Japanese Journal of Ichthyology
Vol. 38, No. 4 1992

魚類学雑誌
38巻4号 1992年

Fine Structure of the Epidermis of the Mudskipper, *Periophthalmus modestus* (Gobiidae)

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Abstract The ultra-structure of the epidermis of the mudskipper, *Periophthalmus modestus*, was examined by both light and transmission electron microscopies. The epidermis is exceptionally not well endowed with mucous or granular cells. Filament-containing cells occur in three distinct layers of the surface, middle and basal epidermis. The surface layer is further subdivided into two layers, an outermost and less superficial one. Two different cell types were identified in the epidermis. Type I cells are flat cells in a single stratum. Type II cells are enormous cells, characterized by having a large vacuole in the cytoplasm. The outermost layer is composed of a free surface of Type I cells and numerous microridges covered with a fuzzy, fibrillar substance. The "fuzz" forms a cuticule-like structure, but keratinization as found in terrestrial animals does not occur. The superficial layer contains Type I cells and intraepithelial blood capillaries. When Type I cells become senescent, numerous intercellular spaces are formed in the plasma membranes of adjacent cells, with the senescent cells finally falling off. Just beneath these cells, however, young cells of Type I are always found. The blood capillaries are usually reinforced with young Type I cells. A large volume of oxygen may be absorbed through the skin using the blood capillary network. The middle layer contains several strata of Type II cells. The special corky structure of these cells seems to play an important role in thermal insulation and protection against ultraviolet light in relation to life out of water. However, by comparison with terrestrial animals, the histological design of the epidermis of this goby appears incomplete, so as to reduce desiccation on land, owing to the epidermis lacking a keratinized stratum. The differentiation of the epidermis seems to be an adaptation for a terrestrial habit in this species.

Mudskippers, mostly belonging to the genera *Periophthalmus* and *Boleophthalmus*, are amphibious, euryhaline, gobiid teleosts. They are widely distributed in intertidal zones, where the water salinity varies remarkably, and feed on small organisms living on the mudflats. It is well known that they spend the greater part of their lives on land and are famous for their air-breathing habit, as they move above the tide line. Thus many physiological studies have been carried out on their terrestrial adaptation and aquatic osmoregulation (Gordon et al., 1965, 1969, 1978, 1985; Tamura and Moriyama, 1976; Tamura et al., 1976; Gregory, 1977; Morii et al., 1978, 1979; Iwata et al., 1981; Iwata and Kakuta, 1983; Iwata, 1988; Lee and Ip, 1987; Ogasawara et al., 1991). In air-breathing fishes, the degree of gas exchange and respiration have been defined mainly by the amount of oxygen absorbed through the skin (Hora, 1935; Whitear, 1952; Gordon et al., 1968; Hughes and Singh, 1970a, b; Johansen et al., 1970; Mittal and Munshi, 1971; Hughes and Munshi, 1973; Hughes et al., 1973, 1974; Ojha and Munshi, 1974; Tamura et al., 1976; Singh, 1976; Niva and Munshi, 1979, 1981; Feder and Burggren, 1985). Although the aerobic respiration and osmoregulation processes of *P. modestus* (Cantor) have been examined from physiological and ethological points of view (Uchida, 1932; Schöttle, 1932; Tamura and Moriyama, 1976; Tamura et al., 1976; Dotsu and Matoba, 1977; Gordon et al., 1978; Iwata et al., 1981; Iwata and Kakuta, 1983; Gordon et al., 1985; Iwata, 1988; Morii and Kasama, 1989), little is known about the skin only, i.e., as a specialized air-breathing organ adapted for an amphibious life style (Harms, 1935; Maekawa et al., 1968). The present study was undertaken to clarify the role of the skin of this particular species of mudskipper by examination of the ultra-structure.

