

# Morphological and Cytochemical Study on the Skin of Korean Eel Goby, *Odontamblyopus lacepedii* (Pisces, Gobiidae)

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The skin of the eel goby, *Odontamblyopus lacepedii*, consists of epidermis, dermis and subcutis. 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 gland cells of acid mucopolysaccharides. The middle layer consists mainly of swollen small or voluminous epidermal cells and shows a web-shaped structure. The thickness of the epidermis depends on the various sizes and the number of layers of the swollen cells. Well-developed lymphatic spaces containing lymphocytes exist in the stratum germinativum and small scales are embedded in the dermis. A large number of blood capillaries are present just below the basement membrane, and a definite area giving AB and PAS positive was present between the basement membrane and scales. Taste buds are distributed on surface of the epidermis at intervals. Considering the structural features of the skin, it may be considered that *O. lacepedii* is more likely to be related to cutaneous respiration as a dual respiratory system.

The eel goby, *Odontamblyopus lacepedii*, belongs to the family Gobiidae and has a long and slender body and is found in shallow estuaries and brackish waters. These habits comprise a place where freshwater and seawater meet and a transitional zone between two aquatic ecosystems. Therefore, these habits sometimes provide a challenging environment for fishes with changes taking place in many environments and require certain adaptation for survival and colonization. *O. lacepedii* inhabits holes in the muddy bottom and its burrows are bamboo tube-shaped, vertical or oblique, extending down into the substrate at the depth of 50 to 90 cm (Kim and Park, 2002). Also, this species can survive for about a day without a constant supply of oxygen as in amphibious mudskipper fishes, *Periophthalmus* and *Boleophthalmus* known as air-breathing fishes. These ecological features of these species are closely related to air-breathing organ as an additional respiratory system (Johansen, 1970; Tamura et al. 1976; Niva et al. 1981; Al-Kadhomiy and Hughes, 1988; Low et al. 1990; Suzuki, 1992; Yokoya and Tamura, 1992; Graham, 1997; Ishimatsu et al. 1998; Park et al. 2000; Zhang et al. 2000; Park, 2002).

The genus *Odontamblyopus* comprises four species in the world (Murdy and Shibukawa, 2001), but the

structure of skin was not well known. The purposes of this paper are to investigate the structure and histochemistry of the skin, and to discuss relationship of the skin and air-breathing organ in Korean eel goby, *O. lacepedii*.

## Materials and Methods

Three specimens were collected from Mujung-ri, Byeollyang-meyon, Seongju-eup, Suncheon-si, Jeollanam-do in the southern estuary of Korea and were 23.5 to 31.1 cm in total length. They were fixed in 10% neutral buffered formaldehyde, and skin fragments of about 5×5 mm<sup>2</sup> from head, dorsum and lateral regions were taken.

These fragments were dehydrated through a standard ethanol series to 100%, cleared in xylene and then embedded in wax (Paraplast, Oxford). Five μm sections were deparaffinized and stained with Ehrlich hematoxylin and counter-stained with eosin, and stained with Masson trichrome for general histology. We demonstrated polysaccharides with alcian blue (AB) at pH 1.0 and 2.5, and periodic acid-Schiff (PAS). We used the PAS technique in combination with AB (pH 2.5), and AB (pH 2.5)-PAS, to distinguish between neutral and acid mucins. For acid mucins, metachromatic reactions with toluidine blue were used. We used high iron diamine (HID) with AB, to assess the nature of the acid mucin. The above staining techniques followed the methods by Humason (1972). For

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visual observations, we examined sections of the skin by light microscopy on hematoxylin and eosin preparations.

