Scanning Electron Micrioscopic Investigation Of "Adhesive Apparatus Epidermis" Of *Glyptothorax Pectinopterus* (McClelland) (Sisoridae)

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Abstract: Surface architecture of the epidermis of adhesive apparatus of *Glyptothorax. Pectinopterus* (McClelland) (Sisoridae) examine by scanning electron microscopy. The hill-stream fishes have developed specialized organs for adhesion in several strategic regions of the body. Adhesive apparatus are the modifications of the skin on ventral surface. The epithelial cells have been demonstrated in the epidermis of adhesive apparatus of *G. pectinopterus,* by scanning electron microscopic methods, to study its structural and functional organization with special reference to adhesion. The epidermis of groove of *G. pectinopterus* is equipped with epithelial cells and mucous cells. The epidermis of ridges of *G. pectinopterus* consists of mainly keratinized cells. The secretion of amount of mucus by the epidermis covering the groove. This might help in maintaining the vacuum creating by the muscles under margins and thus preventing the entrance of water and air.

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Key words: Fish, *G. pectinopterus,* Epidermis, Adhesive Apparatus, SEM.

1. Introduction:

Surface architecture of the epidermis of adhesive apparatus of *G. pectinopterus* examine by scanning electron microscopy. The adhesive apparatus are integumentary modifications, serve as highly specialized organs and are definite in location, form and size, lip may be associated with a specialized adhesive pad or modified as an oral sucker which provide as admirable device for the adhesion of fish to the substratum (Hora, 1921, 1922; Saxena, 1922; Saxena, 1959; Saxena, 1961; Saxena & Chandy, 1966; Ojha & Singh, 1992; and Singh et. al, 1994). The most important characteristics in response to these conditions are the integument modification in the form of an adhesive disc which has become a life saving kit for most of the Hill-stream fishes. Hora (1922, 1923 and 1930) studies large number of fishes with respect to their adaptive modifications, He described the adhesive organ of *Garra annandalei* (cyprimoids) and *Glyptothorax modraspatnam* (silurioide).Some aspect of ultrastructures of the adhesive apparatus of several fishes have been studied before (Das & Nag, 2005, 2006; Singh & Chandy, 1960; Hora, 1922; Bhatia, 1950; Saxena, 1963; Lal et al., 1966; Bose et al, 1971 and Singh & Agarwal NK., 1990, 1991).

2. Material and Methods:

Live adult specimens of *G. pectinopterus* (5-6 cm long, collected 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. Specimens were maintained in laboratory at 25 ± 2^0C. The fish were cold anesthetized, following Mittal & Whitear (1978), for SEM preparation of paired fins. 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^oc at Refrigerator. The tissue were washed in 2-3 changes in phosphate buffer and dehydrated 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. Results:

The adhesive apparatus is situated ventrally in this fish (Fig. 1).

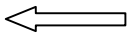

Fig 1: Photograph of the showing well adhesive apparatus of *G. pectinopterus* in ventral side of body (Marked with arrow).

In *G. pectinopterus* the adhesive apparatus is heart shape and is present in the thoracic region behind the posterior lip, in between the base of pectoral fins and is known as thoracic adhesive apparatus (approximate length 16.0mm and width 14.0) (Bisht, I., 1999) (Fig.2) 80, 8) The thoracic adhesive apparatus of *G. pectinopterus* provided with 15-16 longitudinally arranged broad folds or ridges (approximate width 84.0 μm) separated by groove (approximate width 17.4 μm) (Fig. 2).

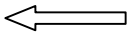

Fig 2: Surface electron microphotograph (SEMPH) of the adhesive apparatus epidermis of *G. pectinopterus* showing well developed longitudinally ridges separated by groove (marked by arrow) (Scale bar 1μm).

The epidermis of groove is nonglandulr and is composed of mainly epithelial cells. In the smooth epidermis, epithelial cells are characterized by microridges. The microridges are compactly arranged. The microridges are numerous filamentous (Fig. 3).

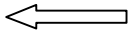

Fig. 3: SEMPH of adhesive apparatus epidermis of *G. pectinopterus* showing the filamentus microridges (marked by arrows) at the surface of epithelia of groove region. (Scale bar- 1 μm).

Interspersed between the epithelial cells, mucous cells are distinguished. The mucous cell opens with well developed mucous cell opening in all of *G. pectinopterus,* the surfaces of epithelial cells of ridges are provided with an organized prominent large, conical unculi. These unculi are backwardly directed and are teeth like in shape (Fig. 4).

