

The Structure of the Skin, Types and Distribution of Mucous Cell of Yangtze Sturgeon (*Acipenser dabryanus*)

La Estructura de la Piel, los Tipos y Distribución de las Células Mucosas del Esturión Yangtze (*Acipenser dabryanus*)

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SUMMARY: The structural characteristics of the skin, types and distribution of mucous cells of Yangtze sturgeon (*Acipenser dabryanus*) were studied at the light microscope level, stained with Haematoxylin-eosin (HE) and Alcian blue-periodic acid Schiff (AB-PAS). The skin of both was composed of epidermis and dermis. The dermis was divided into stratum spongiosum and stratum compactum. The stained color of stratum compactum was stained more deeply than that of stratum spongiosum. The skin thickness displayed differences in the fish at different body positions. The thickest of epidermis layer was on the dorsal region for Yangtze sturgeon, reversely, the thinnest was the mandibular region; Stratum spongiosum on the mandibular region was the thickest, the stratum spongiosum of the maxillary region was not obvious. In summary, keratinized spines, a kind of keratin derivative, are widely distributed in the mandibular, ventral, dorsal, and caudal peduncle skin surface for Yangtze sturgeon, and some pit organs mainly present in the skin surface of the maxillary and ventral regions. In short, the small amount of mucous cells in the skin of Yangtze sturgeon and the type of mucous cell were main Type IV, nevertheless there was a distribution of a few Type III.

KEY WORDS: Yangtze sturgeon; Skin; Morphological structure; Mucous cell.

INTRODUCTION

Fish skin differs from other exposed vertebrate skin most notably at the surface where living epidermal cells are in direct contact with the environment (Henrikson & Matoltsy, 1967). The skin and mucous layer of fish provide the first line of defense against infection by potential environmental pathogens and play a critical role in survivability. Fish skin provides defense, protection, breathing, sensory properties, and maintains the body's internal environment balance (Hawkes, 1974). The skin of extant fishes consists of an epidermis and an underlying dermis with glands, lateral lines and receptors, however differences occur in the structure and thickness of fish skin composition due to the differences of species and their environments (Jiang, 2012; Lei *et al.*, 2012; Shi, 2013; Ragueira *et al.*, 2016). The epidermis of the skin is equipped with different types of cells which are involved in the secretion of surface mucus-mucous cells which are known to contain various biologically active macromolecules, predominantly glycoproteins. Glycoproteins are implicated in important biological functions at immune reactions (Raj *et al.*, 2011).

The River Sturgeon, *Acipenser dabryanus*, (also known as the Yangtze sturgeon) is endemic to China and historically was distributed in the mainstem of the upper Yangtze River and the lower Jinsha River (Zhang *et al.*, 2011). Presently, the species is rare and classified as a Category I protected species (highest level of concern). Decline of this sturgeon was caused by overharvest and construction of the Gezhouba Dam on the mainstem of the Yangtze River (Wei *et al.*, 1997). Previous studies about *A. dabryanus* were mainly focused on its hematopoiesis, genome, stress, etc (Zhou *et al.*, 2014; Wang *et al.*, 2016; Liu *et al.*, 2017), relatively little is known about their skin.

The objective of this paper is to compare the histology of skin, skin type, and distribution of mucous cell in *A. dabryanus*, and it will help to investigate the connection between the mucus immune mechanisms in each species skin and their inhabited environment. This study has important implications for comparing the relation of biological functions to evolution and will provide a valuable reference for resource protection and biodiversity.

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MATERIAL AND METHOD

Live specimens of *A. dabryanus* (mean \pm S.D., standard length, 44.26 ± 2.16 cm; standard weight, 288.94 ± 51.41 g; $n = 10$) were obtained from the Sichuan Fisheries Research Institute. The water temperature was maintained at 17 ± 1 °C and the pH was maintained 7-8. The DO level varied in the range 11 ± 1 mg/L. The fish in the aquarium were acclimated for 2 weeks prior to sampling.

All fish were anesthetized with an overdose of MS-222 (100 mg/L). After the skin of *A. dabryanus* was taken from the maxillary, the mandibular, the dorsal, the ventral, and caudal peduncle regions (Fig 1). The sample size is $2\text{cm} \times 1\text{cm} \times 0.5\text{cm}$. Tissue samples from the fish were fixed in Bouin's fluid for a 24h period. Following fixation, each tissue sample was cut into $1\text{cm} \times 0.5\text{cm} \times 0.5\text{cm}$ size pieces, soaked in decalcifying fluid, and then dehydrated in an ethanol series of ascending concentrations. After drying, samples were cleaned in xylene, embedded in paraffin, sectioned in a transverse plane at 6 μm , and stained with haematoxylin-eosin (H/E) and Alcian blue-periodic acid Schiff (AB (pH=2.5)-PAS).

