

Mucous Cells and Their Structure on the Epidermis of Five Appendages in the Korean Flat-headed Goby, *Luciogobius guttatus* (Pisces; Perciformes)

Jong-Young Park*, Ik-Soo Kim, Yong-Joo Lee¹ and Hyun-A Baek

Faculty of Biological Sciences, Chonbuk National University, Korea and

¹Jeonju National University of Education, Korea

The flat-headed goby, *Luciogobius guttatus*, inhabits tidepools and river mouths, and stays under stones on the dried bottom for the duration of the low tide. To know the relationship of its respiration and habit in this fish, the epidermis of five appendages was observed. The epidermis has three layers: the outermost layer, middle layer and stratum germinativum. The outermost layer is composed of polygonal cells or rather flattened cells, and mucous cells. The unicellular mucous cells showing acid mucopolysaccharides are 11.1 to 16.1 μm in mean height and in one or two rows. The middle layer consists mainly of large epidermal cells that are swollen by adjacent epidermal cells and arranged in a web-shaped structure. The swollen cells are 12.3 to 15.2 μm in mean height and arranged in one to 11 layers. Since the swollen cells occupy the entire height of the epidermis, the epidermis is thick. A large number of blood capillaries are present just below the stratum germinativum. Taste buds are distributed at intervals on the surface of the epidermis. Based on these epidermal structures, it is likely that *L. guttatus* utilizes cutaneous respiration in a dual respiratory systems.

Key words : Epidermis, mucous cell, swollen cell, intertidal fish, *Luciogobius guttatus*

Introduction

The Korean flat-headed goby, *Luciogobius*, consisted of 4 species as *L. grandis*, *L. guttatus*, *L. koma* and *L. saikaiensis* (Kim *et al.*, 2005). They are negatively buoyant and demersal because of absence of swimbladder, perching upright on the substrate when not swimming. They inhabit tidepools and river mouths, and stays under stones on the dried bottom for the duration of the low tide (Choi *et al.*, 2002; Kim *et al.*, 2005). Intertidal fishes including flat-headed gobies can be found emerged under boulders or in crevices during low tides, occasionally in shallow pools in water that does not cover their bodies.

As tide recedes, water levels drop until the substrate itself is exposed (Horn and Riegler, 1981; Horn *et al.*, 1999). Eventually, their habitats cause aquatic hypoxia during low tides.

Therefore, intertidal fishes have an additional air-breathing mechanism to overcome their hypoxia (Bridges, 1988; Graham, 1997; Horn *et al.*, 1999). However, most marine intertidal fishes, unlike freshwater air-breathing fishes, have no specialized or enclosed air-breathing organ (Graham, 1997). In marine fishes including intertidal fishes, the respiration using air must take place across the same surfaces in air as it does in water: the gills, the skin, and perhaps the linings of the opercular and buccal cavities (Graham, 1997; Horn *et al.*, 1999). Marine fishes in the world known as having air-breathing organ, reported in the field or the laboratory, are about

*Corresponding author: park7877@chonbuk.ac.kr

60 species in 12 families (Graham, 1997; Horn *et al.*, 1999). However, *Luciogobius* had not been studied at all. Through the histology of the epidermis in *L. guttatus*, therefore, we are going to get information on the structure and histochemistry of the epidermis and discuss on possibility of cutaneous air respiration.

Materials and Methods

Two males and three females, ranging from 59.8 mm to 71.6 mm in standard length, were collected in June, 2004 by a hand and a small net from Gigo-ri, hangseon-myeon, Hanam-gun, Jeollanam-do, in the southern coast Korea. The specimens were anaesthetized with MS222. For histological examination, the skin fragments, $5 \times 5 \text{ mm}^2$, were fixed in 10% neutral buffered formaldehyde and 5 regions of the skin were taken from the following fins: dorsal fin, pectoral fin, anal fin, caudal fin and sucking disc.

