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Histology and Morphometrics of the Epidermis of the Fins and Sucking Disc of the Mudskipper, *Periophthalmus modestus* (Pisces, Gobiidae)

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The epidermis of the mudskipper, *Periophthalmus modestus*, consists of three layers- the outermost layer, middle layer and stratum germinativum. Extensive fine blood capillaries are present near the superficial layer of epidermis and outermost layer in five fins and a sucking disc. The diffusion distance between the vascular capillaries and the surface of epidermis ranged from 3.6 to 10.9 μm: 3.6 μm in the sucking disc, 10.9 μm in the anal fin and 4.6 to 5.0 μm in the two dorsal fins. Rate of the surface area of respiratory epithelium, the surface area of the fine blood capillaries occupied per surface area of epidermis in 0.1 mm, is 3.7 to 4.4% in two dorsal fins and 1.1% in the anal fin. The middle layer is simpler in structure consisting of small or voluminous cells swollen by epidermal cells, and this layer appeared web-like. Well-developed lymphatic spaces containing lymphocytes existed in the stratum germinativum. The five fins and sucking disc had no epidermal glands.

Mudskippers such as *Boleophthalmus, Periophthalmus, Periophthalmodon* and *Scartelaos* belonging to the Gobiid subfamily Oxudercine are amphibious. They all live in mangrove swamps or on tidal flats, and move or skip briskly on the mudflats, or burrow into the mud. During the periods they are out of water, they breathe mainly air. These amphibious lifestyles allow many researchers to examine their respiratory system closely.

Among the mudskippers, the genus *Periophthalmus* consists of 12 species from Indo-Pacific to Africa, extending to Korea (Murdy, 1989). Of these, the oxygen uptake of *Periophthalmus cantonensis* (as *Periophthalmus modestus* by a review of Murdy) and *P. sobrinus* takes place through water and air, and on land, they rely mainly on the skin (Teal and Garey, 1967; Tamura et al., 1976; Ikebe and Oishi, 1996; Graham, 1997). *P. schlosseri* carries air to their mudflat burrows for their aerial respiration and to their embryos (Ishimatsu et al., 1998). In addition to these physiological studies, the general structures of the skin related to cutaneous air respiration have been reviewed by some workers (Whitear, 1986; Suzuki, 1992; Yokoya and Tamura, 1992; Zhang et al., 2000; Park et al., 2000; Park, 2002). However, these histological investigations were partially focused on restricted body skins such as the head and back, or operculum, abdomen and caudal region, and comparative data on their regional epidermis were not given. Moreover, there are no reports on the epidermis of the fins and sucking disc. Another mudskipper, genus *Boleophthalmus*, performed aerial respiration through only the epidermis of body regions except fins and a sucking disc (Park et al., 2003c).

The purpose of this paper is primarily to study whether a respiratory epithelium related with oxygen uptake is really present in accessory appendages in *P. modestus*. Therefore, we observed the structure of the epidermis from 6 different regions (5 fins and 1 sucking disc), and compared their diffusion distance and the rate of surface area of respiratory epithelium.

Materials and Methods

Two males and one female, ranging from 71.8 to 76.9 mm in standard length, were collected by a casting net from Amtae Island, Sinan-gun, Jeollanam-do, Korea. The specimens were anaesthetized with MS222 (Sigma). For histological examination, the skin fragments, 5×5 mm^2, were fixed in 10% neutral buffered formaldehyde and

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taken from different 6 regions: 5 fins (the pectoral, the first dorsal, the second dorsal, the anal and the caudal fin) and a sucking disc fused with pelvic fins. The skin fragments were cut from their base part. We dehydrated these sections through a standard ethanol series to 100%, cleared in xylene and then embedded in Paraplast (Oxford). We deparaffinized 5 μm sections and stained them with Ehrlich hematoxylin, and HE. For evaluation of the epidermis, we examined sections of the skin by Video Test-Master (VT image analysis program) on hematoxylin and eosin preparations. The distance between the capillary endothelial cell and the epidermis was measured and assumed to represent the diffusion distance.

Results

General morphology

In 6 regional epidermises, five fins and a sucking disc of *Periophthalmus modestus*, the epidermis showed the same structure although there were differences in thickness or number of cell layers. The epidermis had a stratified epithelium consisting of several layers, and could be divided into three layers: stratum germinativum, middle layer and outermost layer (Fig. 1A).

