and cell compositions (B-D). Note: Cp, chromatophore; Cy, cuticular layers, D, dermis, Do, dorsal part; Ep, epidermis; Fl, fusiform layer; L, left head; Ly, lymphocyte; Ma, malpighian cell; Mc, mucous secreting cell; Pn, peripheral nerve; Sb, stratum basal, Sf, sacciform cell; Stc, stratum compactum; Sts, stratum spongiosum; Tb, taste bud; v, ventral part. Scale bar, A = 100  $\mu$ m and B-D = 30  $\mu$ m; Periodic Acid Schiff (PAS)



**Figure 3** A schematic diagram of the head skin in the *Devario regina* showing its structural characterization. Note: Bm, basement membrane; Cp, chromatophore; Ct, cuticle; Cy, cuticular layers, D, dermis, Ep, epidermis; Fl, fusiform layer; M, muscular element; Ma, malpighian cell; Mc, mucous secreting cell; Sb, stratum basal, Sf, sacciform cell; Stc, stratum compactum; Sts, stratum spongiosum; Tb, taste bud.

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