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# Histology of Skin of the Amphibious Fish, *Periophthalmus modestus*

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**Structure of the skin in amphibious fish, *Periophthalmus modestus*, was described in relation to cutaneous respiration. The epidermis has no gland cell. The epidermis consists of three regions: outermost layer of one to five layers of flattened epithelial cells, middle layer of swollen epithelial cells instead of glandular cells and stratum germinativum of cuboidal cells. There are numerous blood capillaries in the outermost layer of the epidermis and diffusion distance between the blood of capillaries and the epidermis is about 1.4 μm. The middle layer of the epidermis appears to be a web-like structure due to the swollen epithelial cells. The stratum germinativum has a well-developed lymphatic space containing lymphocytes. There are numerous blood capillaries and elliptical area with acid mucopolysaccharides in stratum laxum of the dermis. The skin of *Periophthalmus modestus* may be an accessory respiratory organ for oxygen uptake during terrestrial or aquatic life.**

Amphibious fishes such as *Monoterus, Amphinou, Boleophthalmus* and *Periophthalums* live in both aquatic and terrestial habitats during their entire life, and therefore, their respiratory system has been the focus of interest. Of the amphibious fishes, *Monoterus albus* and *Amphinous* have a dual respiratory system for oxygen uptake; that is, water respiration using the dissolved oxygen through the gill and cutaneous respiration using air directly through the skin (Liem, 1967; Mittal and Munshi, 1971). A similar cutaneous respiration was seen in other fishes which live in stagnant and warm reservoirs, swamps or rice field causing periodic drought (Jakubowski, 1958; Johansen, 1970; Mittal and Banerjee 1974; Mittal et. al., 1980; Whitear, 1986; Park and Kim, 1999, 2000).

*Periophthalmus modestus* (Gobiidae), a mud skipper, inhabits brackish water, and has amphibious habit: it moves or skips briskly on mud flat of land or burrows into the mud. It was reported that in *P. modestus* the oxygen uptake is accomplished through the water and air, and on land, it relies mainly on the skin (Tamura et al., 1976). Unfortunately, there has been no histological approach. Based on our study, there is a difference between *Periophthalmus modestus* and other air-breathing fishes in skin structure.

Therefore, this study was carried out in order to obtain information on structure of the skin and to discuss the relationship between the skin and respiration of *P. modestus*.

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## Materials and Methods

Ten specimens were collected from the brackish water of the Mankyong River, Korea, and they ranged from 50.5 mm to 90.4 mm in standard length. The specimens were fixed in 10% neutral buffered formaldehyde. The skin fragments were taken from four regions; the top of the head, and the dorsal, lateral and abdominal regions.

These sections 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 hematoxylin, iron alum hematoxylin, counter-stained with eosin, and Masson trichrome stain (Gurr, 1956) for general histology. Polysaccharides were visualized by either alcian blue reaction (AB) at pH 1.0 and 2.5 (Steedman, 1950; Lev and Spicer, 1964), of periodic acid-Schiff (PAS) (Lilllie and Greco, 1947). Diffusion distance were calculated between the outermost red blood cell and the outermost epidermal layer according to Moitra et al. (1989).

## Results

The skin of *Periophthalmus modestus* could be divided into epidermis (outermost layer, middle layer and stratum germinativum), dermis (stratum laxum and stratum compactum) and subcutis (Fig. 1A).

### *Epidermis*

Epidermis of *Periophthalmus modestus* could be divided into three layers; the outermost layer, middle layer and stratum germinativum (Fig. 1B).