Material and methods

Adult males and females of the mudskipper, *P.*

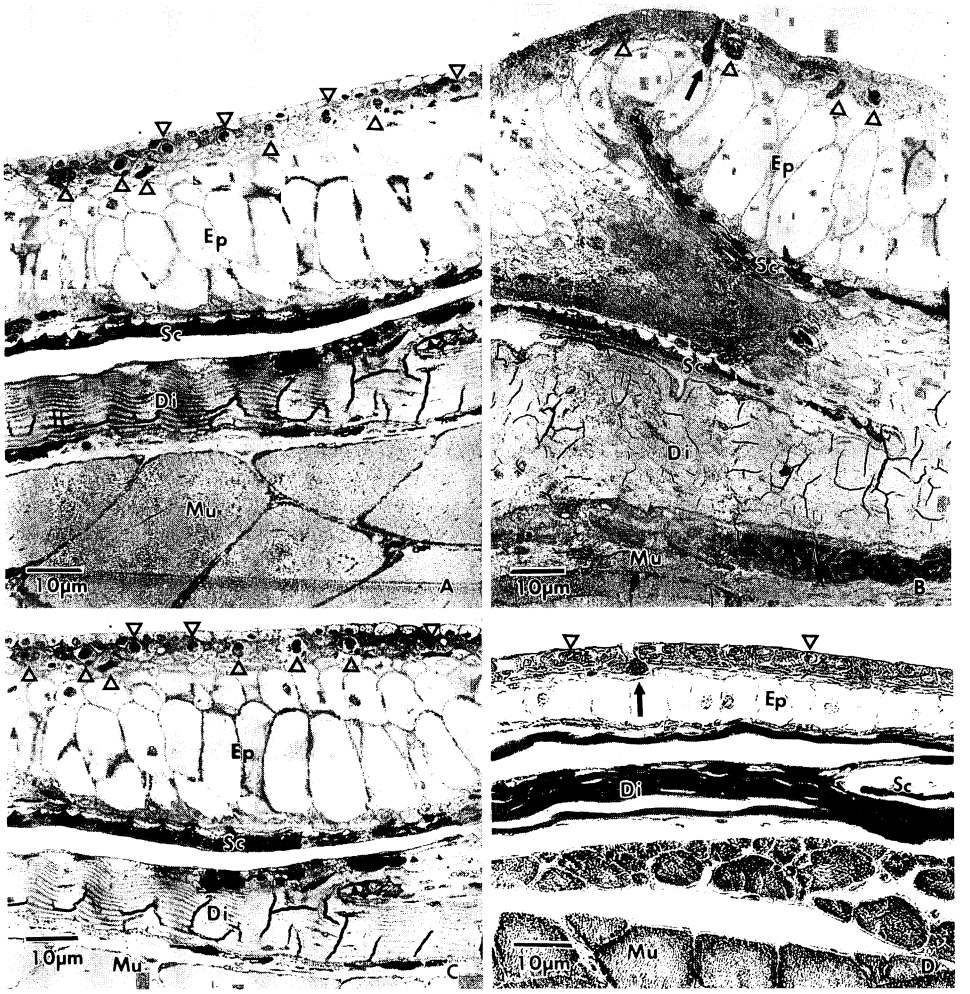


Fig. 1. Cross sections of the dorsal (A), ventral (B, D) and caudal (C) skin in *P. modestus*, showing intraepithelial blood capillaries (arrow heads), sensory-like pit organs (arrow) and structure of the skin. A–C stained with 1% toluidine blue and D with azan. Di, dermis; Ep, epidermis; Mu, muscle; Sc, scale.

modestus (varying between 22.3–77.5 mm in total length), were collected from the estuary mudflats of the Edo River, which flows into Tokyo Bay (salinity ca. 1.2%). For routine histology, pieces of skin, about 5×5 mm, taken from the dorsal, ventral and caudal portions of the body were fixed in Bouin's fluid or 10% neutral formol (pH 7.4, Wako Pure Chemical Industries Ltd.). Paraffin sections were stained with Delafield's hematoxylin and eosin (HE), Heidenhain's azan triple stain (azan) and periodic acid-Schiff (PAS) reaction. For electron microscopy, tiny pieces were immersed in Karnovsky's solution, and post-fixed with 1% OsO_4 for 2 hrs. The blocks were then dehydrated in graded ethanol and embedded in Epon 812. Ultra-sections were cut on an ultra-microtome (Poter-Blum MT-1), stained with uranyl acetate and Reynold's lead citrate and observed with a Hitachi HU-12A electron microscope. Semi-thin sections stained with 1% toluidine blue (pH 7.4) were used for information regarding orientation and gross histology. For scanning electron microscopy, blocks fixed with Karnovsky's solution were dehydrated in graded ethanol, critical point-dried, sputter-coated, and then examined with a JEOL JSTM-20S. Species identification followed Murdy (1989).