We dehydrated these sections through a standard ethanol series to 100%, cleared in xylene and then embedded in wax (Paraplast, Oxford). We deparaffinized $5 \mu\text{m}$ sections and stained them with Harris hematoxylin, Ehrlich hematoxylin, and counter-stained with eosin, and Masson trichrome stain (Gurr, 1956) for general histology. Mucins of gland were demonstrated by alcian blue solution (AB) at pH 1.0 and 2.5 (Steedman, 1950; Lev and Spicer, 1964), and the periodic acid-schiff (PAS) method. In addition, the PAS technique was employed in combination with AB and vice-versa for neutral and acid mucins. Acid mucin was shown by metachromatic reactions with toluidine blue (Tock and Pearse, 1965). Also, high iron diamine (HID) and with AB (Spicer, 1965) were used for nature of the acid mucins.

For evaluations of the epidermis, we took two skin fragments by each region per specimen by Video Test-Master (VT image analysis program) on hematoxylin and eosin preparations. More than 10 sections were used for measurement per two skin fragments.

Results

General structure of *Epidermis*

In 5 different appendage regions, the structures of the epidermis exhibited always a stratified epithelium which consisted fundamentally

of the superficial layer, the middle layer and the stratum germinativum (Figs. 1 and 2). This principal structure is seen in all 5 regions, although there are differences in their thickness or stratum especially in the middle cell layer.

The basal layer, the stratum germinativum was composed of a single layer of either cuboidal cells, or more or less columnar cells (Figs. 2A–2B). Blood vessels located adjacent to the taste bud organs of the epidermis are found in the intra-epithelial layer (Fig. 2A). A number of blood capillaries are present just under the basal membrane.

The middle layer of epidermis is simpler in structure. This layer consists of few round or cuboidal epithelial cells but mainly large epithelial cells (Figs. 2A–2E). The large cells are swollen adjacent to the epithelial cells, called as swollen cell. The swollen cells have an oval nucleus and a homogeneous cytoplasm, and their boundary is clear (Figs. 2A–2F). Occasionally, they appeared to vesicles or vacant acellular structures due to a loss of nucleus. The swollen cell does not show any histochemical reactions for general histochemical stain as well as staining for polysaccharides. The swollen cells are various in height, mean 12.3 to $15.2 \mu\text{m}$ and multi-layered, 1 to 11 layers. The layer of the swollen cell is much in the caudal fin, reaching 3

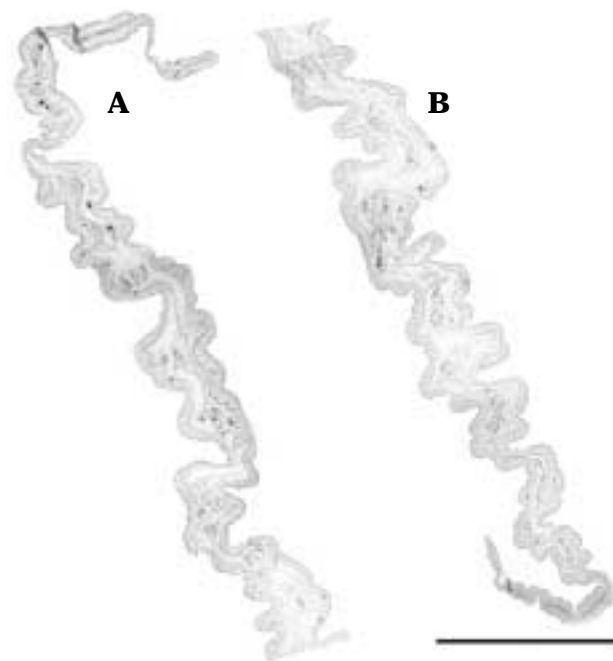


Fig. 1. Base of the Pectoral fin of *Luciogobius guttatus*. A, upper region; B, lower region (Bar = 0.3 mm).

