A COMPARATIVE SURFACE-ARCHITECHTURAL STUDY OF "SNOUT EPIDERMIS" OF SOME OF THE HILL-STREAM FISHES: A SCANNING ELECTRON MICROSCOPIC INVESTIGATION.

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Abstract: Various cellular components in the epidermis of snout of *G. gotyla*, *G. pectinopterus* and *P. sulcatus* have been characterized by using scanning electron microscopy techniques in an attempt to understand their functional significance in relation to friction. The epidermis is differentiated into rough and smooth in *G. pectinopterus* and *P. sulcatus* and is smooth only in *G. gotyla*. The rough epidermis consists of the epithelial cells. The smooth epidermis in addition to these cells type also possesses mucous cells in the all three fishes. The surface of rough epidermis and smooth epidermis of *P. sulcatus* and *G. pectinopterus* are keratinized in nature. In the smooth epidermis the mucous cell apertures are interspersed between the epithelial cells in these fishes. In the rough epidermis, the epithelial cell surfaces are modify into epidermal growth the unculi. The rough epidermis of mid dorsal part of snout possesses epidermal tubercles. The base of each tubercle is broad, some poorly developed taste buds are describe in the snout of *P. sulcatus.*In *G. pectinopterus* large numbers of unculiferus plaques are present all aver the snout. These plaques are separated each other by epidermal furrow. The presence of these plaques is probably an adaptation to the peculiar digging mode of life of this fish as surface can better protect from wear and tear, providing hardness, durability and mechanical strength than that of glandular epidermis. Taste buds assist fish in the location of food and in the analysis of chemical structures in the epidermis of snout of all the three fishes under present investigation may compensate for reduce eyes in the hill-stream fishes.The presence of a very thick coat of mucus over the snout epidermis if compared to that of its general body epidermis in *G. gotyla* that is liable to more frictional force. When the fish swims upstream is significant. This may provide sufficient lubrication to reduce the frictional stress between the body surface and the water current and may protect the epidermis from wear and tear the protective role of epidermis modification acting as first line of defence against the colonization of pathogens.

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1. Introduction:

The epidermis of teleost fishes is generally nonkeratinized. Burgess (1956) studied the epidermis of several species of fishes using histochemical techniques and reported that the protein, keratin is usually not synthesized. Keratinizatuion does, however, occur in some fishes in specialized structures such as the teeth in oral disc of cyclostomes (Dawson, 1963 and Haristry & Potts, 1971) and the breeding tubercles in many teleosts (Collette, 1977, Schwerdtfeges & Bereiterhahn, 1978). The jaws and horny lips in many species of herbivorous minnows (Klub & Wang, 1932). The occipital organs in gonorynchiform genus *Kneria* (Peters, 1967) and the keratinized areas at the surface of flask-shape epidermal organs in *Marulius* (Sasse et. al., 1970). The surface epithelial calls on the frictional surface of the adhesive apparatus in various Indian hill-stream fishes are modifies as spine like processes (Hora, 1922) which are said to be cornified (Saxena., 1959, 1961 and Saxena & Chandi, 1966). Electron microscopic studies on the general body epidermis of *Bagarius bagarius* also give the evidence of the presence of true keratinization (Mittal & Whiter, 1979).

The present investigation has been designed to have a comparative study of the functional organization of the epidermis of snout of *G.gotyla*, *G.pectinopterus* and *P.sulcatus.* Snout epidermis is subjected to more frictional stress if compared with the epidermis covering the general body surface as it is first to come in contact with water current, especially when fish swims upstream and hence correlation has been made in relation to friction.