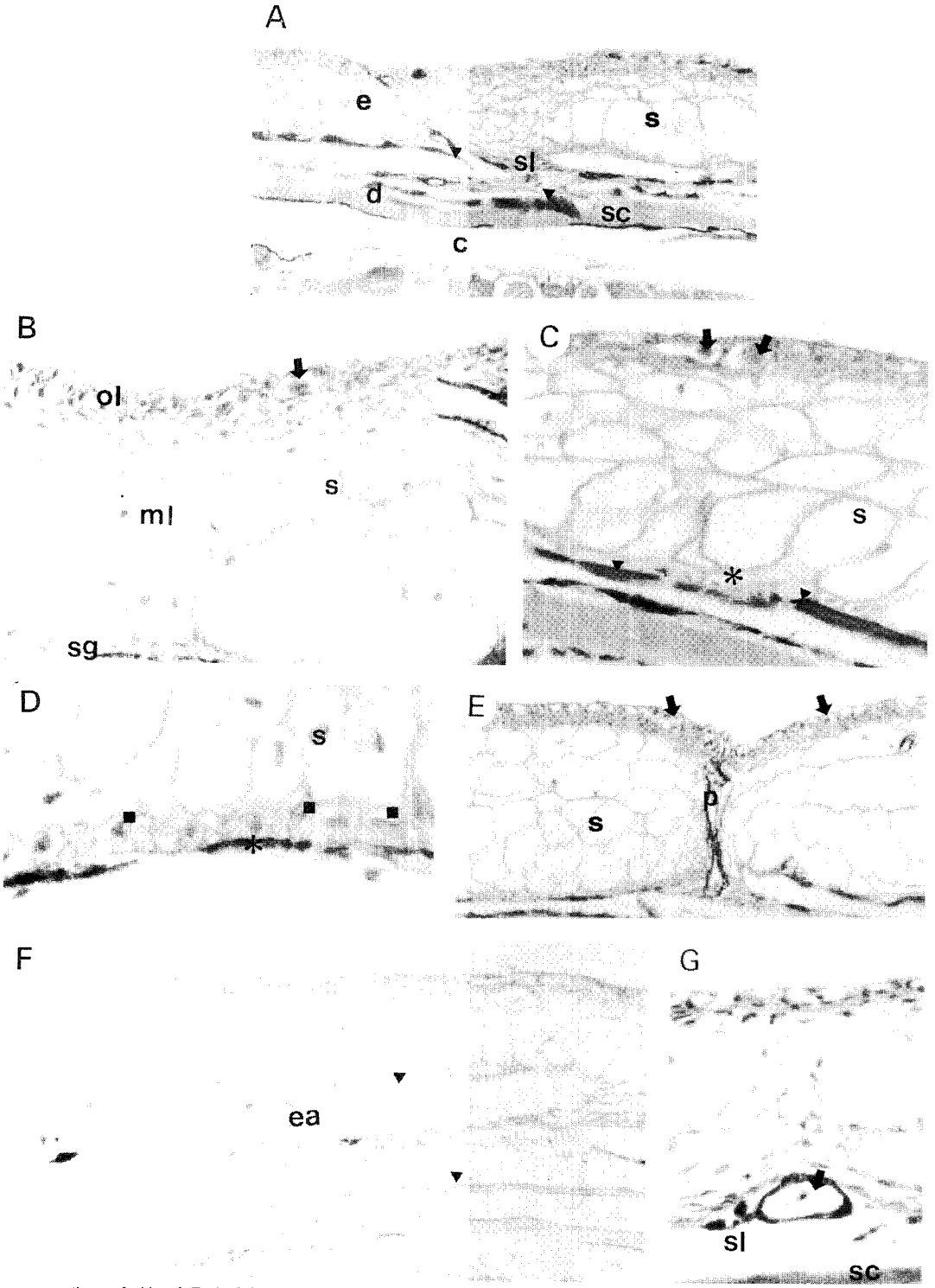


**Fig. 1.** Transverse sections of skin of *Periophthalmus modestus*. A, Dorsal skin is composed of epidermis (e), dermis(d) containing stratum laxum (sl) and stratum compatum (sc), and scale (arrowheads) and subcutis (c). The swollen cells are seen in the middle layer of epidermis. Harris's hematoxylin and eosin. B, The epidermis consisted of outermost layer (ol), middle layer (ml), and stratum germinativum (sg). Numerous blood cells (arrow) are found in the outermost layer of the epidermis. Iron alum hematoxylin and eosin. C, The epidermis is characterized by many blood cells (arrows), the swollen cells(s), lymphocytes (*), Note the scales(arrowheads) nuder the epidermis. The swollen cells lack nucleus probably due to misfixation. Harris's hematoxylin and eosin. D, The swollen cells (s) and the epithelial cells (solid rectangles) have ovoid or spherical nucleus and homogeneous cytoplasm. Note the lymphocytes (*) at stratum germinativum. Iron alum hematoxylin and eosin. E, The middle layer of epidermis exhibits web-like structure with swollen cells (s). Pigment cells (p) and blood cells (arrow). Harris's hematoxylin and eosin. F, The elliptical area (ea) in the stratum laxum of the dermis is alcian blue (pH 1.0) positive. Whereas other epithelial cells are negative in alcian blue staining (pH 1.0). G, A Blood capillary (arrow) with a blood cell is found under the basement membrane. Note the stratum laxum (sl) and stratum compactum (sc) in the dermis. Masson trichrome stain. Scale bar=25 μm.

**Table 1.** Diffusion distance in the airbreathing organs of dual breathing fishes

| Species | Literature | Air-breathing organ | Diffusion distance (μm) |
|---|---|---|---|
| *Mastcembellus pancalus* | Mittal and Munshi (1971) | Skin | 34.0 |
| *Amphipnous cuchia* | Mittal and Munshi (1971) | Skin | 19.0 |
| *Anabas testudineus* | Hughes et al. (1973) | Suprabranchial chamber and labyrinthine organ | 0.21 |
| *Heteropneustes fossilis* | Hughes et al. (1974) | Air-sac | 1.6 |
| *Channa punctata* | Hakim et al. 1978 | Suprabranchial chamber | 0.78 |