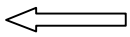

Fig 4: SEMPH of adhesive apparatus epidermis of *G. pectinopterus* showing prominent unculi on the surface of ridges (marked by arrows) (Scale bar – 2 μm).

The base of unculi of both the fishes possesses hexagonal epithelial cells indicating that these unculi are the modification of epithelial cells in the fishes (Fig. 5).

Fig 5: SEMPH of adhesive apparatus epidermis of *G. pectinopterus* showing hexagonal epithelial cells at the base of each unculi (marked by arrows) (Scale bar – 3 μm).

In the adhesive apparatus of well developed, certain, oval/ spherical openings of neuromuscular organs are observed frequently in the surface of epithelial cells (Fig. 6).

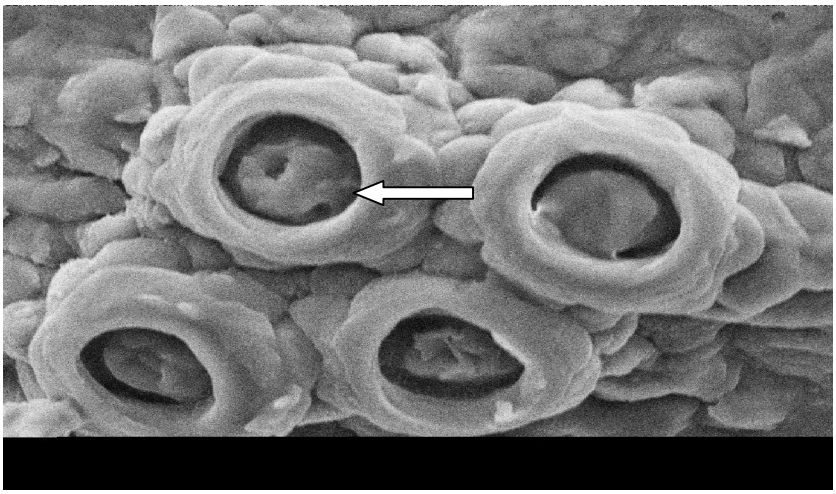

Fig 6: SEMPH of adhesive apparatus epidermis of *G. pectinopterus* showing well developed neuromuscular organs (marked by arrows) (Scale bar – 3 μm).

Fig 7: SEMPH of adhesive apparatus epidermis of *G. pectinopterus* showing developed epidermal growth (marked by arrows) of the epithelial cells (marked by arrows head) (Scale bar – 3 μm).

The tiny conical hook shaped projection arising out of the epithelial cells are present along both the sides of adhesive apparatus these conical structures are called epidermal growth (Fig 7).

4. Discussions:

The epidermis of the adhesive apparatus, and the structures associated with them show considerable structural modifications. These may be considered as adaptations in relation to its peculiar adhesive procuring device, feeding habit, feeding zone and the nature of its rivers. Practically this fish is bottom dweller inhabiting torrential hill-stream with an uneven bed composed of stones, rocks, boulders, gravel and sand, Hora (1922), Saxena (1954), Ojha & Singh (1922) and Singh et al., (1994) think that the central portion of the adhesive pad constitute an effective sucker when the fish rest on the substratum however, the mucogenic epithelia covering the rostral cap and the central region of the adhesive pad are likely to be subjected to wear and tear during frequently adhesion and friction of the fish with the substratum as they are on the ventral side of the body. Thus heavy secretions elaborated by the epithelial cells and the mucous cells could be regarded as an adaptation to lubricate and protect the epithelial cell from abrasion.

Wu and Liu (1940) also thought that the adhesive disc in *Glyptosternum* could be protective than adhesive as the chest region of the fish is liable to friction when fish swims upstream. Bose et al., 1971) reported that the adhesive disc protected the ventral scale less portion from stones and sand particles like a shield. The epidermis covering the ridges is characterized by the presence of spines, whereas in the groove between ridges is devoid of spines. These ultrastructural features allow us to speculate about the possible mechanism operative in the process of adhesion by adhesive apparatus in *G. pectinopterus* when the adhesive apparatus are pressed against the substratum a reduced pressure is created by the musculature attached to the ridges and grooves. The spiny projections might then assist in effective adhesion, especially by unchatring to the organic growth on the submerged rocks. Sinha et. al., (1990) and Singh & Agarwal (1991) suggested that the spines are mainly responsible for adhesion to the substratum. The mucus secretion from the mucous cells causes a weak adhesion and prepares the substratum for subsequent action of the spines. In addition, the mucus seems to afford protection to the spines from abrasion during adhesion. The apparent lack of spines in some of the epidermal cells (located near the base of the ridges) indicates that these structures are often damages and then possibly lost to generate new spines. The factor causing damage to the spines could be the constant mechanical abrasion or reduced mucus secretion from the surrounding mucous gland in altered physiological states.