Samples were observed using a Nikon ECLIPSE Ti-s microscope. The whole mount preparations were utilized to count the number of the mucous cells using a stage micrometer (Object micrometer) with a scale graduate in units of 1/100 (1 division = 0.01 mm). Biovis Image Plus, an advanced image processing and analysis software, was used for measuring dimensions of the skin. Samples of 10 randomly selected sites of skin were analyzed for each estimation. Estimations were based on the data obtained from a sub-sample of ten fish. Data was pooled separately and results were expressed as mean value \pm standard deviation (S.D.) throughout.

RESULTS

The skin of *A. dabryanus* was composed of epidermis, dermis and hypodermis (Fig. 2-1).

Epidermis of the *A. dabryanus*. The *A. dabryanus* epidermis, obviously are divided into three principal layers: the superficial layer, the middle layer and the basal layer. The main structural component of the epidermis consists of stratified irregular epithelial cells arranged in several tiers. The basal layers composed of columnar basal cells covering the basement membrane. Its nucleus, located in the upper part of basal cells, is columnar (Fig. 2-2). The long axis of the basal cells were perpendicular to the basement membrane. The middle layer encompasses multilayer irregular epithelial cells and mucus cells. The nucleus of epidermis cell is round or oval in the intermediate layer, which is located in the center of the cell. The nuclei of epithelial cells, which is close to mucus cells, were crescent or irregular. The epidermis and the dermis are separated by a relatively thick basement membrane. Some pit organs are present in the skin surface of the maxillary and ventral regions (Fig. 2-3). In addition, the maxillary region had more pit organs than ventral and mandibular regions, while none were observed in the dorsal and caudal peduncle regions. The depth of the pit organ in the maxillary region was (169.71 ± 31.54 μm) and (192.23 ± 61.00 μm) in the ventral region. The structure of the pit organ formed a 'cave' due to the depression from the edges to the center. The pit organ was composed of willow leaf shape supporting cells and pear-shaped receptor cells. The receptor cell was strongly stained; in contrast, the supporting cell was weakly stained (Fig. 2-4). Differences occurred in the skin thicknesses from samples taken at different positions (Table I): from thick to thin on the epidermis, in order: dorsal with (133.30 ± 41.31 μm),

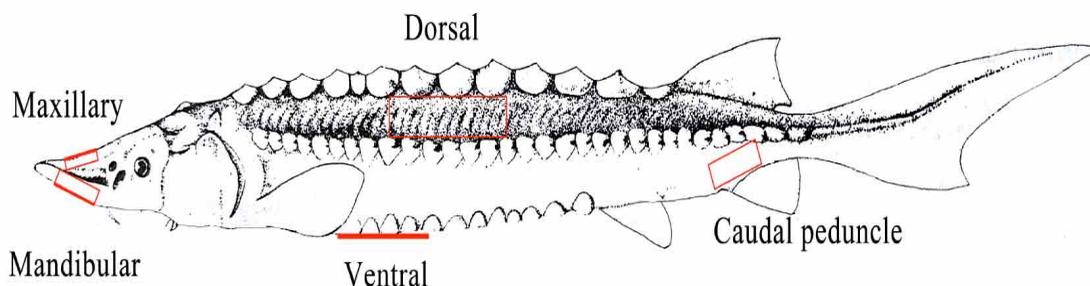


Fig. 1. The schematic diagram of sampling site.