| *Boleophthalmus boddaerti* | Biswas et al. 1981 | Opercular chamber | 1.22 |
| *Lepidocephalichthys guntea* | Moitra et al. 1989 | Intestine | 2.6 |
| *Periophthalmus modestus* | Present authors | Skin | 1.4 |

The outermost layer of epidermis is composed of one to five layers of flattened epithelial cells (Fig. 1B). There are numerous blood capillaries in between these cells. Red blood cells present extending from inner to outermost regions of this layer. Pigment cells are situated around the blood capillaries. Diffusion distance between the blood of capillaries and the outermost layer is about 1.4 μm.

The middle layer of epidermis (Fig. 1A-G) has no epidermal gland and is simpler in structure. This layer consists of few round or cuboidal epithelial cells and larger and voluminous epithelial cells. The larger cells are swollen adjacent to the epithelial cells. Due to the swollen cells, the middle layer exhibits a web-like structure in appearance. The swollen cells have an oval nucleus and a homogeneous cytoplasm, and their boundary is clear (Fig. 1B-E). Occasionally, they appear to be a vesicle or a vacant acellular structure due to a loss of nucleus by improper fixation. The swollen cells are variable in height, reaching 10 μm to 45 μm. A thick region of the middle layer is filled with the swollen cells, arranged in several layers. Therefore, the thickness of the skin is mainly determined by the size of the swollen cells in the middle layer.

The stratum germinativum consists of a single layer of cuboidal cells with an oval or spherical nucleus and a homogeneous cytoplasm (Fig. 1C-D). In between the cuboidal cells, there are small ovals or round lymphatic space containing small lymphocytes (Fig. 1C-D). The lymphocytes have a deeply stained nucleus and faintly stained small cytoplasm. They are PAS- and AB-positive.

*Dermis*

The dermis is separated well by thin basement membrane, and can be divided into a thin stratum laxum of loose connective tissue and a thick stratum compactum of dense connective tissue (Fig. 1A, C, F).

