

Surface Architecture of the Opercular Epidermis and Epithelium Lining the Inner Surface of the Operculum of a Walking Catfish, *Clarias batrachus*

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Abstract In *Clarias batrachus*, the opercular epidermis (OE) and epithelium lining the inner surface of the operculum (EISO) showed significant differences in their surface architecture, such being correlated with different conditions at the two locations. The surfaces of the epithelial cells had characteristically intricate patterns of microridges, which were mostly short-ridged with narrow spaces between them (OE) or high-ridged and widely spaced (EISO). These patterns were believed to be associated with the relative extent of surface secretory vesicles, present for the secretion and renewal of the extra-cellular cuticular coat at these locations. Extensively distributed on the OE, wide mucous cell openings enable increased mucus secretion, considered to be an adaptation resulting from the greater need for lubrication, protection and inhibition of invasion and proliferation of pathogenic microorganisms, than at the EISO, where mucous cell apertures were few and narrow. Taste buds were generally situated at papillate, epidermal elevations that projected from the OE surface. This possibly enhances gustatory function, being an adaptation compensating for reduced vision. The absence of taste buds in the EISO suggested that a gustatory function does not occur in that region.

Fish epidermis exhibits different patterns of surface architecture (Whitear, 1990). These patterns, considering comparable anatomical sites of various teleosts, are species specific (Fishelson, 1984). Although reports exist on the surface organization of epithelia derived from the epidermis at different locations, including for example, oral epithelium (Lane and Whitear, 1982) and gill epithelium (Ojha and Hughes, 1988), there are none on the surface relief pattern of the epithelium lining the inner surface of the operculum (EISO) or its comparison with the opercular epidermis (OE).

The present investigation was undertaken to clarify details of the surface sculpture of the OE (usually concealed beneath a thick layer of mucus) and EISO of a walking catfish, *Clarias batrachus*, and to correlate the structural modifications at these sites with their functional and adaptive significance. A member of the Clariidae (Siluriformes) (Greenwood et al., 1966), *C. batrachus* is a popular aquaculture species in Southeast Asia, where it inhabits muddy and marshy waters. Having the capacity to breath air by using its auxiliary breathing organs, the fish is very resistant to anoxic conditions, enabling very

dense populations to be maintained in ponds. The air breathing habit allows the fish to come out of the water to make extensive land excursions, frequently at night, in search of food or to migrate to other water bodies (Sterba, 1963; Welcomme, 1988).

Materials and Methods

Live specimens ($n=5$) of *Clarias batrachus* (Linnaeus) (length 140 ± 10 mm, weight 35 ± 1 gm) were collected from ponds at Varanasi, India. The fish were maintained in the laboratory at controlled room temperature ($25 \pm 1^\circ\text{C}$), being fed minced goat liver on alternate days. After acclimatization to the laboratory conditions for at least 15 days, the fish were cold anaesthetized (Mittal and Whitear, 1978). Pieces of the operculum were excised, rinsed in physiological saline, cut into conveniently-sized pieces (approximately 4×4 mm) and fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 4 h at 4°C . Fixed tissues were washed with 0.1 M cacodylate buffer (pH 7.4) and dehydrated in a cold (at 4°C) ethyl alcohol series, of ascending concentra-

tions, mixtures of ethyl alcohol and acetone in the ratios 3:1, 1:1 and 1:3, and anhydrous acetone. Tissues were critical point dried using a CPD 750 dryer (EMscope Laboratories Ltd., England) with liquid carbon dioxide as the transitional fluid. Pieces of the operculum with the OE or EISO facing upwards were then attached to stub, coated with gold using a SC 500 sputter coater (EMscope Laboratories Ltd., England) and examined with a JSM-840A (JEOL, Japan) or 515 SEM (Philips, England) scanning electron microscope.

