

Study on the Respiratory Organ of Spined Loach, *Iksookimia longicorpa* (Pisces, Cobitidae), in Relation to the Air-breathing System

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To observe the respiratory system in relation to the air-breathing organ in *Iksookimia longicorpa*, micro-anatomical investigation was performed on the epidermis and on intestinal tract fragments. The epidermis was distinguished by two types of skin glands, a small mucous cell and a large club cell. The mucous cell was acid sulfomucins (some sialomucins), but the club cell did not give any histochemical tests for mucosubstances. The presence of a well defined lymphatic system with small lymphocytes was established in the stratum germinativum layer of the epidermis. A large number of blood capillaries run very close to each other just below the basement membrane. The straight intestinal tract is divided into an intestine and rectum, which consisted of a mucosa (epithelial layer), lamina propria-submucosa, muscularis, and serosa. The intestine and rectum have shorter mucosal folds and a thinner wall. The majority of the epithelial mucous cells contain acid sulfomucines. Based on the above results, *I. longicorpa* adapts to poor dissolved oxygen conditions by using an additional respiratory system using air through the epidermis, not the intestines.

Key words : skin, acidic sulfomucins, mucous cell, club cell, intestine, *Iksookimia longicorpa*

Introduction

The respiration of most fishes is performed through the gills using the dissolved oxygen in water. However, some fish live in warm and stagnant reservoirs, ponds and ricefields undergoing periodic drought which causes a reduction of dissolved oxygen in the water. Such water bodies can be inhabited by fishes which can derive their oxygen from such modified organs using air as the intestine (Cobitidae), the skin (Cobitidae, Anguillidae, Nototeridae, Gobiidae), the branchial chambers (Anabatidae, Osphromenidae,

Channidae), the swim bladder (Dipnoi), the labyrinthine organ (*Anabas*), and others (Liem, 1967; Johansen, 1970; Tamura *et al.*, 1976; Niva *et al.*, 1981; Munshi and Hughes, 1986; Whitear, 1986; Moitra *et al.*, 1989; Itazawa and Hanyu, 1991; Ishimatsu *et al.*, 1998; Park and Kim, 1999, 2001; Zhang *et al.*, 2000; Park, 2002; Park *et al.*, 2003a, b). Among the air-breathing fishes, the family Cobitidae was known as performed through the skin or the intestine: the skin in *Misgurnus fossilis*, *M. anguillicaudatus*, and *Iksookimia koreensis* (Jakubowski, 1958; Park and Kim, 1999; Park, 2002), the intestine in, *M. fossilis*, *Lepidocephalichthys guntea*, *M. anguillicaudatus* and *M. mizolepis* (Koyama 1958a, b; Johansen 1970;

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Moitra *et al.*, 1989; Itazawa and Hanyu 1991; Park and Kim, 2001; Park *et al.*, 2003b).

Among Cobitid fish, whereas, the *Misgurnus* has two modified organs, the skin and the intestine. The Korean endemic a spined loaches, *I. longicorpa*, inhabits the rocky or stony bottoms of mountain streams causing hypoxia during the dry season of summer. These habits are very similar to those of *Misgurnus* of the same family. Therefore, we are going to get any information whether *I. longicorpa* to take up air use both the epidermis and intestine, or separately.

Material and Methods

The observed 10 specimens were collected from the Seomjin River, Korea and ranged from 76.5 mm to 99.5 mm in standard length. The dorsal epidermis and intestine fragments for histological examination were fixed in 10% neutral buffered formaldehyde.

These fragments were dehydrated through a standard ethanol series to 100%, cleared in xylene and then embedded in wax (Paraplast, Oxford). 5 μm sections were deparaffinized and stained with Harris's hematoxylin, iron alum hematoxylin, counter-stained with eosin, and Masson trichrome stain (Gurr, 1956) for general histology. For blood cell giemsa method was used.

Mucin of gland were demonstrated by alcian blue solution (AB) at pH 1.0 and 2.5 (Steedman, 1950; Lev and Spicer, 1964), and periodic acid-Schiff (PAS) method with or without prior digestion with diastase/saliva (Lillie and Greco, 1947). In addition, 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, Aldehyde fuchsin and with AB (Spicer and Meyer, 1960), and high iron diamine (HID) and with AB (Spicer, 1965) were used for nature of the acid mucins.

Acetylation and deacetylation for the confirmation of hydroxyl group was performed following

Lillie (1954), Methylation and demethylation (Spicer, 1960) were done to confirm the acidic nature of the mucins. For evaluations of the epidermis and intestine, we used Carl Zeiss vision (LE REL. 4.4).