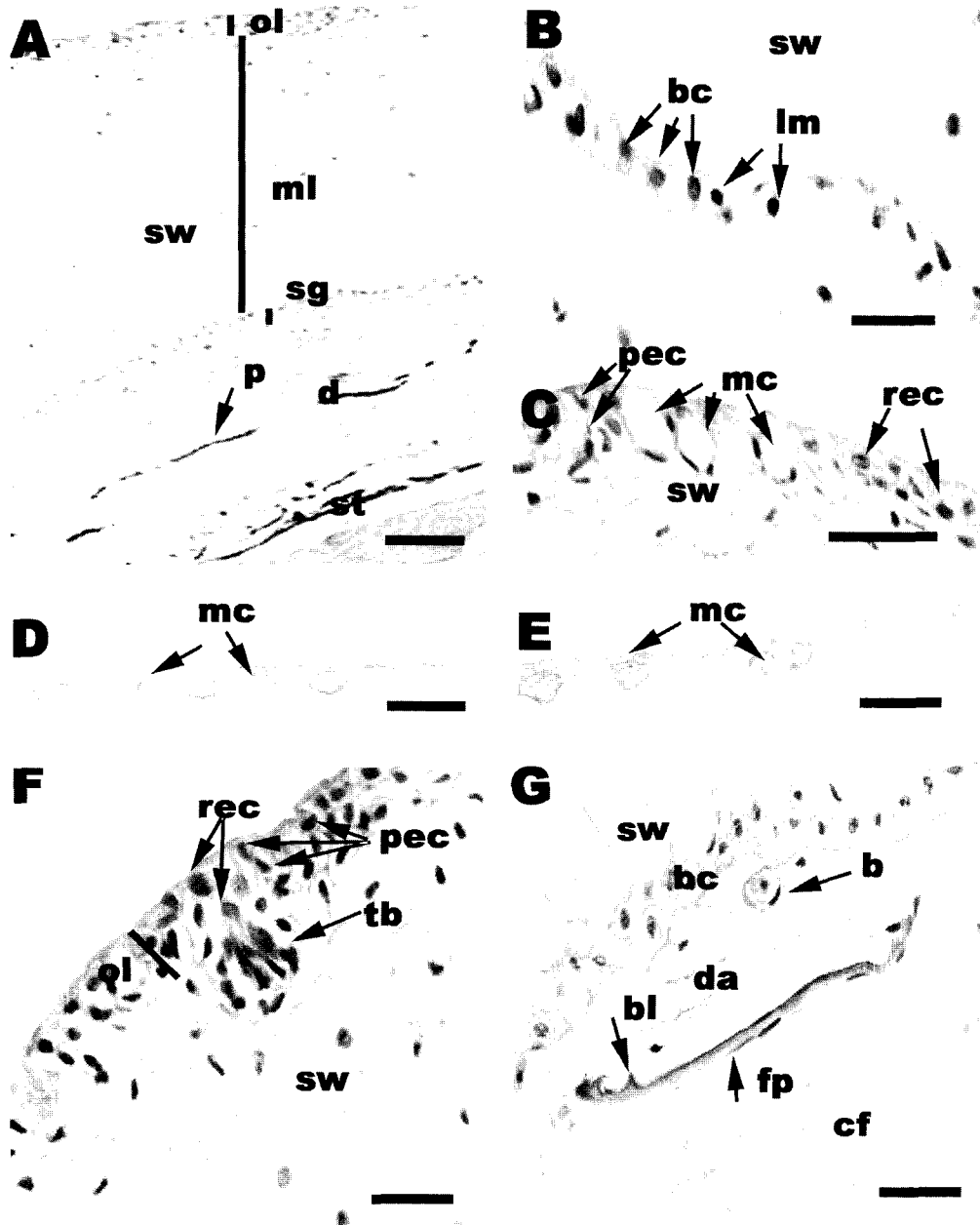
## Results

The skin of *O. lacepedii* comprises 3 layers, epidermis,

dermis and subcutis (Fig. 1A).

### Epidermis

Epidermis of *O. lacepedii* could be divided into three layers: Stratum germinativum, middle layer and outermost



**Fig. 1.** Transverse sections of skin of *Odontamblyopus lacepedii*. A, The skin of the head is divided into epidermis consisting of 3 layers, i.e. outermost layer, middle layer and stratum germinativum, dermis and subcutis. There are swollen cells and pigment cells. Ehrlich haematoxylin and eosin. B, The stratum germinativum of the epidermis in the dorsum. This layer has cuboidal basal cells and lymphocytes. Ehrlich haematoxylin and eosin. Scale bar=25  $\mu$ m. C, The outermost layer of the epidermis of the head. This layer has mucous cells and epithelial cells. Ehrlich haematoxylin and eosin. Scale bar=25  $\mu$ m. D, The mucous cells of the head. These cells are weakly positive with PAS-AB (pH 2.5). E, The mucous cells of the lateral region. They are weakly positive with HID-AB (pH 2.5). F, The taste bud is present in the epidermis. Ehrlich haematoxylin and eosin. G, A definite area in the dermis is PAS-AB (pH 2.5) positive. The scale consist of an upper bony layer and inner fibrillary plate. Scale bar=15  $\mu$ m. b, blood cell; bc, basal cell; bl, upper bony layer; cf, collagen fiber; d, dermis; da, definite area; fb, inner fibrillary plate; lm, lymphocyte; mc, mucous cell; ml, middle layer; ol, outermost layer; p, pigment cell; pec, polygonal epithelial cell; rec, round epithelial cell; sg, stratum germinativum; st, subcutis; sw, swollen cell. Scale bars=13  $\mu$ m (B), 25  $\mu$ m (D-F), and 50  $\mu$ m (A).

layer (Fig. 1A).