Observations

Opercular epidermis.—The surface of the OE comprised epithelial cells, which appeared irregularly polygonal in outline (Fig. 1). The surface architecture of the epithelial cells was, in general, uniformly characterized by narrowly-spaced, short, narrow microridges, which sometimes branched, and were irregularly interwoven to form intricate patterns (Fig. 2). The boundaries between adjacent epithelial cells were demarcated by a poorly defined, double row of microridges.

Many mucous cell openings, seen as wide, rounded apertures, often containing blobs of mucus, were interspersed between the epithelial cells (Fig. 1). Generally, such apertures occurred where the boundaries of three or more epithelial cells met (Figs. 1 and 2).

Taste buds were irregularly distributed, generally projecting from the epithelial surface, since the OE at these locations was elevated into papillate structures (Fig. 3). The gustatory processes of the taste buds, when viewed dorsally, appeared granular. The epithelial cells were concentrically arranged at the papillate, epidermal elevations (Fig. 3).

Epithelium lining the inner surface of the operculum.—The EISO, unlike the OE, was not covered by a thick layer of mucus. The surface sculpture of the epithelial cells of the EISO (Fig. 4) showed a strikingly different pattern to that of the OE epithelial cells, the majority of the former being characterised by wide, high-ridged, elongated, microridges that were almost uniform in breadth (Fig. 5). Such microridges showed either extensive linear arrangements or branched and interlocked to form intricate maze-like (sometimes fingerprint-like) patterns (Figs. 5 and 6). Papillate microridges, either restricted to central portions or distributed over the

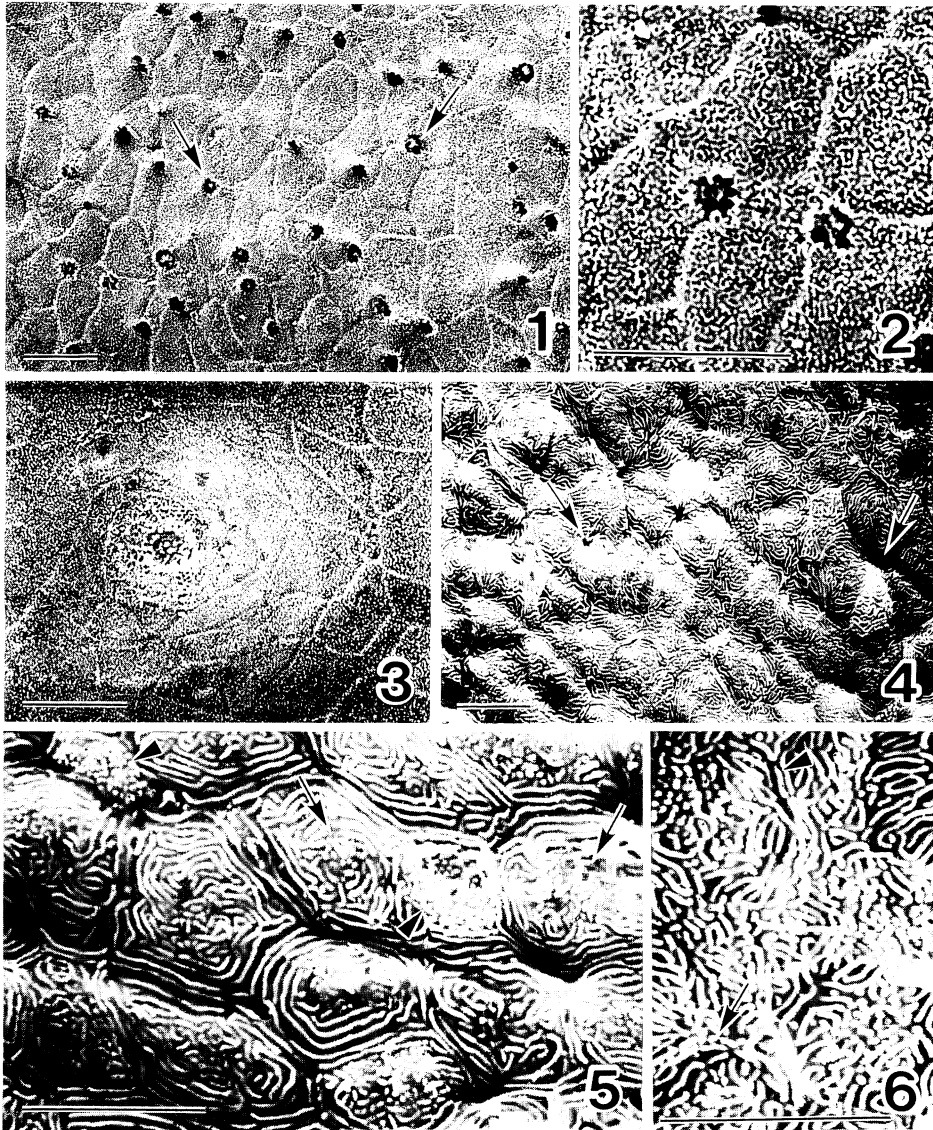
cell surface, were also discernible on some epithelial cells (Fig. 5). The spaces between the ridges were generally broad. The boundary between adjacent epithelial cells was demarcated by a well-defined, double row of ridges, often continuous, (Fig. 5) but in some cases discontinuous or zipper-like (Fig. 6).