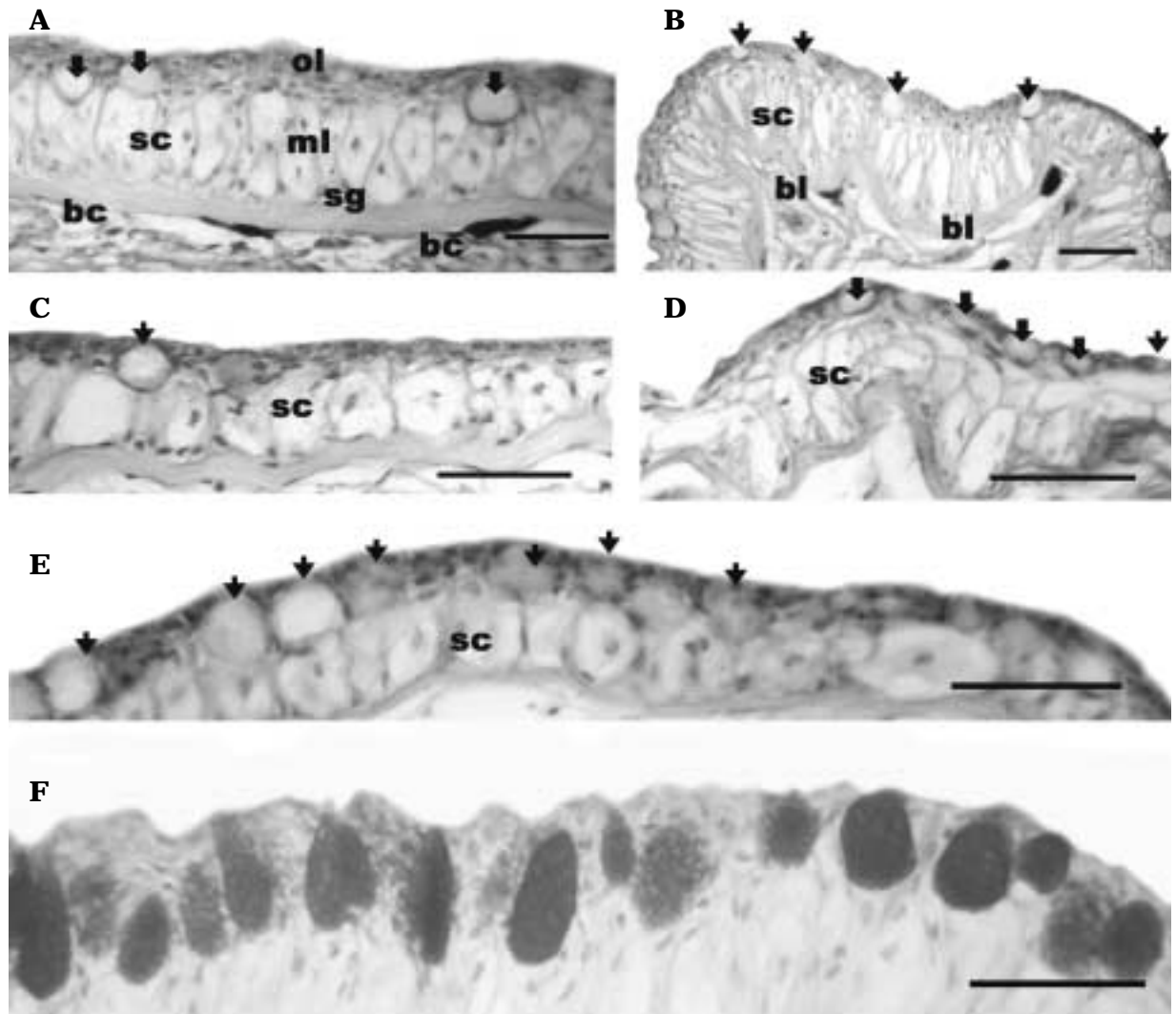


Fig. 2. Transverse sections of the epidermis of *Luciogobius guttatus* (Bars indicate 50 μm). A, The base of the dorsal fin. Ehrlich haematoxylin and eosin. The epidermis consisted of 3 layers; outermost layer (ol), middle layer (ml) and stratum germinativum (sg). The swollen cells (sc) occupy the height of the middle layer and plenty of blood capillaries (bc) are found in the dermis. Arrows, mucous cell. B, The base of the caudal fin. Ehrlich haematoxylin and eosin. C, The base of the anal fin. Ehrlich haematoxylin and eosin. D, The base of the pectoral fin. Ehrlich haematoxylin and eosin. E, The base of the sucking disc. Ehrlich haematoxylin and eosin. F, The base of the sucking disc. The mucous cells show deep purple with PAS reaction. Abbreviation; bc, blood capillaries; ml, middle layer; ol, outermost layer; sc, swollen cell; sg, stratum germinativum; arrow, mucous cell.

to 11 layers, and less in other appendages, similarly 1 to 7 layers (Table 1). Therefore, the thickness of the epidermis is mainly determined by the size or number of stratum of the swollen cells arranged in the middle layer. By these stratified swollen cells, the middle layer shows a web-shaped structure in appearance (Fig. 2).