The stratum laxum just under the basement membrane is composed of thin collagen fiber bundles that are Masson trichrome reaction positive (Fig. 1G). The layer is characterized by rich blood vessels, pigment cells, and elliptical area (Fig. 1A, F, G). The elliptical area is found between the basement membrane and the scale, and is weakly positive in PAS, AB, and AB-PAS reactions (Fig. 1F). As the elliptical area deeply penetrates into the epidermis, the thickness of the epidermis is reduced. The scale is in the elliptical area and divided into the upper bony layer and the inner fibrillary plate (Fig. 1A, C). The stratum compactum consists of a thick and compact collagen fiber bundle which contains pigment cells and blood capillaries. They are positive in PAS staining.

*Subcutis*

Of the skin structure, this layer is the innermost and thinnest, and is found in between the stratum compactum of the dermis and the muscle (Fig. 1A). This layer contains blood vessels and is filled with fat cells showing empty spaces which are negative in hematoxylin/eosin staining.

## Discussion

The histological characteristics of the skin of dual breathing fishes vary with species but are in general as follows: 1) A thick skin, consisting mainly of epidermis, 2) Existence of a well-differentiated are large skin gland cells, 3) A well-developed vascular system, 4) Intraepithelial capillaries, 5) Existence of elliptical area or definite area, 6) Absence or reduction of scale. These characteristics are found in amphibious fishes or fishes living in environments such as stagnant and warm reservoirs, swamps or rice field causing periodic drought (Jakubowski, 1958; Liem, 1967; Johansen, 1970; Mittal and Munshi, 1971; Mittal and Banerjee 1974; Whitear, 1986; Park and Kim, 1999, 2000).

In *Periophthalmus modestus*, the skin has a thick epidermis, ranging from 130 μm to 188 μm in thickness. In air breathing fishes, this value is higher than *Heteropneustes fossilis* (98 μm), *Mastacembelus pancalus* (44 μm), *Amphipnous cuchia* (119 μm) and *Monopterus albus* (75 μm) (Liem, 1967; Mittal and Munshi, 1971), whereas it is lower than *Misgurnus fossilis* (182-397 μm) (Jakubowski, 1958) and *M. anguillicaudatus* (169-255 μm) (Park and Kim, 1999).

The epidermis of *P. modestus* has numerous blood capillaries in its outermost layer. The diffusion distance between the blood capillaries and the skin is about 1.4 μm. This value is higher than that (0.21 μm) reported for other air-breathing organs (suprabranchial chamber and labyrinthine organ), and similar to that reported for air sac (1.6 μm) (Table I). However, this value of *P. modestus* is the highest among other fishes

air-breathing by skin. Therefore, we think that the skin of *P. modestus* has respiratory epithelium to make up for the deficient oxygen, and that the gas exchange probably occurs between the air taken from the skin and the blood circulating in the outermost surface of the epidermis. In this species, Tamura et al (1976) experimentally proved the proportion of total oxygen consumption in aquatic and terrestrial habitats and reported that its respiration on land relies mainly on its skin.

The skin of air-breathing fishes has several kinds of epidermal gland cells for partial oxygen uptake (Jakubowski, 1958; Liem, 1967; Mittal and Munshi, 1971; Johansen, 1970; Mittal and Banerjee 1974; Whitear, 1986; Park and Kim, 1999, 2000). However, the skin of *P. modestus* is devoid of the gland cells, and the swollen epithelial cells exist in the middle layer of the epidermis instead. Such presence of swollen cells was confirmed in *Periophthalmus kohlreuteri* undergoing cutaneous respiration (Whitear, 1986).