2. Materials and Methods:

Live adult specimens of *G. gotyla* (7-9 cm long) were collected from Kosi River at Kakrighat, Distt Nainital, *G. pectinopterus* (5-7 cm long) from west Ramganga River at Chaukhutiya, Distt. Almora, and *P. sulcatus* (6-7 cm long) from east Ramganga River at Thal, Distt. Pithoragarh respectively water current was very fast having velocity 0.5 to 2.0 m/sec. in Kosi, 1.5 to 2.5 m/sec. in west Ramganga and 2.0 to 3.0 m/sec. in east Ramganga (Bhatt & Pathak, 1991). Specimens were maintained in laboratory at 25 ± 2^{0}C. The fish were cold anesthetized, following Mittal & Whitear (1978), for SEM preparation of snout. Tissue were excised and rinsed in 70 % ethanol and one change saline solution to remove debris and fixed on 3% Glutaraldehyde in 0.1M phosphate buffer, at p^{H} 7.4 for one night at 4^{0}C at Refrigerator. The tissue were washed in 2-3 changes in phosphate buffer and dehydration in the graded series of ice cold Acetone (30%, 50%, 70%, 90%, and 100% approximate 20-30 min.) and critical point dried, using Critical Point Dryer (BIO-RAD England) with liquid carbon dioxide as the transitional fluid. Tissues were glued to stubs, using Conductive Silver Preparation (Eltecks, Corporation, India) Coated with gold using a sputter Coater (AGAR, B 1340, England) and examined in a Scanning Electron Microscope (Leo, 435, VP, England). The results were recorded using Kodak T-MAX 100 professional film (Kodak Ltd., England).

3. Result Analysis

The skin of snout is scale less in all three fishes and the epidermis is both types keratinized and mucogenic. The snout epidermis of *G. gotyla* is glandular and smooth. The epidermis of snout of *P. sulcatus*, however, possesses smooth surface and rough surface at irregular intervals and the snout epidermis of *G. pectinopterus* possesses a large number of plaques distributed at irregular intervals (Fig. 1).

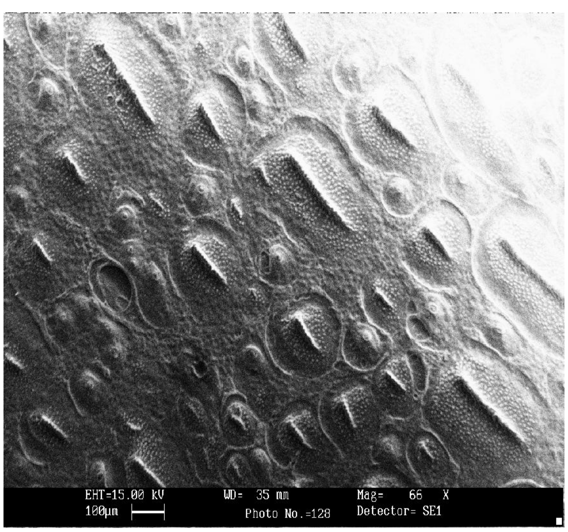

Figure 1. SEMPH of snout epidermis of *G. pectinopterus* showing large number of numerous plaques separated from epidermis furrows (Marked by arrows) (Scale bar-100 µm).

The epidermis of snout of *G gotyla* and smooth epidermis of snout of *P. sulcatus* is resembles with each other. Epidermis possesses epithelial cells and mucous cells apertures intersperse between the epithelial cells in both the fishes. The free surface of epithelial cells is bearing a series of microridges. The microridges of the cells of epidermis of *G. gotyla* appear smooth and uniform in width. They are concentrically arranges (Fig. 2).

In *P.sulcatus,* microrigdes are not interconnected to each other but are elongated filamentous (fig. 3). The filaments are smaller than the filaments of general body epidermis of this fish.

Figure 2. SEMPH of the snout epidermis *of G. gotyla* showing microridges (Marked by arrows) (Scale bar- 3 µm).

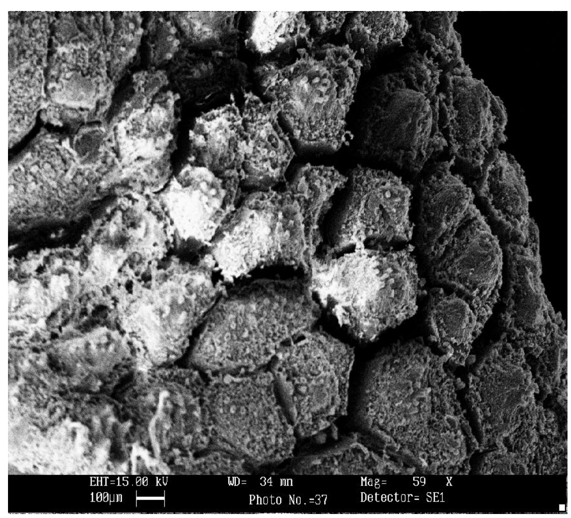

Figure 3. SEMPH of the snout epidermis of *P. sulcatus* of showing numerous filamentous microridges (Marked by arrows) (Scale bar- 100 µm).