The stratum germinativum consisted of a single layer of cuboidal cell, or more or less columnar cells. Small oval or round lymphatic spaces having small lymphocytes

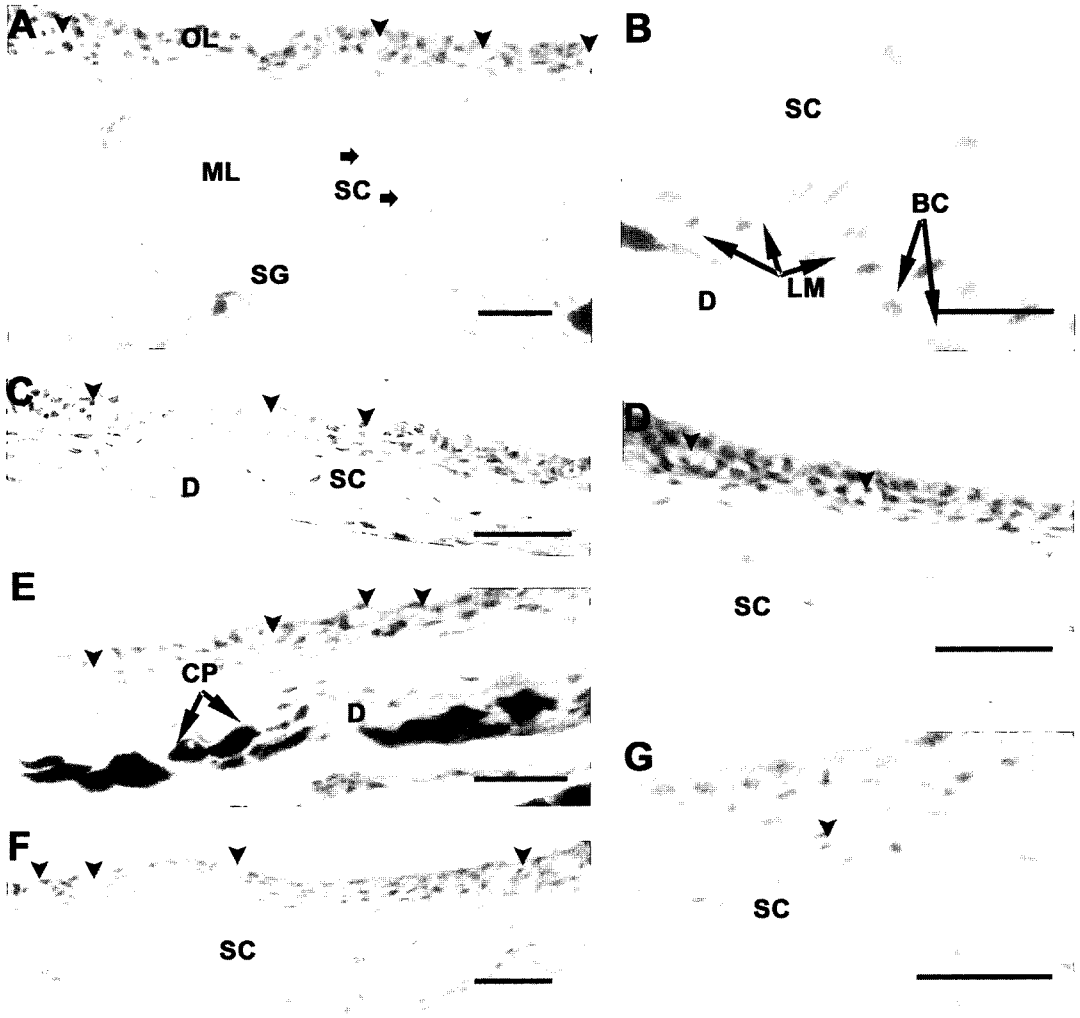

Fig. 1. Transverse sections of the epidermis of the base of 5 fins and a sucking disc from *Periophthalmus modestus* with Ehrlich haematoxylin and eosin. A, The epidermis of the sucking disc consists of outermost layer (OL), middle layer (ML) and stratum germinativum (SG). Blood capillaries (arrowheads) are found in the outermost layer and swollen cells (SC) in the middle layer. B, The stratum germinativum consists of basal cells (BC) and lymphocytes (LM). Swollen cells are seen in the middle layer and dermis (D) is situated beneath the stratum germinativum. C, In the 1st dorsal fin, numerous blood capillaries (arrowheads) are situated near the outermost layer and there are swollen cells (SC) and dermis (D). D, In the pectoral fin, blood capillaries (arrowheads) and swollen cells (SC) are found in the epidermis. E, In the 2nd dorsal fin, numerous blood capillaries (arrowheads) are found near the outermost layer and chromatophores (CP) are present in the dermis beneath the epidermis. F, Numerous blood capillaries (arrowheads) and swollen cells (SC) in the epidermis of the caudal fin are found. G, Small blood capillary (arrowhead) and swollen cells (SC) are present in the anal fin. Scale bars=25 μm.

are randomly present between basal cells. These lymphocytes had a strongly stained nucleus and weakly stained small amount of cytoplasm (Fig. 1B).

The middle layer was characterized by having simpler structure which consisted of larger spindle-shaped or round cells. The larger, swollen cells had spherical centric nucleus with homogenous cytoplasm, and their boundary was clear (Fig. 1A, 1D and 1G). The cytoplasm was not stained with HE. Occasionally, they appeared as vesicles or vacant acellular structures due to a loss of the nucleus (Fig. 1F and 1G). The large cells were variable, ranging from 7.0 to 61.0 μm in height. Because of a stratified middle layer composed of 3 to 15 layers of the swollen cells, this layer showed a web-like structure. A thicker epidermis had larger swollen cells and more numerous swollen cell layers. Blood capillaries and pigment cells were present.