The outermost layer is composed of two types of cell, epidermal cell shaped with round or polygonal cells arranged in 1 to 3 layers, and mucous

gland cell (Fig. 2). The unicellular mucous cells are spherical in shape and their nuclei are basal, spherical or oval with a thin rim of slightly basophilic cytoplasm. The mucous cells open to the exterior by a short narrow neck that opens on the surface by a wide pore. The mucous cells with mean 11.1 to 16.1 μm in height are arranged in 1 to 2 rows. Number of the distributed mucous cell are about 0 to 7 per 100 μm , much in anal fin and similar in other appendages (Table 1).

Table 1. The thickness of the epidermis, height of mucous cell and the swollen cell in 5 appendages of the epidermis of *Luciogobius guttatus*

Regions	Thickness of the epidermis (μm)		Height of the mucous cell (μm)		¹⁾ No. of mucous cell	Height of the swollen cell (μm)		²⁾ Stratum of swollen cell
	mean \pm SD	Min. ~ Max.	mean \pm SD	Min. ~ Max.		mean \pm SD	Min. ~ Max.	
Base of dorsal fin	38.6 \pm 13.5	22.5 ~ 69.3	14.3 \pm 3.1	8.8 ~ 18.6	1 ~ 4	15.2 \pm 6.4	6.8 ~ 32.9	1 ~ 4
Base of pectoral fin	25.0 \pm 8.3	8.7 ~ 17.6	11.6 \pm 2.3	8.7 ~ 17.6	0 ~ 5	12.3 \pm 4.8	5.2 ~ 26.4	1 ~ 5
Base of anal fin	40.0 \pm 16.2	19.0 ~ 87.5	12.7 \pm 3.2	5.6 ~ 17.6	0 ~ 7	15.2 \pm 6.5	4.7 ~ 42.6	1 ~ 7
Base of caudal fin	53.4 \pm 13.1	29.2 ~ 80.2	16.1 \pm 3.6	8.9 ~ 23.1	0 ~ 5	15.2 \pm 11.3	4.8 ~ 41.2	3 ~ 11
Base of sucking disc.	25.7 \pm 12.2	6.9 ~ 48.4	11.1 \pm 3.9	6.2 ~ 18.6	1 ~ 5	13.1 \pm 6.6	5.2 ~ 26.7	1 ~ 4

Min, minimum; Max, maximum; ¹⁾Number of mucous cell per 100 μm in the middle layer; ²⁾Stratum of swollen cell arranged in the middle layer.

Table 2. A summary of the histochemical tests performed to show the nature of the mucous cell in *Luciogobius guttatus*

Techniques employed	Mucous cell
Hematoxylin & eosin	\pm B
Weigerts iron Haematoxylin	+++B
Masson trichrome	-
PAS	++R
PAS after digestion in malt diastase/PAS	++R
Alcian blue (1.0)	++B
Alcian blue (2.5)	++B
Alcian blue/PAS	++B, BR, R
PAS/Alcian blue	++B, BR, R
Toluidine blue	γ -meta
Acetylation/PAS	-
Methylation/AB	-
Methylation/Saponification/AB	+B
Aldehyde fuchsin	+R
Aldehyde fuchsin/Alcian blue (2.5)	++BR
High iron diamine	+N
High iron diamine/alcian blue	+++BN

B, blue; BN, bluish black; BR, bluish red; G, green; N, black; PN, pink; R, red; +, increasing intensity of reaction; \pm , fairly present; -, absent.

Histochemistry of mucous cell

The mucous cells were predominantly distributed in the outermost layer of the skin but were sparse in the middle layer. The mucous cells gave a deep red color reaction with PAS technique, which is diastase resistant, γ -metachromasia with toluidine blue, blue with the AB at pH 1.0 and 2.5 (Table 2). The mucous cells, giving a red color with aldehyde fuchsin and black color with high iron diamine, were likely to be sulfomucins. In mild methylation/AB and acetylation/PAS techniques they were negative, and in methylation/saponification-AB they were stained blue. Their nuclei were purple or red color with AB-PAS reaction and Masson trichrome stain.