Interseperated between the epithelial cells mucous cells apertures distinguished on the snout epidermis of both the fishes. The mucous cell apertures generally are rounded and occurred at border of each epithelial cell in *G. gotyla*. In *P. sulcatus*, mucous cells apertures are rare comparatively and occurred at the border of the three and four epithelial cells (Fig.4, 5).

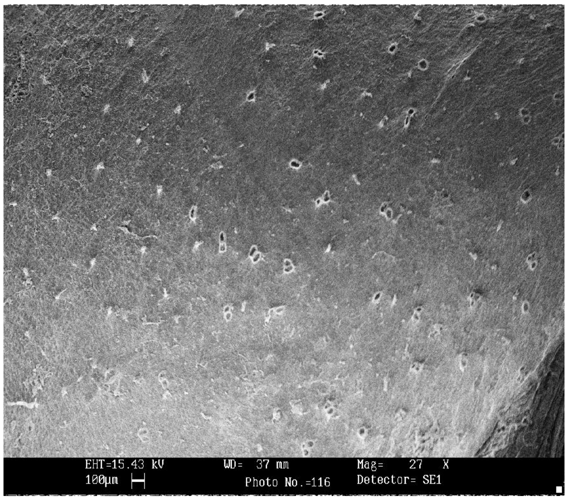

Figure 4. SEMPH of the snout epidermis *of G. gotyla* showing mucous cells openings (Marked by arrows) (Scale bar- 100 µm).

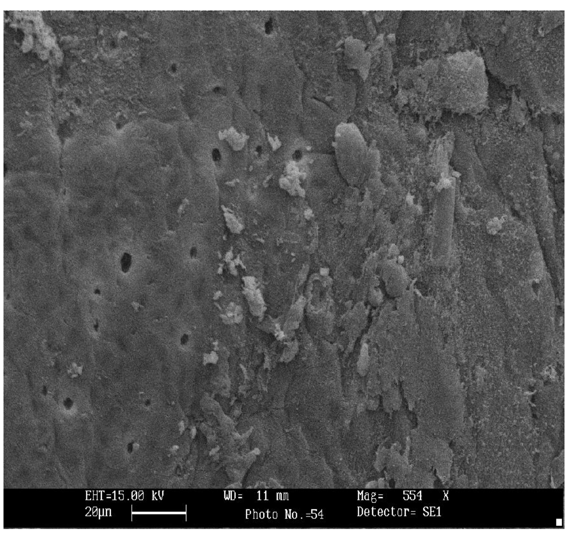

Figure 5: SEMPH of the snout epidermis *of P. sulcatus* showing mucous cells opening (Marked by arrows) (Scale bar-30 µm).

The rough epidermis of snout of *P. sulcatus* bears only epithelial cells. Surface of these epithelial cells are modifies into epidermal growth the unculi (Fig. 6). These unculi are short and stumpy structures. In both these fishes the epidermis of mid-dorsal part of snout possesses epidermal tubercles. These type of structures are absent in dorso-lateral part of snout. The base of each tubercle is broad. Some poorly developed taste buds are described in the snout of *P. sulcatus*, each bud rounded in shape, and formed by the concentrically arranged epithelial cells (Fig.7).