The outermost layer had villi lining up the layer and was made up of 2 to 5 layers of flattened epithelial cells (Fig. 1A and 1D). A large number of fine blood capillaries were situated in between these cells and accompanied by dermal collagen. The red blood cells were very close to, or more or less far to the surface of this layer according to regional epidermis. Pigment cells accompanied the blood capillaries intruding into the epidermis. A few lymphocytes were present. Neither mucous cells nor grand cells were found in 6 regional epidermises.

Thickness of the epidermis

The thickness of the epidermis depended on the layer and size of the swollen cells in the middle layer. In the thickness of the 5 fins and a sucking disc, the pectoral fin was the thickest, averaging 98.6 μm (range 32.1 to 148.0 μm) (Table 1), and its middle layer had 3 to 15 layers of swollen cells, reaching average of 19.1 μm (7.0 to 40.5 μm) in height. In contrast, two dorsal fins were the thinnest, averaging 44.4 μm (21.9 to 64.7 μm) in the first dorsal fin and 45.3 μm (11.8 to 83.5 μm) in the second dorsal fin. In the middle layer of the two dorsal fins, the size of the swollen cells was similar or less than the pectoral fin, but the number of layer was less than the pectoral fin, 3 to 9 layers. The sucking disc was thick, averaging 84.5 μm (17.5 to 147.7 μm), whereas the caudal and anal fins were thin, averaging 63.9 μm (34.1 to 126.8 μm) and 59.4 μm (23.7 to 87.9 μm), respectively.

Surface area of the fine blood capillaries and epidermis

The value of the surface area of the fine blood capillaries was the highest in the two dorsal fins, average 206.3 μm^2 (83.2 to 400.7 μm^2) in the first dorsal fin and 164.0 μm^2 (85.0 to 311.8 μm^2) in the second dorsal fin, but was the lowest in the anal fin, 70.0 μm^2 (40.0 to 198.2 μm^2) (Table 1). The other fins and the sucking disc showed similar values, with average 132.7 to 144.2 μm^2 as in Table 1.

In the surface area of the epidermis, the pectoral fin and sucking disc was higher 9939.8 and 9225.1 μm^2, respectively average. Two dorsal fins showed the lowest value: average 4614.1 μm^2 (2481.9 to 7208.9 μm^2) in the first dorsal fin and 4976.3 μm^2 (2477.4 to 8011.3 μm^2) in the second dorsal fin. The values were closely related to their thickness of the epidermis.