The stratum germinativum consists of a single layer of cuboidal cells with an oval or spherical nucleus (Fig. 1A, B). Small ovals or round lymphatic spaces with small lymphocytes are present between basal cuboidal cells. The nucleus of the lymphocyte is smaller than that of the cuboidal cell, round and condensed (Fig. 1B), and its cytoplasm is also very small.

The middle layer consists of few round or cuboidal epithelial cells and voluminous epithelial cells (Fig. 1A-C, G). The voluminous cells are swollen adjacent to the epithelial cells, called swollen cells. The swollen cells have oval nucleus and homogeneous cytoplasm, and their boundary is clear (Fig. 1A-C). However, they look like vesicles or vacant acellular structures without nucleus by improper fixation. Between these swollen cells, there are a few cuboidal cells. The swollen cells do not show any histochemical reactions for general histochemical stain as well as staining for polysaccharides. The swollen cells vary in height (15.0–110.0  $\mu\text{m}$ ) and consist of multilayers in the middle (2–28 layers). There are more layers of swollen cells in head (4–28 layers) than in the dorsum and lateral regions (2–8 layers). Therefore, thickness of the skin is mainly determined by the size and the number of layers of swollen cells in the middle layer. Because of these features, the middle layer shows a web-like structure in appearance.

The outermost layer is composed of two types of cells: an epidermal cell shaped with round or polygonal cells and a mucous gland cell (Fig. 1C, F). These cells are arranged in 1–5 layers, mostly 2–3 layers. The 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 are mean of 10.8  $\mu\text{m}$  in height, ranging from 10.0 to 12.5  $\mu\text{m}$ , and are distributed approximately 4–5 per 100  $\mu\text{m}$  in number on epidermis of the three regions. The mucous cells are highly vacuolated and of basophilic secretory matter. In histochemical reaction, the mucous cells show blue with AB at pH 2.5, but in PAS, AB (pH 1.0), HID, toluidine blue staining, they are negative. However, they give a blue color with PAS-AB (pH 2.5) (Fig. 1D) and blue color with HID-AB (pH 2.5) staining (Fig. 1E).

Thickness of the epidermis depends on the strata and size of the swollen cells in the middle layer. The thickness of the epidermis shows high value in the head with a mean of 225.3  $\mu\text{m}$  (range 118.8–297.0  $\mu\text{m}$ ) and low value in the dorsum, with a mean of 117.2  $\mu\text{m}$  (range 59.0–188.1  $\mu\text{m}$ ). The lateral region shows mean 110.6  $\mu\text{m}$  (range 89.1–128.7  $\mu\text{m}$ ).

The epidermis has taste buds, which is typically composed of sensory cells and is a bottle-shaped expansion (Fig. 1F).

#### Dermis

The dermis consists mostly of bundles of coarse

collagenous fibers arranged compactly in several layers (Fig. 1A, G). The collagen bundles are arranged parallel to each other. The dermis is the thickest in the head with a mean of 163.3  $\mu\text{m}$  (range 118.8–217.8  $\mu\text{m}$ ) and the thinnest in the lateral line with a mean of 103.9  $\mu\text{m}$  (range 59.4–118.8  $\mu\text{m}$ ). The dorsum was mean of 105.6  $\mu\text{m}$  ranging 108.9 to 118.8  $\mu\text{m}$ . This layer is weakly PAS positive, and develops deep green color in Masson trichrome-stained preparations for collagen.

The scale is embedded in the dermis with a defined area formed between the basement membrane and the scale. This area is weakly positive with AB and PAS (Fig. 1G). The scale is divided into the upper bony layers, with ridges of striae arranged on its free surface, and lower fibrillar plate, composed of parallel collagen fibers (Fig. 1G). The upper bony layers were basophilic in nature, and gave a red color with PAS and metachromasia with toluidine blue. The lower fibrillar plate was eosinophilic, weakly PAS positive, alcian blue negative and gave strong orthochromasia with toluidine blue.