Results

1. Epidermis

The epidermis of *I. longicorpa* is composed of the outermost layer, middle layer and stratum germinativum (Fig. 1A). The average thickness of the epidermis is approximately 135.9 μm (range 116.4 ~ 169.6 μm).

The stratum germinativum is composed of a single layer of cuboidal cells. Round or oval lymphatic spaces containing one or two small lymphocytes are situated between the cuboidal cells (Figs. 1A and C). The lymphocytes are found with deeply stained nuclei, surrounded by small amounts of faintly stained cytoplasm inside these spaces (Fig. 1C). The lymphocytes stain purple in AB and blue in PAS. The lymphocytes penetrate the middle layer, often reaching up to the outer cell layers of the epidermis. Blood vessels located adjacent to the sense organs of the epidermis are found in the intraepithelial layer.

The middle layer is composed mainly of voluminous club cells and a few flask or spherical mucous cells and a few lymphocytes (Figs. 1A and B). In between these skin glands the elongated spindle shaped epidermal cells are found vertically. The cell boundary of these cells is usually not clear as it greatly expanded or stretched out due to the collateral pressure of these glands. The club cells arranged in 2 to 5 layers are oval or spherical in shape (Figs. 1A and B). They are usually uninucleate with having an oval nucleus, sometimes binucleate having two nuclei very close to each other. Some club cells have a few vacuole in their cytoplasm (Figs. 1A and B). The club cell is an average of 41.1 μm (\pm 9.8) in height (range 23.7 to 62.5 μm). The thickest part of the epidermis is the middle layer.

The outermost layer is composed of polygonal cells and a few flattened cells arranged in 2 to 6 rows (Figs. 1A and B). There are small spherical or flask-shaped unicellular mucous glands in this layer (Fig. 1A). The mucous cells have a large spherical body and a short narrow, and a basal, spherical or oval nucleus with a thin rim of slightly basophilic cytoplasm. Their size are mean $14.7 \mu\text{m}$ (± 1.7) in height (range 12.9 to $17.6 \mu\text{m}$), smaller and fewer than club cell. They have highly vacuolated and basophilic secretory matter. These cells give a deep red color reaction with the PAS technique, which is diastase resistant, -metachromasia with toluidine blue, blue with the AB at pH 1.0 and 2.5. The mucous cells,

showing a red color with aldehyde fuchsin and black color with high iron diamine, are likely to be sulfomucins. In mild methylation/AB and acetylation/PAS techniques they are negative, and in methylation/saponification-AB they are stained blue. Their nuclei are a purple or red color with AB-PAS reaction and Masson trichrome stain. A few lymphocytes are present between the epidermal and mucus cells.

The pit organs consisted of single or in groups of two to three are distributed on the surface of the skin (Fig. 1B). Each pit organ is a pear-shaped structure, sunk below the epithelial cells and lie directly on the basement membrane. In the intraepithelial layer there are blood vessels.