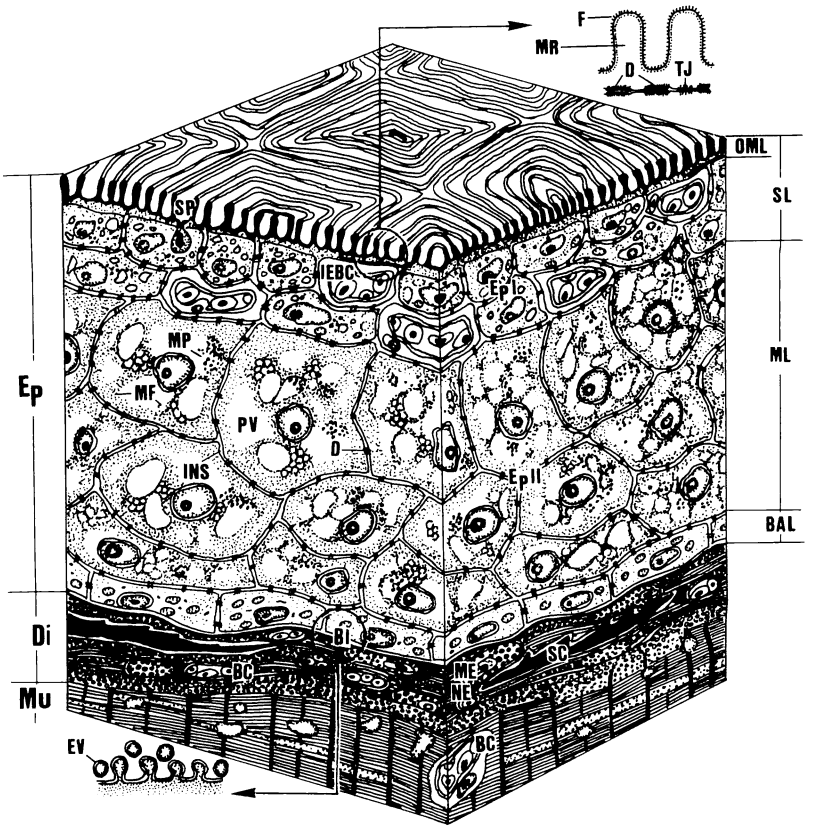


Fig. 2. Schematic illustration showing the ultra-structure of the skin of *P. modestus*. Epidermis (Ep) is divided into surface (SL), middle (ML) and basal (BAL) layers. The surface of microridges (MR) developed in the outermost layer of the epidermis (OML) is covered with a fuzzy, fibrillar substance (F), and desmosomes (D) and tight junctions (TJ) are seen in the terminal web. The surface layer contains Type I cells (EpI) and intraepithelial blood capillaries (IEBC). The middle layer consists of several strata of Type II cells (EpII), characterized by having an enormous vacuole (PV). Endoplasmic vesicles (EV) occur in the basal lamina (Bl). BC, blood capillary; Di, dermis; INS, intercellular space; ME, melanophore; MF, microfilament; MP, metallic particle; Mu, muscle layer; NE, ending of unmyelinated nerve bundle; SC, scale; SP, sensory-like pit organ.