Rate of surface area of respiratory epithelium

Rate of the surface area of respiratory epithelium, the surface area of the fine blood capillaries occupied per surface area of epidermis in 0.1 mm, was higher in the dorsal fins: average 4.4% (range 1.0 to 9.0%) in the dorsal fin and 3.7% (1.6 to 8.6%) in the dorsal fin (Table 1). The dorsal fins had a large number of fine blood capillaries, although they showed the lowest value in the thickness and surface area of the epidermis. The pectoral fin and sucking disc with higher values in the thickness and surface area were lower, 1.7% (0.7 to 4.2%) and 1.5% (0.6 to 2.6%). The anal fin was much less, 1.1% (0.6-3.5%). The caudal fin was more or less high, 2.0% (0.9 to 5.7%).

Diffusion distance

The diffusion distance between the blood capillaries and the surface was variable (Table 2). In 6 regional epidermis,

Table 1. General features of regional epidermis of *Periophthalmus modestus*

Regions	Thickness of epidermis (μm)		Surface area (μm^2)/Unit length (0.1 mm)				Rate of surface area of respiratory epithelium (%)*	
			Blood capillaries		Epidermis			
	$\bar{\chi}$	Range	$\bar{\chi}$	Range	$\bar{\chi}$	Range	$\bar{\chi}$	Range
Base of 1st dorsal fin	44.4	21.9- 64.7	206.3	83.2-400.7	4614.1	2481.9- 7208.9	4.4	1.0-9.0
Base of 2nd dorsal fin	45.3	11.8- 83.5	164.0	85.0-311.8	4976.3	2477.4- 8011.3	3.7	1.6-8.6
Base of pectoral fin	98.6	32.1-148.0	144.2	42.9-344.1	9939.8	3593.7-14210.5	1.7	0.7-4.2
Base of anal fin	59.4	23.7- 87.9	70.0	40.0-198.2	6634.0	5620.0- 7801.1	1.1	0.6-3.5
Base of caudal fin	63.9	34.1-126.8	132.7	61.0-238.8	8445.0	4180.7-22229.5	2.0	0.9-5.7
Base of sucking disc	84.5	17.5-147.7	138.1	54.3-302.1	9225.1	5580.4-12646.8	1.5	0.6-2.6

*Surface area of capillaries/epidermis×100

Table 2. Diffusion distance of regional epidermis of *Periophthalmus modestus*

Regions	Diffusion distance (μm)	
	$\bar{\chi}$	Range
Base of 1st dorsal fin	5.0	0.5-12.5
Base of 2nd dorsal fin	4.6	1.5-11.0
Base of pectoral fin	5.0	1.0-17.1
Base of anal fin	10.9	4.3-17.9
Base of caudal fin	7.8	2.8-31.4
Base of sucking disc	3.6	0.8-15.1

the blood capillaries of the sucking disc was situated close to the surface, averaging 3.6 μm (range 0.8 to 15.1 μm), but that of the anal fin with much smaller surface area of blood capillaries was present in the inner region, far from the surface, average 10.9 μm (4.3 to 17.9 μm). Two dorsal fins with the highest value of the respiratory epithelium was average 4.6 to 5.0 μm and the pectoral fin was average 5.0 μm (1.0 to 17.1 μm) in diffusion distance. The value of the caudal fin was 7.8 μm (2.8 to 31.4 μm).

Discussion

General structures on epidermis of *Periophthalmus* known as cutaneous air-breathing fish skin were well documented by some workers: 1) in the stratum germinativum, the presence of well-defined lymphatic spaces containing small lymphocytes, 2) in the middle layer, simpler structure and the existence of thick swollen cells, 3) in outermost layer, the existence of abundant intraepidermal capillaries near its surface, 4) reduction of scale, and 5) the absence of the mucous and any glandular cells (Whitear, 1986; Suzuki, 1992; Yokoya and Tamura, 1992; Park et al., 2000; Park, 2002). These observations on the epidermis structure of *P. modestus* was performed either in the dorsal, ventral and caudal portions (Suzuki, 1992), or in the head, trunk and mandible (Yokoya and Tamura, 1992), and the top of head, dorsal, lateral and ventral portions (Park et al., 2000). The above structural features were the same in 6 different epidermises including 5 fins and a sucking disc.