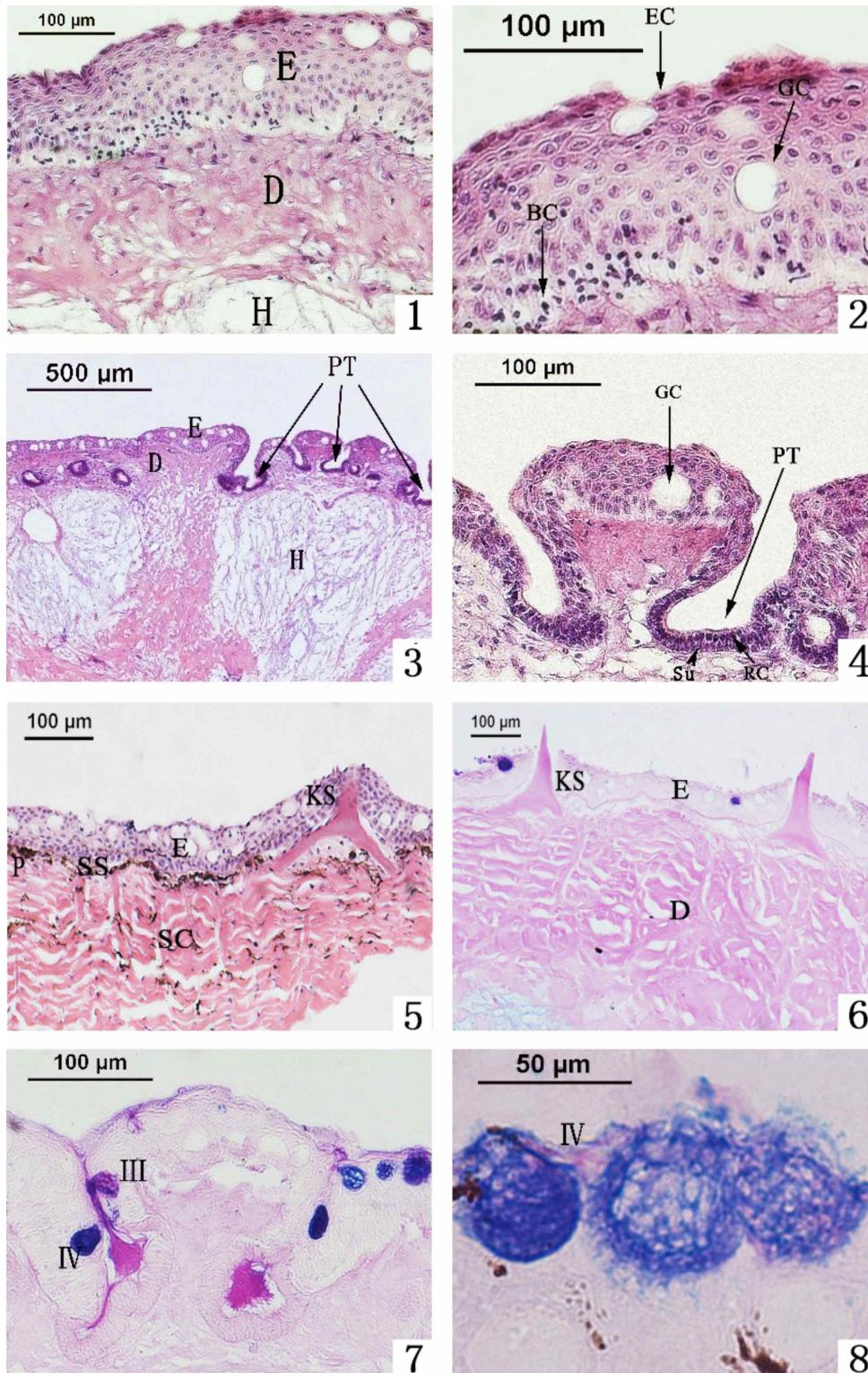


Fig. 2. Observation of the structure of the skin about *Acipenser dabryanus*. Note:1-5 for HE staining; 6-8 for AB-PAS staining
 Explanation: 1, 2, 6 The ventral skin of *A. dabryanus*; 3, 7 The maxillary skin of *A. dabryanus*; 4 The pit organ of *A. dabryanus*; 5,8 The dorsal skin of *A. dabryanus*. E: Epidermis; D: Dermis; H: Hypodermis; SS: Stratum spongiosum; SC: Stratum compactum; EC: Epithelial cells; P: Pigment; KS: Keratinized spines; BC: Basal cell; GC: Goblet cell; PT: Pit organ; Su: Supporting cell; RC: Receptor cell; ?: Type? mucus cell; ??: type ? mucus cell.

maxillary with (98.12 ± 39.99 mm), ventral with (74.51 ± 29.31 mm), caudal peduncle with (53.61 ± 14.63 mm), mandibular with (47.43 ± 18.81 mm).