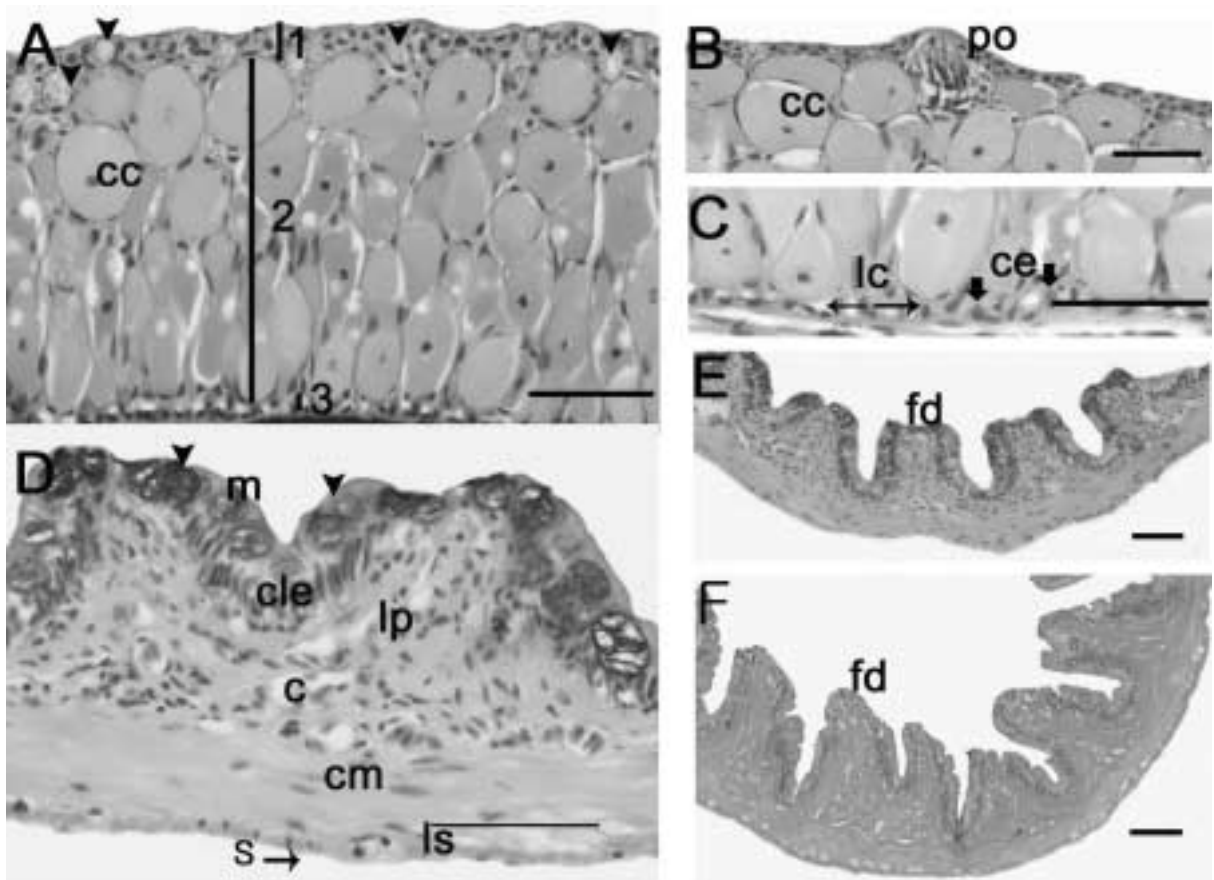


Fig. 1. Structures of the epidermis (A to C) and intestinal tract (D to F) of *Iksookimia longicorpa* stained with Ehrlich haematoxylin and eosin (bars indicate $50 \mu\text{m}$). A-C. The dorsal skin; D and E, the rectum close to the anus; F, the intestine. (Abbreviations: 1, outermost layer; 2, middle layer; 3, stratum germinativum; c, capillary; cc, club cell; ce, cuboidal epithelium; cle, columnar epithelium; cm, circular muscle; fd, folder of intestine; lc, lymphocyte; lp, Lamina propria-submucosa; ls, longitudinal muscle; po, pit organ; s, serosa; arrowhead, mucous cell.

2. Intestinal tract

The intestinal tract is a straight tube which could be divided into two regions, intestine and rectum. The tract consisted of four layers: mucosa, lamina propria-submucosa, muscularis, and serosa (Figs. 1D to F).

Mucosa

The mucosal epithelium of the intestinal tract consists of the following cells: columnar epithelial cells, mucus-secreting cells, lymphocytes, and granulocytes (Fig. 1D)

The columnar epithelial cells are tall and cylindrical with oval nuclei situated either centrally or toward the base of the cells (Fig. 1D). The mucous cells are spherical, flask-shaped, or ovoid, and interspersed among the columnar epithelial cells. The cells are highly vacuolated and basophilic haematoxylin and eosin preparations. The number of the mucous cells is about 10~11 in the intestine and 12~15 in the rectum per 100 μm . The mucosa with longitudinal folds has the lamina propria-submucosa and the lining epithelium along the entire length of the intestinal tract (Figs. 1D to F). The folds of the intestine are more or less complicated, and the height of the folds was mean 151.3 μm (± 33.7), ranging 79.0~199.2 μm . The folds of the rectum close to the anus become low and slightly wavy to straight, mean 53.2 μm (± 16.7) measuring 34.5 to 78.3 μm . Lymphocytes surrounded by small amounts of faintly stained cytoplasm are seen. Granular cells are seen in the mucosa and lamina propria-submucosa, but less in the muscularis and serosa.

Lamina propria-submucosa

The tissue lying below the mucosa consists of a lamina propria and submucosa with no intervening muscularis mucosa (Fig. 1D). The lamina propria contained thin collagen bundles in a mesh-like appearance and extended into the mucosal folds. The submucosa is composed of a stratum compactum lying between the lamina propria and the inner circular muscle layer of the muscularis (Fig. 1D). The stratum compac-

tum consists of bundles of coarse and uniform collagenous fibres which are arranged compactly in several layers. This layer has granular cells, lymphocytes, blood vessels and nerve cells (Fig. 1D).