All epidermis of 6 different regions had abundant fine blood capillaries near its surface (the outermost layer), or its inner region (the middle layer). As in Table 1, diffusion distance between blood of the capillaries and the surface of skin ranged from 3.6 to 10.9 μm. Of the 12 species of the genus *Periophthalmus*, the diffusion distance was reported only in the back of *P. modestus* (average 1.4 μm) and *P. magnuspinnatus* (average 1.5 μm) (Park et al., 2000; Park, 2002), in the opercular pouches of *P. vulgaris* (average 1.0 to 3.2 μm) (Singh and Munshi, 1969). Based on the above results, cutaneous gas exchange of *P. modestus* may occur more closely to the surface of body epidermis rather than in appendages. The diffusion distance was also reported in other air-breathing fishes: the suprabranchial chamber and labyrinthine organ of *Anabas testudineus* (0.21 μm), the intestine of *Misgurnus mizolepis* (0.7 μm) and *M. anguillicaudauts* (11.2 μm), the suprabranchial chamber of *Channa punctata* (0.78 μm) and the opercular chamber of *Boleophthalmus boddaerti* (1.22 μm), and the epidermis of *Liobagrus mediadiposalis* (169 μm) (Hughes et al., 1973, 1974; Hakim et al., 1978; Biswas et al., 1981; Moitra et al., 1989; Park et al., 2000; Park and Kim, 2001; Park et al., 2003a,b).

The rate of surface area of respiratory epithelium in 5 fins and sucking disc was from 1.1 to 4.4%. Among them, the two dorsal fins with the thinnest epidermis had much more fine blood capillaries, averaging 3.7 to 4.4%, and on the mudflat, the dorsal fins toward the upper region might be exposed to the air for a long time. On the other hard, the pectoral, anal fin and sucking disc are the lower region of body which is easily immersed in the water or soft mud, and their values were lower, 1.1 to 1.7%. Therefore, it can be surmised that the rate of respiratory epithelium surface seems to be closely related to their amphibious pattern rather than to the thickness of the epidermis or diffusion distance. For *P. cantonensis* (*P. modestus*), Tamura et al. (1976) reported that the proportion of oxygen uptake by the skin was 48% in water. For *P. sobrinus*, Teal and Carey (1967) measured similar proportions of skin, 50 to 60% of total aerial respiration.

In general, the known air-breathing fishes have several types of gland cells such as mucous, club, or sacciform cell. In addition to their secretory function, they have a thick epidermis which is important in the epidermal structures for air-breathing fishes such as *Monopterus, Conger, Heteropneustes, Mastacembelus, Liobagrus, Misgurnus, Lepidocephalichthys, Boleophthalmus* and *Scartelaos* (Jakubowski, 1958; Liem, 1967; Johansen, 1970; Mittal and Munshi, 1971; Mittal and Banerjee, 1974; Whitear, 1986; Al-Kadhomity and Hughes, 1988; Low et al., 1990; Park and Kim, 1999; Park et al., 2000; Park, 2002; Zhang et al., 2000). However, the epidermis of the appendage and body of *P. modestus* has a simpler structure without any gland. Instead of gland, this species has a thicker epidermis consisted of swollen cells. The swollen cells enlarged epidermal cells with desmosome in *P. kohlreuteri* (Whitear, 1986) and *P. magnuspinnatus* (Park, 2002).

A well-defined lymphatic space containing small lymphocytes was arranged in a defined region of the stratum germinativum of the epidermis. These lymphatic spaces were reported in the body skin of *P. modestus* and *P. magnuspinnatus* (Park et al., 2000; Park, 2002) and in other air-breathing fishes such as *Heteropneustes, Mastacembelus* and *Amphipnous* (Mittal and Munshi, 1971). Mittal and Munshi (1971) reported that these lymphatic spaces play a role in the protection of the

epidermis.

In regional epidermises of *Boleophthalmus pectinirostris* known as mudskipper fishes, all of the body epidermises can absorb air through their epidermis which is rich with blood capillaries and dermal bulges, except for the fins and sucking disc (Park et al., 2003c). However, unlike the genus *Boleophthalmus*, it is evident that *P. modestus* can take oxygen from all epidermis of the body regions as well as the accessory appendages containing the fins and sucking disc.

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