Thickness of the epidermis

The thickness of the epidermis depends mainly on the thickness of the middle layer, ie, the size and the stratum of the swollen cells, particularly their size or number (Table 1). In the 5 appendages, the thickness of epidermis was thicker in the caudal fin (mean 53.4 $\mu\text{m} \pm 13.1$) and anal fin (mean 40.0 $\mu\text{m} \pm 16.2$), which have large swollen cell (mean 15.2 $\mu\text{m} \pm 6.5$) and numerous strata (1 to 11 layers) in the middle layer. Whereas the pectoral fin and the sucking disc have a thinner epidermis, mean 25.0 $\mu\text{m} \pm 8.3$ and mean 25.7 $\mu\text{m} \pm 12$, respectively. The pectoral fin is mean 12.3 $\mu\text{m} \pm 4.8$ in the height and 1 to 5 rows and the sucking disc is mean 13.1 $\mu\text{m} \pm 6.6$ and 1 to 4 rows.

Discussion

The air-breathing fishes are dual respiratory system, which use a combination of aerial and aquatic respiration (Graham, 1997; Randle and Chapman, 2005). Among them, the skin related to cutaneous air respiration have been known as the following structures: a thicker epidermis due to several types of glands, intra-epithelial blood capillaries, abundant blood capillaries in the superficial dermis, a well-developed vascularization, reduction or absence of scales, and a definite area with acid mucopolysaccharides in the dermis (Jakubowski, 1958; Liem, 1967; Johansen, 1970; Mittal and Munshi, 1971; Mittal and Banerjee, 1974; Mittal *et al.*, 1980; Whitear, 1986; Suzuki, 1992; Yokoya and Tamura, 1992; Park and Kim 1999, 2000; Park *et al.*, 2000; Park, 2002a, b; Park *et al.*, 2003a, b; Zhang *et al.*, 2003).

Luciogobius guttatus had a thick epidermis

that consisted of unicellular mucous cell in the outermost layer and voluminous swollen cells giving web-shaped structures in the middle layer as the above know air-breathing fishes. In addition, plenty of blood capillaries are situated under the stratum germinativum.

In *L. guttatus*, the mucous cells were present in the outermost layer of the epidermis and acid mucopolysaccharides in nature. Acidic mucopolysaccharides were of characters appearing in the cutaneous respiratory fishes such as *Monopterus*, *Mastacembelus*, *Amphipnous*, and *Misgurnus*, and *Boleophthalmus* (Mittal and Munshi, 1971; Mittal and Banerjee, 1974; Mittal *et al.*, 1980; Park and Kim, 1999; Park *et al.*, 2003a).

The middle layer of the epidermis consisted mainly of swollen cells which are varied in the size and the stratum. These the swollen cells which are varied were reported only in the skin of amphibious mudskipper fishes, some *Periophthalmus* and *Boleophthalmus* (Whitear, 1986; Al-Kadhomy and Hughes, 1988; Low *et al.*, 1990; Yokoya and Tamura, 1992; Suzuki, 1992; Zhang *et al.*, 2000; Park, 2002; Park *et al.*, 2003a). These stratified swollen cells occupied the entire height of the epidermis, which it shows web-structure in appearance as in the mudskipper fishes.

L. guttatus has no scale. Absence and reduction of the scales was mainly found in cutaneously respiratory fishes such as *Misgurnus*, *Periophthalmus*, *Heteropneustes*, *Mastacembelus*, *Iksookimia*, *Amphipnous*, *Monopterus*, and *Liobargrus* considered as an adaptation for movement or burrowing (Liem, 1967; Mittal and Munshi, 1971; Whitear, 1986; Park, 2002; Park *et al.*, 2003a, b).

L. guttatus have no intra-epithelial blood capillaries. But *L. guttaus* has plenty of blood capillaries just beneath the basal layer of the epidermis. Liem (1967) had been experimentally demonstrated in the epidermis of *Monopterus albus* with only large mucous cells, no intra-epithelial blood capillaries. Although the blood vessels are situated in the dermis, oxygen obtained through the mucous cell of the epidermis is sufficient to be able to diffuse to the deeper dermis (Jakubowski, 1958; Liem, 1967; Mittal and Munshi, 1971; Whitear, 1986; Graham, 1997; Horn *et al.*, 1999).