Dermis of the *A. dabryanus*. The epidermis is bonded to a deeper skin layer known as the dermis. The dermis is divided into stratum spongiosum and stratum compactum (Fig. 2-5). The stratum spongiosum is a loose connective tissue and contains pigment cells, which are mainly melanophores. The pigment cells were mainly distributed between epidermis and dermis or dermis and hypodermis and it is very sparse in stratum compactum of dermis. The irregular pigment cells were deeply stained by HE (Fig. 2-5). In quantity, there were the most pigment cells in the dorsal region, while there were few pigment cells in the maxillary, mandibular, ventral and caudal peduncle skin. There were sharp keratinized spines stretched out from the stratum spongiosum of dermis to the skin surface (Figs. 2-5, 6). The stratum spongiosum of dermis was discontinuous, and it presented with keratinized spines. Keratinized spines all increase the thickness of the stratum spongiosum (Table II). The keratinized spines were the most frequent in dorsal skin, in the mandibular and caudal peduncle skin. They were least frequent in ventral skin, and absent in maxillary skin. From thick to thin on the stratum spongiosum, in order, was the mandibular region with (38.17 ± 6.44 mm), the dorsal region with (33.51 ± 14.99 mm), the caudal peduncle with (17.46 ± 11.10 mm), and the ventral region with (15.56 ± 9.32 mm) (Table I). The maxillary region had few keratinized spines and stratum spongiosum. From thick to thin on the stratum compactum,

in order, was the dorsal region with (563.43 ± 202.53 mm), the caudal peduncle with (488.97 ± 222.58 mm), the mandibular region with (281.10 ± 150.37 mm), the ventral region with (261.48 ± 56.41 mm), and the maxillary region with (90.15 ± 33.62 mm) (Table I).

Mucous cells. The mucous cells have a typical ‘goblet cell’ appearance and the cells were full of viscous granule. The mucous cells of each kind of fish skin were divided into four types by AB-PAS staining: type I mucous cells contained PAS-positive neutral mucopolysaccharides and were stained red; type II mucous cells contained PAS-negative acid mucopolysaccharides and were stained blue; type III mucous cells contained mainly neutral mucopolysaccharides, together with small quantities of acid mucopolysaccharides, the cell were stained purple-reddish; type IV mucous cells contained mainly acid mucopolysaccharides, together with small quantities of neutral mucopolysaccharides, the cell were stained bluish violet.

In the skin of the *A. dabryanus*, there were both types III and IV mucous cells, with type IV being the most numerous (partial acid mucopolysaccharides) and only a few type III mucous cells were observed (partial neutral mucopolysaccharides) (Fig. 2-7, 8). The mucous cells displayed an uneven distribution and quantity in the different sample parts. The quantity of mucous cells was seen in the lowest amounts in the caudal peduncle, which contained an occasional a type IV mucous cell. Mucous cells were less frequent in ventral skin, but the cells identified were type

Table I. The Thickness of the skin in different parts of *A. dabryanus* (Mean±SD).

Parts	Thickness of epidermis(m)	Thickness of dermis(m)	
		Stratum spongiosum	Stratum compactum
Maxillary	98.12±39.99		90.15±33.62
Mandibular	47.43±18.81	38.17±6.44	281.10±150.37
Dorsal	133.30±41.31	33.51±14.99	563.43±202.53
Ventral	74.51±29.31	15.56±9.32	261.48±56.41
Caudal peduncle	53.61±14.63	17.46±11.10	488.97±222.58

Table II. The comparison of stratum spongiosum of each parts of the *A. dabryanus* skin with and without keratinized spines (Mean±SD).

Parts	Thickness of Stratum spongiosum(m)	
		Parts
Mandibular	with keratinized spines	122.60±55.42
	without keratinized spines	38.17±6.44
Dorsal	with keratinized spines	148.25±29.95
	without keratinized spines	33.51±14.99
Abdomen	with keratinized spines	33.30±16.37
	without keratinized spines	15.56±9.32
Caudal peduncle	with keratinized spines	79.02±39.60
	without keratinized spines	53.61±14.63

IV mucous cells. Mucous cells were more frequent in maxillary region, where there were many type IV mucous cells and few type III mucous cells. Mucous cells were the most frequent at the dorsal region, where one type IV mucous cell was observed at a distance of about 0.18 mm, and one type III mucous cell was observed at a distance of about 8.46 mm. In addition, there were mucous stores in the depression of the pit organ at the maxillary and ventral skin. With an irregular shape and size, these mucous cells' core were round or oval and some were located in the upper part of epidermis, and some in the bottom of epidermis.