Mucous cell openings in the EISO, in contrast to those of the OE, were relatively few and narrow (Figs. 4 and 5). Taste buds were absent.

Discussion

The surface architecture of the superficial layer of epithelial cells in the OE and EISO of *Clarias batrachus* is characterized by specialized structures which, in other fishes, have been described as cytoplasmic folds (Merrilees, 1974), microvilli (Harris and Hunt, 1975), microfolds (Hunter and Nayudu, 1978) or ridges (Iger et al., 1988). Insofar as these structures appeared as microridges under SEM and microvilli under TEM (due to the lower resolution by the latter), the term "microvillar ridges" was used by Mittal et al. (1980). The use of the term "microridges" for these structures by Whitear and Mittal (1986), Whitear (1990) and Suzuki (1992) seems appropriate and is continued here.

The present investigation showed interesting differences in the form of the microridges on the surface of the epithelial cells of the OE and EISO in *C. batrachus*. The surface epithelial cells in this fish are mucogenic in nature, synthesizing and secreting glycoproteins, thus contributing to overall surface epithelial secretion (Banerjee and Mittal, 1975; Garg and Mittal, 1990) and forming an extra-cellular cuticular coat. Microridges have invariably been observed in teleosts having a mucogenic epidermis/epithelia, where the surface epithelial cells are involved in the secretion of an extra-cellular, cuticular coat (Whitear, 1986). Whitear (1990) suggested that the form of the microridges is related to the process of secretion at the cell apex. Further, she suggested the surface sculpting to be a consequence of the arrival of new membranes, as vesicles carrying secretions fused with the apical plasma membrane. Accordingly, high ridges would indicate the rapid arrival of secretory vesicles at the surface. In *C. batrachus*, the occurrence of conspicuous, high-ridged, wide microridges at the surface of the epithelial cells of the EISO, compared to short-ridged, narrow microridges at the OE, may thus be associated with a faster



Figs. 1-6. Scanning electron micrographs of the opercular epidermis (Figs. 1-3) and the epithelium lining the inner surface of the operculum (Figs. 4-6) of *Clarias batrachus*. Scale bar = 10 μ m.

Fig. 1. Mosaic of irregularly polygonal epithelial cells with prominent boundaries, interspersed with rounded, mucous cell apertures (arrows).

Fig. 2. Narrow, short ridged microridges. Note the mucous cell apertures discernible at the boundary of three or more epithelial cells.

Fig. 3. Taste bud at the apex of a papillate epidermal elevation.

Fig. 4. Compare the arrangement of the epithelial cells with that in Figure 1. Note the high-ridged, wide microridges and few mucous cell apertures (arrows).

Fig. 5. Microridges forming a maze or finger print-like pattern. Note the papillate microridges either restricted to the central portion (arrows) or widely distributed over the surface of the epithelial cells (arrow heads), and the cell boundaries demarcated by a double row of continuous microridges.