References

- Al-kadhomy, N.K. and G.M. Hughes. 1988. Histological study of different regions of the skin and gills in the mudskipper, *Boleophthalmus boddarti* with respect to their respiratory function. *J. Mar. Biol. Ass. U. K.*, 68 : 413~422.
- Bridges, C.R. 1988. Respiratory adaptations in intertidal fish. *Amer. Zool.*, 28 : 79~96.
- Choi, Y., J.H. Kim and J.Y. Park. 2002. Marine fishes of Korea. Kyo-Hak Publishing Co. Ltd., 645 pp (in Korean).
- Graham, J.B. 1997. Air breathing fishes: Evolution, diversity, and adaptation. Academic Press, San Diego, 299 pp.
- Gurr, G.T. 1956. A practical manual of medical and biological staining techniques. Interscience, New York. 126 pp.
- Horn, M.H., K.L.M. Martin and M.A. Chotkowski. 1999. Intertidal fishes. Academic Press, San Diego, 399 pp.
- Horn, M.H. and K.C. Riegler. 1981. Evaporative water loss and intertidal vertical distribution in relation to body size and morphology of stichaeoid fishes California. *J. Exp. Mar. Biol. Ecol.*, 50 : 273~288.
- Jakubowski, M. 1958. The structure and vascularization of the skin of the pond-loach (*Misgurnus fossilis* L.). *Acta. Biol. Cracoviensia*, 1 : 113~127.
- Johansen, K. 1970. Air breathing in fishes. In: Hoar W.S. and D.J. Randall (eds), *Fish Physiology IV*. Academic Press, New York, pp. 361~411.
- Kim, I.S., Y. Choi, C.L. Lee, Y.J. Lee, B.J. Kim and J.H. Kim. 2005. Illustrated book of Korean fishes. Kyo-Hak Publishing Co., Korea, 613 pp (in Korean).
- Lev, R. and S.S. Spicer. 1964. Specific staining of sulphated groups with alcian blue at low pH. *J. Histochem. Cytochem.*, 12 : 309.
- Liem, K.F. 1967. Functional morphology of the integumentary, respiratory, and digestive systems of the synbranchoid fish, *Monopterus albus*. *Copeia* (1967) : 375~388.
- Low, W.P., Y.K. IP and D. J.Y. Lane. 1990. A comparative study of the gill morphometry in the mudskippers-*Periophthalmus chrysospilos*, *Boleophthalmus boddaerti* and *Periophthalmodon schlosseri*. *Zool. Sc.*, 7 : 29~38.
- Mittal, A.K. and J.S.D. Munshi. 1971. A comparative study of the structure of the skin of certain air-breathing fresh-water teleosts. *J. Zool. Lond.*, 163 : 515~532.
- Mittal, A.K. and T.K. Banerjee. 1974. Structure and keratinization of the skin of a fresh-water teleost *Notopterus notopterus* (Notopteridae, Pisces). *J. Zool. Lond.*, 174 : 314~355.
- Mittal, A.K., M. Whitear and S.K. Agarwal. 1980. Fine structure and histochemistry of the epidermis of the fish, *Monopterusuchia*. *J. Zool. Lond.*, 191 : 107~125.
- Park, J.Y. 2002a. Morphology and histochemistry of the skin of the spined cobitid fish, *Iksookimia koreensis*, in relation to respiration. *Folia Zool.*, 51 : 241~247.
- Park, J.Y. 2002b. Structure of the skin of an air-breathing mudskipper fish, *Periophthalmus magnuspinnatus*. *J. Fish Biol.*, 60 : 1543~1550.
- Park, J.Y. and I.S. Kim. 1999. Structure and histochemistry of skin of mud loach, *Misgurnus anguillicaudatus* (Pisces, Cobitidae), from Korea. *Korean J. Ichthyol.*, 11 : 109~116.