Pigment cells and blood capillaries are present just beneath the basement membrane or sometimes randomly in this layer (Figs. 1A and 1G).

#### Subcutis

This layer is present under the dermis (Fig. 1A). There are the nerves and blood vessels in this layer. This layer has numerous empty spaces composed of fat cells in hematoxylin/eosin stained preparations.

#### Discussion

The skin of *O. lacepedii* exhibited several histological features; 1) the thick epidermis had mucous gland cells, voluminous swollen cells and well-developed lymphocytes; 2) the dermis had a defined area with acid mucopolysaccharides and abundant blood vessels; and 3) there were small scales embedded in the dermis. These characteristics of the skin were closely related to the air-breathing fishes of cutaneous respiratory system, mainly occurring in amphibious fishes (Whitaker, 1986; Al-Kadhomy and Hughes, 1988; Low et al. 1990; Yokoya and Tamura, 1992; Suzuki, 1992; Zhang et al. 2000; Park and Lee, 2002; Park, 2002).

The mucous cells were present in the outermost layer of the epidermis and acid mucopolysaccharides in nature. Acidic mucopolysaccharides of the mucous cell have also been reported in other cutaneous respiratory fishes such as *Monopterus*, *Mastacembelus*, *Amphiprion*, and *Misgurnus*, and *Boleophthalmus* (Mittal and Munshi, 1971; Mittal and Banerjee, 1974; Mittal et al. 1980; Park and Kim, 1999; Park et al., 2001; Park and Lee, 2002). According to Mittal and Munshi (1971), the mucous cells act as a barrier to infection and desiccation and to prevent abrasion.

The middle layer of the epidermis had swollen cells

varying in size. The presence of swollen cells is very interesting, and was 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 and Lee, 2002; Park, 2002). Due to the various size and number of strata of the swollen cells, the epidermis becomes thick, ranging from 110.6 µm to 225.3 µm. Also, the thick epidermis was characteristics of the skin in cutaneous respiratory fishes. The swollen cell has been known to have desmosome in cutaneous air-breathing fishes such as *P. magnuspinnatus* (Park, 2002) and *P. kohltreuteri* (Whitear, 1986). Otherwise, the epidermis of the genus *Periophthalmus* consisted of the swollen cells and intraepidermal blood capillary without mucous cells, and that of the *Boleophthalmus* had the swollen cells and mucous cells (Al-Kadhomy and Hughes, 1988; Zhang et al. 2000; Park and Lee, 2002; Park, 2002). However, intraepidermal blood capillary in *O. lacepedii* did not exist.

Well-defined lymphatic spaces containing small lymphocytes were present in the stratum germinativum layer of the epidermis. These well-defined lymphatic spaces were known in other cutaneous respiratory fishes such as *Heteropneustes*, *Mastacembelus*, *Amphipnous*, *Iksookimia*, *Misgurnus*, and *Periophthalmus* (Mittal and Munshi, 1971; Park and Kim, 2000; Park et al. 2001; Park, 2002). According to Mittal and Munshi (1971), the lymphatic space seems to be related to the protection of the epidermis.

Embedded small scales and elliptical area or definite areas with acid mucopolysaccharides exist in the superficial layer of the dermis. The small scales or absence of the scales were mainly found in cutaneous respiratory fishes such as *Misgurnus*, *Periophthalmus*, *Amphipnous* and *Monopterus*, considered as an adaptation for movement or burrowing (Liem, 1967; Mittal and Munshi, 1971; Whitear, 1986; Park et al. 2001; Park, 2002). The definite area was found in the epidermis of other cutaneous respiratory fishes, *Amphipnous*, *Misgurnus* and *Periophthalmus* (Mittal and Munshi, 1971; Park et al. 2001; Park, 2002), but its function was not known. Moreover, *O. lacepedii* can live for much longer without a supply of oxygen or in insufficient waters causing a decrease of dissolved oxygen, as in amphibious fishes, *Periophthalmus* and *Boleophthalmus*.

On the basis of the ecological aspects and structural characteristics of the skin, it may be considered that *O. lacepedii* seems to be related to a cutaneous respiratory system for adaptation to its environments.

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