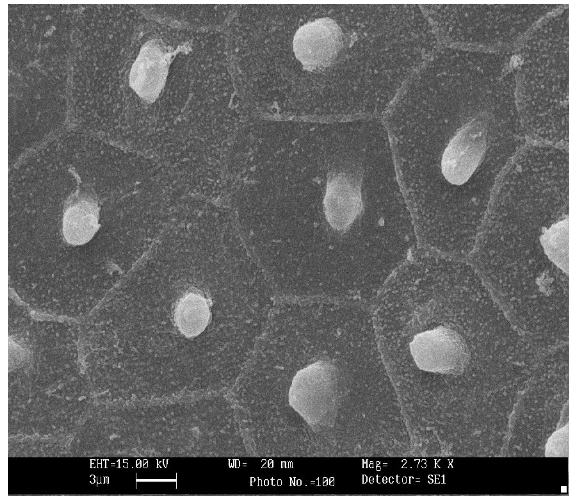

Figure 6: SEMPH of the snout epidermis *of P. sulcatus* showing unculi, the modified epidermal growth (Marked by arrows) (Scale bar- 3 µm).

Figure 7. SEMPH of the snout epidermis *of P. sulcatus* showing taste buds (Marked by arrows) (Scale bar- 10 µm).

In *G. pectinopterus*, large number of unculiferous plaques are present all over the snout (Fig 1). The number of plaques is less than the plaques of general body epidermis as well as are smaller in size. These plaques are separated from each other by epidermal furrow (Fig.1).

The surface cells of the epidermal plaques are keratinized while those in the furrow are mucogenic (Mittal and Munshi, 1970). Each plaque have a centrally placed ridge of 6 to 8 overlapping cells (Fig. 8). The rest of the plaques are covered by polygonal unculiferous epithelial cells (Fig. 9). The unculi may be stout, conical and curved at the tip. These are smaller in size than the unculi of general body epidermis.

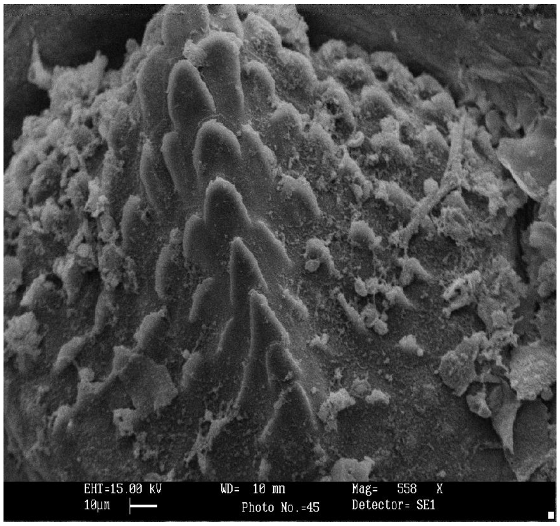

Figure 8: SEMPH of the snout epidermis of *G. pectinopterus* of showing single plaque with centrally placed ridge (Marked by arrows) (Scale bar- 10 µm).

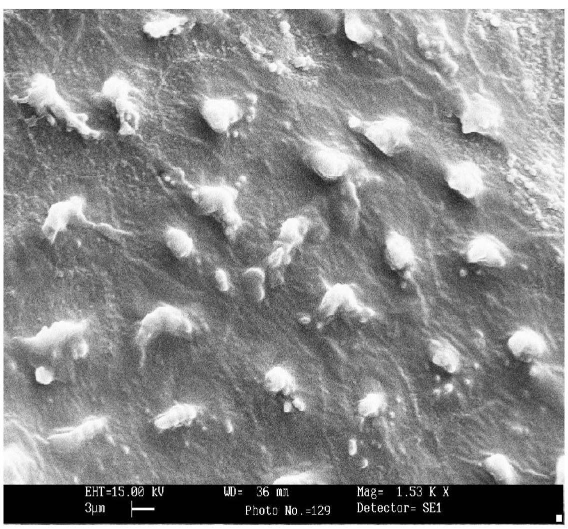

Figure 9. SEMPH of the snout epidermis of *G. pectinopterus* of showing unculiferus epithelial cells (Marked by arrows) (Scale bar- 3 µm).

At distal end of each unculus, the surface appears rough and exhibits vertically-oriented microvillous projection (fig. 10). In each unculus these projections gradually decline from the peripheral to central region. Thus, each appears much-like a tooth that has a characteristic sharp edge at the margin and deep depressions centrally (fig. 11).