- Park, J.Y. and I.S. Kim. 2000. Structure and cytochemistry of skin in spined loach, *Iksookimia longicorpus* (Pisces, Cobitidae). Korean J. Ichthyol., 12 : 25 ~ 32.
- Park, J.Y., I.S. Kim and S.Y. Kim. 2000. Histological study on skin of the amphibious fish, *Periophthalmus modestus*. Korean J. Biol. Sci., 4 : 315 ~ 318.
- Park J.Y., Y.J. Lee, I.S. Kim and S.Y. Kim. 2003a. A comparative study of the regional epidermis of an amphibious mudskipper fish, *Boleophthalmus pectinirostris* (Gobiidae, Pisces). Folia Zool., 52 : 431 ~ 440.
- Park J.Y., I.S. Kim and S.Y. Kim. 2003b. Structure and histochemistry of the skin of a torrent catfish, *Liobagrus mediadiposalis*. Environ. Biol. Fish., 66 : 3 ~ 8.
- Randle, A.M. and L.J. Chapman. 2005. Air-breathing behavior of the african anabantoid fish *Ctenopoma muriei*. J. Fish Biol., 67 : 292 ~ 298.
- Spicer, S.S. 1965. Histochemistry manual. The university of Kansas Medical Center, Kansas City, 54 pp.
- Steedman, H.F. 1950. Alcian blue 8G: a new stain for mucin. Quart. J. Micr. Sci., 1 : 477 ~ 479.
- Suzuki, N. 1992. Fine structure of the epidermis of the mudskipper, *Periophthalmus modestus* (Gobiidae). Japan. J. Ichthyol., 8 : 379 ~ 396.
- Tock, E.P.C. and A.G.E. Pearse. 1965. Preservation of tissue mucins by freeze-drying and vapour fixation. J. R. Microsc. Soc., 84 : 519 ~ 537.
- Whitear, M. 1986. The skin of fishes including cyclostomes: epidermis. In: Bereiter-Hahn J., A.G. Matoltsy and K. S. Richards (eds), Biology of the integument Vol. 2 Vertebrates. Springer Verlag, New York, 9 ~ 64.
- Yokoya, S. and O.S. Tamura. 1992. Fine structure of the skin of the amphibious fishes, *Boleophthalmus pectinirostris* and *Periophthalmus cantonensis*, with special reference to the location of blood vessels. J. Morphol., 214 : 287 ~ 297.
- Zhang, J., T. Taniguchi, T. Takita and A.B. Ali. 2000. On the epidermal structure of *Boleophthalmus* and *Scartelaos* mudskippers with reference to their adaptation to terrestrial life. Ichthyol. Res., 47 : 359 ~ 366.

Received: August 1, 2005

Accepted: September 9, 2005

한국산 미끈망둑 *Luciogobius guttatus* (농어목) 5개 부속지의 표피구조 및 점액세포 박종영* · 김익수 · 이용주¹ · 백현아

전북대학교 생물과학부 · ¹전주교육대학교 과학교육과

조간대와 강하구에 출현하는 미끈망둑 *Luciogobius guttatus*은 썰물 동안에 건조하기 쉬운 자갈 바닥 아래에서 서식하는 특징을 보인다. 이러한 서식처를 선호하는 미끈망둑의 호흡체계를 조사하기 위해 지느러미 및 흡반을 포함하는 5개의 부속지의 표피구조를 관찰하였다. 표피는 모두 맨바깥층 (outermost layer), 중간층 (middle layer), 기저층 (stratum germinativum)으로 구성된다. 맨바깥층은 다형세포 또는 편평세포, 그리고 점액세포로 구성되어 있으며, 특히 단일세포성의 점액세포는 11.1~16.1 μm 의 크기로 1~2층으로 배열되어 있으며, 산성다당류로 확인되었다. 중간층은 이웃한 표피세포로 팽대해진 팽대세포 (swollen cell)로 구성되어 있으며, 12.3~15.2 μm 의 크기로 1~11층으로 배열되어 마치 거미망구조를 보인다. 이러한 팽대세포로 인해 상피 두께의 대부분을 차지한다. 많은 모세혈관이 기저막 아래에 분포하고 있으며, 미뢰 (taste bud)가 표피에 존재한다. 이러한 표피의 특징은 미끈망둑이 이중호흡체계인 피부호흡 (cutaneous respiration)과 밀접한 관계가 있는 것으로 생각된다.