DISCUSSION

The structure of the skin. The main function of skin covering the external surfaces of vertebrates is to protect the organism from its environment and to preserve the consistency (Henrikson & Matoltsy). The main function of the thickness of skin is its protection from infection. The teleost skin is where the epidermis is close to the dermis. It is practical to subdivide teleost epidermis into basal, mid, and surface layers. The dermis may be divided into a stratum spongiosum and a stratum compactum (Hawkes). Some adaptations of fish to the stresses that exist in aquatic environments are seen in specialized body surface structures, such as scales. *A. dabryanus* have no scales, but keratinized spines are present in the skin surface of *A. dabryanus*. In vertebrates, the epidermis undergoes some degree of keratinization, but this is not true for most fish (Raj *et al.*). *A. dabryanus* inhabits the shallow water, where it forages upon the algal growth on the rocks and gravel bottom, and often inhabits in the pebble region. In contrast, the *A. dabryanus*, experiences friction with the faster waters and rocky substrate and has skin that is rough with plenty of keratinized spines. These fish are also covered with 5 rows of bone plates, to protect themselves (Zhang *et al.*). These keratinized spines were similar in structure to that of contact organs. More cells skin cutting derivatives, analogous dermal structures, known as contact organs, are present on the scales or fin rays. The main shape of these contact organs was a swept-back hook (Zhang & Shen, 1999a). *Xenodexia ctenolepis* has been known to have contact organs all over the body of males, females, and juveniles (Zhang & Shen, 1999b). *Trachidermus fasciatus* has many protuberances in both male and female. The head, back, body side and caudal peduncle skin surface has many keratinized spines in the Yellow River Roughskin Sculpin, but the Luanhe River Roughskin Sculpins had numerous mounds and no sharp keratinized spines (Jiang). Contact organs have three functions: stimulation of the females in breeding; provide tactile stimulation, and as tactile receptors. In addition,

contact organs may have some hydrodynamic significance correlated with adaptation to torrential currents (Wiley & Collette, 1970).

The skin of fish contains many sensory organs. The structure of a fishes' sensory organs were usually constituted by supportive cells and sensory cells (Mukai *et al.*, 2008; Dezfuli *et al.*, 2009), such as the pit organ of the *A. dabryanus*. Sensory cells were considered an important part of the sense organs, because they could convert mechanical stimulation into an electrical signal. Nerve fibers connected at the bottom of the sensory cells could transfer stimulation to the nerve center. Some pit organs are present in the skin surface of the *A. dabryanus*. The pit organ, also known as superficial neuromasts. The structures of pit organ formed a 'cave' and have mechanical, thermal and electrical pain functions (Jiang). Superficial neuromasts abundance is higher in fish that inhabit still-water or in fish that are not excellent swimmers (Marshall, 1971). Schellart and Engelmann showed that superficial neuromasts detect the velocity of water flow and are highly sensitive to a vibrations in still-water (Engelmann *et al.*, 2000).

Many pit organs were observed in the *A. dabryanus*'s rostral, and began to appear and develop even in larvae, before initial feeding began (Shi). The pit organ could also assist in the detection of the velocity of the water flow (Jiang). Liang (1996) confused ampullary organs with pit organs when studying the feeding behavior of Chinese sturgeon. Shi believed that ampullary organs mainly existed in cartilaginous fishes, and there were pit organs at the ventral part of the Chinese sturgeon's and *A. dabryanus*'s snout. Ampullary organs were different in structure to other sense organs. The surface of the ampullary organ is very smooth and does not have sensory hairs. The nucleus of the Chinese sturgeon receptor cells are round in shape are completely surrounded by supporting cells (Shi).

Types and distribution of mucous cell. Kitzan & Sweeny (1968) were the earliest to research the mucous cells of fish. Based on the difference in PAS staining reaction, the goblet cells were divided into three types by light microscope and electron microscope observation: stains bright red (I), stains pink (II), stains deep red (III) (Kitzan & Sweeny). The standard classified types in accordance to PAS-positive degrees was confused, and does not accurately reflect the contents of mucous cells. Uribe & Sibbing (1984) showed that carp mucous cells, when stained with Alcian Blue (AB), Periodic Acid Schiff (PAS) and High Iron Diamin (HID), classified into three types I: Sacciform mucous cells, II: Pyriform cells, III: Goblet cells. The sacciform mucous cells are all heavily stained with HID and thus contain sulfo mucines. Pyriform cells lining the orobuccal as well as the pharyngeal lumen

appear to contain sialo mucines as they are almost HID negative. The same applies to the intestinal goblet cells (Uribe & Sibbing). The classifications of mucous cells are defective by the differences in size and shape, irrespective of the contents of mucous cells and their secretion. The function of the mucous cells are closely related to mucopolysaccharides. Mucous cells form different shapes at different positions, and are steric, however the paraffin sections were steric space. Some studies have found that the main components of mucus are neutral or acidic mucopolysaccharides, while others suggest proteins (Zaccone, 1973). Through comparison of various methods, the method which divided mucous cells into four types by AB-PAS staining is scientific and practical.