The epidermal furrow region, possesses epithelial cells, the free surface of each epithelial cell is bear filamentous microridges (Fig.12).

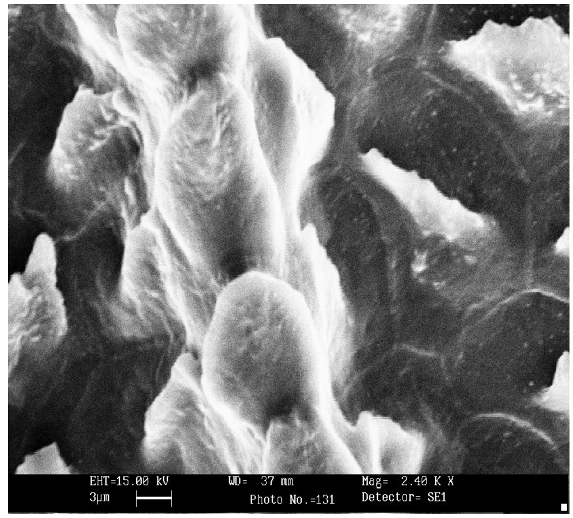

Figure 10. SEMPH of the snout epidermis of *G. pectinopterus* of showing microvillous projection of unculi (Scale bar –3 µm).

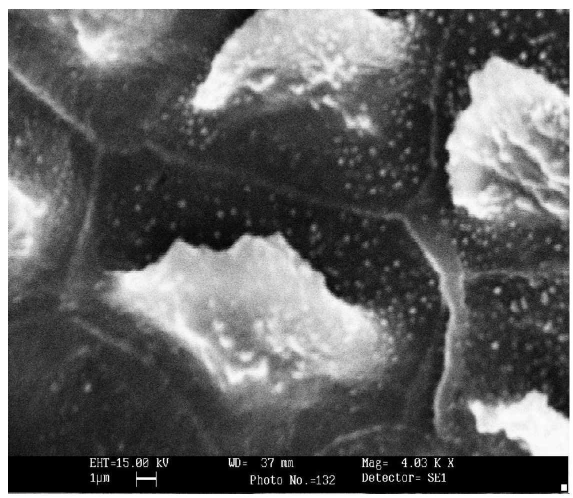

Figure 11. SEMPH of the snout epidermis of *G. pectinopterus* of showing polygonal tooth like unculi (Scale bar -1 µm).

4. Conclusions

Fish have a wide range of protective skin adaptations, which enable them to occupy habitats ranging from rocky bottom surface to turbulent water.

The skin of snout is scale less in all three fishes.

In *G.gotyla*, the mucous cell openings are much here if compared with that of its general body epidermis.

The protective function of the slime secreting cells in the non-keratinized epidermis of *G.gotyla* is probably taken up by the dead keratinized superficial layer in the epithelium of *P. sulcatus* and *G.pectinopterus*.

The mucus secreted by the mucous cells over the skin performs wide variety of functions. We concentrate, here, mainly on its role in adapting the fish to its peculiar mode of life in mountain torrents. The presence of a very thick coat of mucus over the snout epidermis if compared to that of its general body epidermis in *G. gotyla* that is liable to more frictional force. When the fish swims upstream is significant. This may provide sufficient lubrication to reduce the frictional stress between the body surface and the water current and may thus protect the epidermis from wear and tear. The protective role of epidermis modification acting as first line of defence against the colonization of pathogens has also been suggested in *Salmo trutta* (Pickering 1974 and Pickering & Richards, 1980). The hill-stream fishes usually feel difficulty in respiration due to the restriction of gill slits to the sides, especially when feeding on algal slime or are attached to rocks and stones (Hora 1922) the presence of thick mucus coat may significantly help in respiration by providing extra oxygen to the fish in *G. gotyla* and *P.sulcatus*.