Muscularis

The muscularis under the lamina propria-submucosa has a circular inner layer and a thinner longitudinal outer layer (Fig. 1D). Between two the muscle layers are small amounts of connective tissue containing blood vessels and fibres. The thickness of the muscularis is 48.8 μm (± 9.2) in the intestine and 31.7 μm (± 5.3) in the rectum.

Serosa

Peripheral to the muscularis, there is a serosa consisted of a thin layer of connective tissue covered by a single layer of squamous cells (Fig. 1D).

Discussion

The modification of the epidermis known as dual breathing fishes, have been documented as the following structures: a thicker epidermis due to several types of glands, intraepithelial 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; Park and Kim, 1999; Park, 2002; Park *et al.*, 2003a, b; Zhang *et al.*, 2003). However, among the above results, the epidermis of *Iksookimia longicorpa* have no intraepithelial blood capillaries, unlike the *Periophthalmus* and *Liobagrus*. Nevertheless, breathing is possible through the blood vessels in the dermis because diffusion of oxygen take place readily across the mucous coat of the epithelium (Jakubowski, 1958; Liem, 1967; Mittal and Munshi, 1971; Whitear, 1986; Graham, 1997; Horn *et al.*, 1999). Whereas,

I. longicorpa has mucous cell. Liem (1967) had been experimentally demonstrated in the epidermis of *Monopterus albus* with only large mucous cells devoid of intraepithelial blood capillaries. According to the above reports, the oxygen of *I. longicorpa* can obtain through the mucous cell is sufficient to be able to diffuse to the deeper dermis.

The structure of the intestinal tract of *I. longicorpa* was similar to that of most teleost as it consists of a mucosa, lamina propria-submucosa (submucosa), muscularis and serosa (Bullock, 1967; Reifel and Travill, 1979; Anderson, 1986; McLeese and Moon, 1989; Tibbetts, 1997). In particular, the intestinal tract of *Lepidocephalichthys guntea* (Moitra *et al.*, 1989), *Misgurnus mizolepis* (Park, 2002), and *M. anguillicaudatus* (Park *et al.*, 2003b) of the family Cobitidae has numerous blood capillaries within the mucosal epithelium. These fishes known as air-breathing fishes have a respiratory epithelium in order to make up for the deficient oxygen supply, and gas exchange probably occurs between the air taken from the intestine and blood circulating at the intestinal mucosa. However, the intestinal tract of *I. longicorpa* has no intracapillaries. Eventually, the mucous cell of the intestinal tract in *I. longicorpa* lubricates passage of food materials and protects the gut lining, no relation to oxygen uptake and intestinal respiration.

Seeing the above results, the air-breathing organ of *I. longicorpa* was performed by the epidermis, not the intestinal tract, unlike *misgurnus* of the same family Cobitidae using both the epidermis and the intestinal tract.

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공기호흡과 관련된 왕종개(미꾸리과어류)의 호흡기관에 관한 연구 박 종 영

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왕종개 *Iksookimia longicorpa*의 공기호흡에 관한 호흡체계를 조사하기 위해 표피 (epidermis)와 소화관 (intestinal tract)를 관찰하였다. 표피는 작은 점액세포 (mucous cell)와 곤봉세포 (club cell)로 이루어진 두 종류의 선세포 (gland cell)을 가진다. 점액세포는 대부분 acid sulfomucin으로 구성되었으나 곤봉상세포는 점액물질에 대한 어떠한 반응도 보이지 않았다. 기저층에는 작은 림파구를 포함하는 lymphocytes가 존재하며, 다량의 모세혈관이 기저층 바로 아래부분에 존재하고 있다. 장 (intestinal tract)은 거의 일직선형태이며, 크게 장 (intestine)과 직장 (rectum)으로 구성된다. 이러한 장은 점액층 (mucosa), 점막하층 (laminal propria-submucosa), 근육층 (musculary), 장막 (serosa) 층으로 구성되었다. 이러한 장은 짧은 fold와 얇은 벽으로 구성되었으며, 점막층은 acid sulfomucin의 점액을 가지고 있다. 공기호흡하는 어류들의 변형된 공기호흡 기관의 특징들과 비교해 볼 때 왕종개는 하천의 가뭄으로 인해 용존산소가 부족할 경우 장에서는 이루어지지 않고 표피에서만 공기를 흡입하는 것으로 생각된다.