The epidermis of adhesive apparatus indicate that, they are in a state of undergoes the processes of keratinization. The keratinization, in the epithelial cells epidermis increases as the move towards the surface in *G. pectinopterus.* The 'unculi' at the surface of ridges facilitate firm adhesion with the substratum and act as frictional device to provent the fish from slipping. At the same time, as revealed from the study of thoracic myology the pressure applied on the adhesive apparatus during adhesion may also expel water and air thereby creating a partial vacuum, within the grooves of successive rigdes (Saxena & Chandy 1966).

Hill-stream fish is inhabitant of the fast flowing cold water streams. This fish is bottom dwellers inhabiting torrential hill-streams with anuneven bed composed of stones, rocks, boulders, gravel or sand. The spine border on the tubercles are very well marked in the upper liable folds of respective lips like, the epithelial cells at the base of the tubercles are very clear. The disc comes in contact with the substratum, first which not only anchor to the substratum but also act as mechano-sensory organs. This process is following by the secreting of mucus is significant as chest region of the fish is liable friction especially when it swims upstream.

This may provide reduce friction between body surface and water currents that protecting the epidermis from wear and tear during locomotion. The adhesive apparatus in differs from other hill-stream fishes in the prominent and orientation of ridges and groves. In *G. telchelta* (Bhatia, 1950; Lal et. al, 1966; Singh et. al., 1990; Singh et. al., 1991; Das et. al., 2006 and 2008) adhesive apparatus comprise the longitudinal to long body axes. In other species *Schizotharax plogiostomus, crossocheilus latius latius* (Sing & Agarwal, 1990) adhesive apparatus consists of transverse band behind scrapping plate and provided with numerous tuberculated projections. While in *Bhavania annandalai* (Hora, 1922,), *Garra annadalei* (Hora, 1923), *Garra mullya* (Saxena, 1962) and *Garra gotyla* (Singh & Agarwal, 1990) it is complex with marked anterior labial fold, posterior labial fold , callous portion of disc and posterior free margin of disc. The present study also reported the occurrence of long spines on morphological ridges but in *P. sulcatus,* there is further subridge and subroove patterns on morphological ridges itself and spines are largely papulated on subridges region only. Secretary activities of epithelial cells and mucous cells in the non-keratinized epidermal grooves may provide protection to the fish from various harmful factors in the environment (Mittlal et. al., 1995 a). In addition, mucus secretion has been associated with diverse activities such as recognition of cells, reception of chemical information and the maintenance of a suitable environment (Mittal et. al., 1995). The interspaces between the tubercles provide continuous flow of water for aeration. The microridges (MR) provide structural integrity to epithelium of flat circular part and increase

the surface area and also prevent mechanical abrasions (Aslon, 1945). The thoracic adhesive apparatus of *G. pectinpterus* operate on a frictional principle. The unculi at the surface of prevent the fish from slipping. When observed in light microscopic the adhesive apparatus of *G. pectinopterus* show the presence of long curved, keratinized epidermal spines (Lal et. al., 1966; Sing et al., 1990) SEM investigation also showed in incidence of long spine containing keratinized major constituent, The spines are hook like structures. These are a further significant factor in the adhesion process at least in *G. pectinopterus*, the unculi present in the epidermis at ridge of adhesive apparatus prevent skidding of the fish. The present of well developed but few mucous cell in epidermis of the grooves is again interesting in the sense that their secretions might form a thin film of mucus along the margin and may thus additionally assist in maintaining the vacuum preventing the entrances of water and air. The microridges are very well marked. They help in retaining of the mucus. The copious amount of mucus secretion by the epidermis covering the grooves in all the three fishes is significant. This might help in maintaining the vacuum created by the muscles forming a film of mucus along its margins and thus preventing the entrances of water and air.

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