Observations

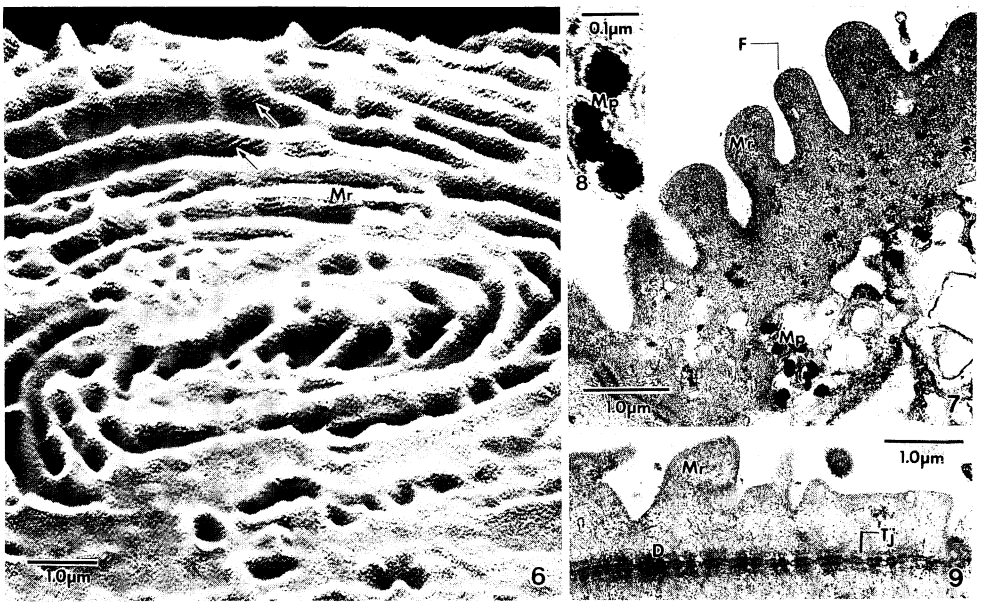
Light microscopy. The epidermis of this species can be divided into three layers: surface, middle and basal. The surface layer is composed of a single stratum of squamous cells, generally oval or pear-shaped in section, and blood capillaries (Fig. 1A–D). The capillaries were distributed over almost the whole skin area, but were rarely found in the ventral region (Fig. 1A, B and C). The capillaries were detected upon staining with acid dyes such as eosin, orange G and/or toluidine blue, but were negative to PAS reaction (Fig. 1A–D). A sensory-like cell with a flask-shaped pit was sporadically encountered in the ventral skin region (Fig. 1B, D). The middle layer is made up of several strata of enormous cells, that are characterized by having a large vacuole in the cytoplasm. However, these cells are variable both in size and shape. The cytoplasm is not stained with HE, azan and toluidine blue, or PAS (Fig. 1A–D). In the ventral surface, the scales penetrate into the basal membrane and expand deeply into the epidermal layer (Fig. 1B). The basal layer consists of a stratum of germinal cells, being either polyhedral or rhombic in shape (Fig. 1A, C). Neither mucous nor granular cells were demonstrated in any of the three layers.

Fig. 3. Type II cell during its young phase. M, mitochondrion; Mr, microridge; N, nucleus; R, free ribosome; rER, rough-surfaced endoplasmic reticulum; Tj, tight junction; V, vacuole.

Fig. 4. Tight junction (Tj) and strong interdigitation found in the terminal web.

Electron microscopy. A schematic illustration of the ultra-structure of the skin is shown in Fig. 2. As in other bony fishes, the skin consists of two layers of epidermis and dermis. Two different cell types were identified in the epidermis: Type I cells are flat in shape and have a large, centrally located nucleus. The cytoplasm contains a large number of microfilaments measuring about 7 nm in diameter, a number of rough-surfaced endoplasmic reticula, a considerable number of free ribosomes, and a few mitochondria and vacuoles (Fig. 3). The Type II cells are distinguished by a large cytoplasmic vacuole with poor cell

Fig. 5. Type II cell found in the mid-epidermal layer. Type II cells have an enormous vacuole in the cytoplasm, and a large number of microfilaments (Mf) gather in the peripheral cytoplasm. Autophagic vacuoles (arrows) occur in intercellular spaces and a residual body (arrow head) is seen in the cytoplasmic vacuole. D, desmosome; M, mitochondria; N, nucleus.



Figs. 6, 7, 8, 9. Outermost layer of Type I cell. Fuzzy substance (F) forms stripes (arrows) on the surface of microridges (Mr). Numerous dense bodies, metallic in nature (Mp), are apparent in the free surface layer and the cytoplasm. Tight junctions (Tj) and a considerable number of desmosomes (D) are seen in the terminal web.