Each species has differences in the quantity and distribution of mucous cells on different parts of the body's skin. Deep-water fish mucous cells are much more abundant in outer regions of the body. This may be an adaptation in relation to its peculiar bottom-scooping habits, disturbing bottom mud more frequently in search of food, thus requiring efficiency in the fish keeping its body surface clean (mucus has remarkable power to precipitate mud held in suspension). In conclusion, the relationship between the abundance of mucous cells in a species and environment is extremely close (Pickering, 1974; Singh & Mittal, 1990). In addition, the number of mucous cells in the scaleless area of a fishes' body was more than the lepidote area, as seen in Harris & Hunt (1975) study that shows that fins have significantly fewer mucous cells than the rest of the body. The types and counts of *A. dabryanus* skin mucous cells were lower than Dabry's sturgeon digestive tract mucous cells (Yang *et al.*, 2018), it was inferred that it was relative to their life habits and skin surface structure. *A. dabryanus* is a potamodromous freshwater fish that does not travel long distances (Zhuang *et al.*, 1997), and the skin of *A. dabryanus* were covered with not only 5 rows bone plates, but also covered with keratinized spines to prevent fish body damage commendably. A high quantity of mucous cells in dorsal skin had protective effects. The thickness of the skin epidermis in each part of the fish is consistent with the number of mucous cells, since the thicker epidermis is, the more mucous cells are needed to secrete mucus to lubricate. There are many keratinized spines and mucous cells on the dorsal skin, but the number of keratinized spines in the rest is inversely proportional to the number of mucous cells. Therefore, it is speculated that skin fish have important protective effects. The mucous cells of maxillary and ventral skin were observed around the pit organs, even more so for pit organs that contained mucus. This suggested that as a part of the pit organs, the mucus of maxillary and ventral skin could assist the detection of the velocity of water flow. This indicates that the type and distribution of mucous cells were relative with species and

external environmental. It well knew that the structure of the skin was one of presentation for ambient environment.

In general, a higher concentration of the mucous cells are present in the epidermis of the anterior regions than in the posterior regions, because the mucus would tend to migrate backwards on the fish when they moved forward through the water. The posterior regions of the body would receive mucus not only from the mucous cells in that region but also from more anterior regions (Pickering). The anterior region of the fish would only receive the mucus from its own epithelium and might consequently require a higher concentration of mucous cells. Li *et al.* (2010) reported that the distribution of mucous cells in *Silurus asotussk* showed that the density of mucous cells was the highest in the ventral skin and the lowest in the dorsal skin, suggesting that this density prevents injury to the *Silurus asotus* ventral skin. Another example is in the *Anguilla Anguilla*, where their anterior regions had fewer mucous cells than posterior regions (Lin *et al.*, 2008).

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RESUMEN: Se estudiaron las características estructurales de la piel, los tipos y la distribución de las células mucosas del esturión Yangtze (*Acipenser dabryanus*) con microscopio de luz, teñidas con hematoxilina-eosina (HE) y azul alción-ácido de Schiff (AB-PAS). La piel estaba compuesta por epidermis y dermis. La dermis se dividía en estrato esponjoso y estrato compacto. El grosor de la piel mostró diferencias en los peces en diferentes posiciones del cuerpo. La capa más gruesa de la epidermis se observó en la región dorsal del esturión Yangtze; a la inversa, la más delgada en la región mandibular. El estrato esponjoso en la región mandibular era el más grueso, el estrato esponjoso de la región maxilar no era visualizado. En resumen, las espinas queratinizadas,

un tipo derivado de la queratina, estaban ampliamente distribuidas en la superficie de la piel del pedúnculo mandibular, ventral, dorsal y caudal en el esturión Yangtze, y algunos órganos en fosas, presentes principalmente en la superficie de la piel de las regiones mandibular y ventral. En resumen, la pequeña cantidad de células mucosas en la piel del esturión Yangtze y el tipo de célula mucosa eran células principales tipo IV, sin embargo, se observaron algunas células tipo III.

PALABRAS CLAVE: Esturión Yangtze; Piel; Estructura morfológica; Célula mucosa.

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