Fig. 6. Prominent microridges forming intricate patterns. Note the discontinuous (arrow head) or zipper-like (arrow) double row of microridges at the cell boundaries.

rate of arrival of secretory vesicles at the surface of the former.

Microridges are thought to be involved in the retention of mucus, in addition to facilitating the spreading of the latter over cell surfaces (Sperry and Wassersug, 1976). Fishelson (1984) related the variations in microridge patterns to locomotory activity and suggested that in faster swimming fishes, the most developed ridges served to trap mucus on the epithelial surface. The view that the development of microridges is an adaptation for retaining mucus at the surface, however, does not seem tenable. If a fish has the capacity to retain mucus owing to the development of surface structures such as microridges, the presence of numerous mucous cells in the epidermis would seem to be of little point, it being apparently more economic to retain rather than to synthesize mucus. According to Whitear (1990), any functioning of microridges to retain mucus on the epithelial surface is contradicted by the ease with which the mucus cover is lost during preparation for microscopy. Furthermore, she suggested that the microridges themselves could not have hydrodynamic properties because they are in life buried in extracellular, cuticular secretions. Modifications in the pattern of microridges can also be caused by various intrinsic, e.g. hormonal (Schwerdtfeger, 1979a, b), or extrinsic factors e.g. temperature (Ferri, 1982), salinity (Ferri, 1983), mercury salts (Pereira, 1988), organic pollutants (Iger et al., 1988) handling and ectoparasites (Whitear, 1990).

C. batrachus dwells mostly at the bottom of ponds, often in muddy and marshy waters. In this fish, profuse mucous secretion at the OE, indicated by the presence of a large number of wide mucous cell openings and a thicker layer of mucus on the OE surface than on that of the EISO, may be related to the greater demand for mucus on the external surface of the operculum than on the inner surface. Mucous secretions protect the fish from abrasion during burrowing in bottom mud (Liem, 1967), precipitate mud that is held in suspension (Hora, 1934) and, in addition, have been accorded an important physiological role in the lubrication and protection of the fish, including the inhibition of invasion and proliferation of pathogenic microorganisms from the surrounding environment (Mittal et al., 1994).

Taste buds assist fish in the location of food and in the analysis of the chemical nature of the surrounding water. The presence of such sensory structures in

the OE of *C. batrachus* may compensate for the reduced eyes in this species, and the consequently restricted visibility in muddy turbid water. Furthermore, the presence of taste buds on the papillate epidermal elevations in *C. batrachus* is perhaps an adaptation which enhances contact with the surrounding water. Atema (1971) considered the extreme development of taste buds over the body of *Ictalurus natalis* as an adaptation to compensate for reduced vision, and Schemmel (1967) showed that in cave-dwelling populations of the Mexican characin, *Astyanax mexicanus*, which has lost its vision, the distribution of external taste buds is more extensive than in river-dwelling *A. mexicanus*, which has normal vision. The absence of taste buds in the EISO of *C. batrachus* points to the lack of any gustatory functions in this region.

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ヒレナマズの一様 *Clarias batrachus* における鰓蓋表皮および内面上皮の表層構造

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Clarias batrachus の鰓蓋表皮と鰓蓋内面被覆上皮の表層構造は、互いに著しく異なっている。表皮は丈が短くて間隙が狭い微小堤から、一方、内面上皮は高くて広い微小堤から成る。これは、本種が泥水生活をするので、表皮の保護や病原体の侵入阻止に小皮層の分泌・更新を要するため、表皮に広域分布する粘液細胞の開口部が広く、自由面への分泌物の到達が容易になることと関係している。これに比し、内面上皮のほうは開口が少なくて狭い。表皮には、味蕾が乳頭状の表皮隆起に存在して味覚能を高め、泥水中の視覚不全を補っていると推定される。一方、内面上皮には味蕾が存在しないので、その機能を欠くことが示唆される。