The elliptical area or definite area with acid mucopolysaccharides exists in the stratum laxum layer of the dermis, known as the semiterrestrial ecological habits of fish (Mittal and Munshi, 1971). In addition, a well-defined lymphatic space containing small lymphocytes was present in the stratum germinativum layer of the epidermis. A few lymphocytes are found in the outermost layer of epidermis. It is concluded that the skin of *P. modestus* is modified as an accessory respiratory organ for oxygen uptake to adjust for their amphibious life in water or on land.

## References


Biswas N, Ojiha J, and Munshi JSD (1981) Morphometric of the respiratory organs of an estuarine goby, *Boleophthalmus boddaerti*. *Jpn J Ichthyol* 27: 316-326.

Gurr GT (1956) A Practical Manual of Medical and Biological Staining Techniques. Interscience, New York.

Hakim A, Munshi JSD, and Hughes GM (1978) Morphometrics of the respiratory organs of the Indian green snake-headed fish, *Channa punctata*. *J Zool* 184: 519-543.

Hughes GM, Dube SC, and Munshi JSD (1973) Surface area of the respiratory organs of the climbing perch, *Anabas testudineus*. *J Zool* 170: 227-243.

Hughes GM, Singh BR, Guha G, and Munshi JSD (1974) Respiratory surface areas of an air-breathing siluroid fish, *Saccobranchus* (= *Heteropneustes*) *fossilis* in relation to body size. *J Zool* 172: 215-232.

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 WS and Radall DJ (eds.), Fish Physiology. IV, Academic Press, New York, pp 361-411.

Lev R and Spicer SS (1964) Specific staining of sulphated groups with alcian blue at low pH. *J Histochem Cytochem* 12: 309.

Liem KF (1967) Functional morphology of the integumentary, respiratory, and digestive systems of the synbranchoid fish, *Monopterus albus*. *Copeia* 1967: 375-388.

Lillie RD and Greco J (1947) Malt diastase and ptylin in place of saliva in the identification of glycogen. *Stain Technol* 22: 67-70.

Moitra M, Singh OK, and Munshi JSD (1989) Microanatomy and cytochemistry of the gastro-respiratory tract of an air-breathing cobitid fish, *Lepidpcephalichthys guntea*. *Jpn J Ichthyol* **36:** 227-231.

Mittal AK and Munshi JSD (1971) A comparative study of the structure of the skin of certain air-breathing fresh-water teleosts. *J Zool* 163: 515-532.

Mittal AK and Banerjee TK (1974) Structure and keratinization of the skin of a fresh-water teleost *Notopterus notopterus* (Notopteridae, Pisces). *J Zool* 174: 314-355.

Mittal AK, Whitear M, and Agarwal SK (1980) Fine structure and histochemistry of the epidermis of the fish, *Monopterus cuchia*. *J Zool* 191: 107-125.

Moitra M, Singh OK, and Munshi JSD (1989) Microanatomy and cytochemistry of the gastro-respiratory tract of an air-breathing cobitid fish, *Lepidpcephalichthys guntea*. *Jpn J Ichthyol* 36: 227-231.

Park JY and Kim IS (1999) Structure and histochemistry of skin of mud loach, *Misgurnus anguillicaudatus* (Pisces, Cobitidae), from Korea. *Korean J Ichthyol* 11: 109-116.

Park JY and Kim IS (2000) Structure and cytochemistry of skin in spined loach, *Iksookimia longicorpus* (Pisces, Cobitidae). *Korean J Ichthyol* 12: 25-32.

Steedman HF (1950) Alcian blue 8G: a new stain for mucin. *Quart J Microscop Sci* 91: 477-479.

Tamura SO, Mori H, and Yuzuhira M (1976) Respiration of the amphibious fishes, *Periophthalmus modestus* and *Boleopthalmus chinensis* in water and on land. *J Exp Biol* 65: 97-109.

Whitear M (1986) The skin of fishes including cyclostomes: epidermis. In: Bereiter-Hahn J, Matoltsy AG, and Richards KS (eds.), Biology of the Integument Vol 2. Vertebrates, Springer Verlag, New York, pp 9-64.


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