The absence of gland cells at non-glandular epidermis in *P.sulcatus* and *G. pectinopterus* and their presence at glandular epidermis in all the three fishes are significant and show an inverse relationship between the degree of keratinization and the abundance of types of various slime secreting glands. Mittal and Banerjee (1974) in *Bagarius bagarius,* Agarwal (1979) in *Sisor rabdophorus*, Benerjee & Mitttal (1978) in *Natrex piseator* and Agarwal & Shah, (1987) in *Garra gotyla* also observed similar relationship. The protective function of slime secreting cells in the glandular epidermis of *G.gotyla* and *P.sulcatus* is probably taken up by the dead keratinized superficial layer in non-glandular epidermis of *p. sulcatus* and *G.pectinopterus*. The snout epidermis of *G.pectinopterus* is hard and provided large number of unculiferous plaques this is probably and adaptation to the peculiar digging mode of life of this fish as keratinized surface can better protect from wear and tear, providing hardness, durability and mechanical strength than that of glandular epidermis.

Sense of taste is an important property in fish for distinguishing from a variety of food available to them in a aquatic environment (Hara, 1994). Taste buds assist fish in the location of food and in the analysis of chemical nature of surrounding water. The presence of such sensory structures in the epidermis of snout of all the three fishes under present investigation may compensate for reduced eyes in the hill-stream fishes and the consequently restricted visibility in muddy turbid water. Furthermore, the presence of taste buds in outer epidermal layers is perhaps an adaptation which enhances contact with the surrounding water. Atema (1971) and Garg et al., (1995), considered the development of taste buds over the body of *Ictalurus natalis* and *Clarias batrachus* as an adaptation to compensate with reduce vision and Schemmel (1967) showed the incave dwelling populations of the Mexican characin, *Astganax Mexicans,* which has lost its vision, the distribution of external taste buds is more extensive than in river–dwelling *A.mexicans*, which has normal vision.

Similar structures are also observed on the snout, lips and barbel epithelium of *Gadus mothua* Linnaeus, 1758 (Harvey & Batty, 1998) on the head, lips and gill rakers of *Damia rerio* Hamilton, 1822 (Hansen et al., 2002) on the lips and mouth of some blennid amd gobiid fishes (Fishelson & Dalarea, 2004) and mouth cavity of *Rita rita* (Yashpal et al., 2006).

The free surface of the epithelial cells at different locations (e.g., skin, lips, mouth cavity and gills) of different fishes, is characteristically differentiated into series of microridges, and referred to as cytoplasmic folds, microvilli, microfolds, microvillar ridges, ridges or microridges (Garg et al, 1995). The microridges are organizes in different ways to form intricate patterns and are thought to be involves in various functions, e.g., absorptive or secretary activities, to aid in laminar flow, holding mucus secretions to cell surface, to provide reserve surface area for stretching, to facilitate the spread of mucus away from mucous cells, to provide mechanical protection, to enhance mechanical flexibility (Whitear, 1990; Olson, 1995).

The form of microridges corresponds to type and rate of secretion at the cells apex. Some variations in surface pattern may reflect the stage of maturation of a particular cell, or group of cells that have recently reaches the surface furthermore, the development of microridges are then a consequence of the arrival of new membranes, as vesicle carrying the secretion fuse with the apical plasmalemma and high ridges would indicate a rapid sequence of arrival of secretory besides at the surface (Whitear, 1990). In the snout epidermis of *G.gotyla*, presence of prominent microridges could thus be considered to reflect high secretory activity of epithelial cells than the *P.sulcatus*, in which the microridges are poorly developed in snout epidermis.

Compactly arranges microridges, often interconnected with microbridges, may also be considered to provide rigidity to the free surfaces of the epithelium, in order to protect against physical abrasions, when maneuvering the fish (Mittal et al., 2004).

The presence of plaques over the epidermis of snout of *G. pectinopterus* may be associates with a localized increase in the thickness of the epidermis. The primarily function to provide mechanical protection and also provide protection against pathogenesis, they might also help to prevent ectoparasitism.

Eiras stofella & Charvet Atmeida (1998, 2000), Eiras stofella & Fank-de-carvalho (2002) and Kumari et al., (2005), observed taste buds in the gill rakers and the pharyngeal side of gill arche of olypphagous *Procholodus scrofa* and the omnivorous *Eugerres brasiloanus and Cathorps spixii* respectively. They suggested that the chemical receptors might be used to help in food selection at swallowing.

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