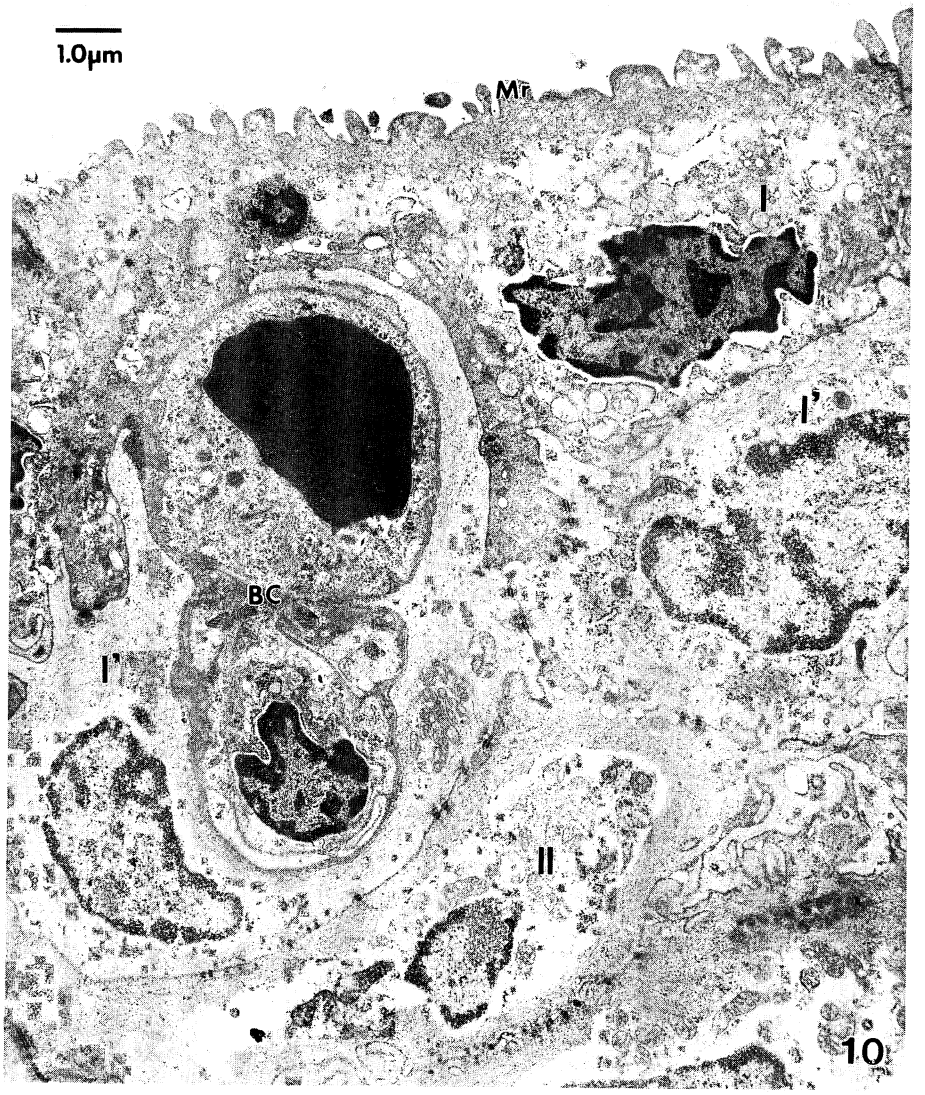


Fig. 10. Superficial layer of the epidermis. The superficial layer consists of Type I cells and blood capillaries (BC). Young Type I cells (I′) are usually located under the senescent Type I cells (I), with blood capillaries always being reinforced by young Type I cells. Mr, microridges; II, Type II cell.

organelles, such as a pycnotic nucleus and few mitochondria. The peripheral cytoplasm near the plasma membrane is filled with numerous tonofilaments, and the plasma membranes of the adjacent cells are strongly interdigitated with certain desmosomes (Fig. 5).

1) Epidermis. The surface layer is further subdivided into two layers, the outermost and the superficial layers. The former is composed of free surface Type I cells. Numerous microridges covered by a fuzzy, fibrillar substance are developed on the free surface (Figs. 6, 7 and 9). The fuzz forms stripes on the free surface (Fig. 6). Numerous dense bodies, metallic in nature, are occasionally detected in the free surface and the cytoplasm of Type I cells (Figs. 7 and 8). The free surface and the plasma membrane appear to make contact with tight junctions and a considerable number of macula adherents (Fig. 9).

The superficial layer comprises Type I cells and