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Original Article

Comparative ultrastructural study of general body epidermis of the hill-stream fishes; *Botia almorhae* (Teleosti: Botiidae), *Homaloptera brucei* (Teleostei: Balitoridae) and *Schizothorax richardsonii* (Teleostei: Cyprinidae)

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Abstract: The aim of the present study is to provide a basis for better knowledge of the surface architecture of the GBE of some hill-stream fishes. The skin of the hill- stream fishes, on the dorsal surface of the body just behind the head, is densely set with scales and composed of an epidermis and a dermis supported by a hypodermis. Noticeable differences exhibited in the patterns of microridges on epithelial cells, distribution of mucous cells and presence of tubercles on the general body epidermis of the hill-stream fishes may be considered as modifications relating to possible difference in the functional requirement at the different locations. The skin has long been recognized to protect the organisms from deleterious environmental impacts (physical, chemical, microbiological). It is also well-known to be crucial for the maintenance of temperature, electrolyte and fluid balance.

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Introduction

The hill-stream fishes are well-adapted to specialized conditions of their life in torrential environment, where velocity of water current is high. Fishes living in hill-streams show several important modifications and may be conveniently divided into two groups. The members of one group are temporary inhabitants of the hill-streams and migrate upwards only at certain periods of their life for specific purposes such as spawning. These species move up by muscular effort and do not exhibit special modifications. Whereas other live permanently in the rivers and streams of the hills such as members of the families Cyprinidae (e.g. *Garra, Schizothorax, Schizothorax, Barbus* and *Crossocheilus),* Balitoridae (e.g. *Balitora*), Psilorhynchidae (e.g. *Psilorhynchus*), Nemacheilidae (e.g. *Nemacheilus*), and Sisoridae (e.g. *Glyptothorax pectinopterus* and *Pseudocheneis sulcatus*) that have specialized organs to live in such an environments. Some fishes in the hill-streams of India are represented by the genera belonging to the families Cyprinidae, Botiidae and Balitoridae. These fishes show a remarkable uniformity in their body contours. Dorsally their body is slightly arched, while ventrally it is usually flat from snout to anus. The aim of the present study is to provide a basis for better knowledge of the surface ultrastructural of the general body epidermis (GBE) of three hill-stream fishes, including *Botia almorhae*, *Homaloptera brucei* and *Schizothorax richardsonii.*

Materials and Methods

The live *B. almorhae* (127-177 mm in total length) were collected from the Kosi River at Kakrighat of District Nainital (1200 m. above sea level (asl)), *H. brucei* (76-101 mm in total length) from West Ramganga at Chaukhutia in District Almora (1200 m asl) and *S. richardsonii* (152-203 mm in total length) from the Kosi River at Hawalbagh in District Almora (1194 m asl) Uttarakhand. The water current was very fast having the velocity between 0.5 to 2.0 m/sec (Bhatt and Pathak, 1991) and the river bed is rocky.

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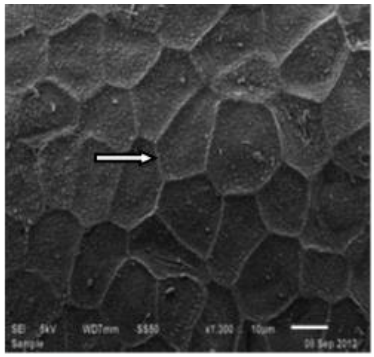
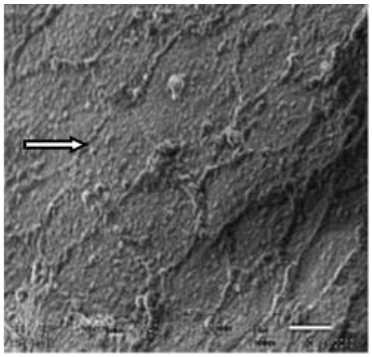


Fig. 1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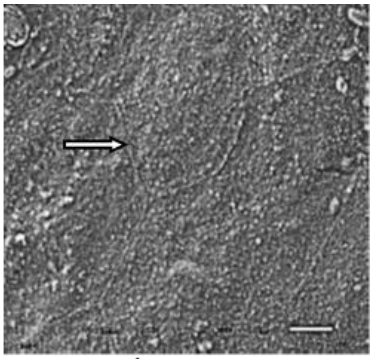


Fig. 3

Figure 1. SEMPH (Scanning electron microphotograph) of the GBE of *Botia almorhae* showing polygonal epithelial cells (marked by arrow) (Scale bar=10 μm).
Figure 2. SEMPH of the GBE of *Homaloptera brucei* showing polygonal epithelial cells (marked by arrow) (Scale bar=5 μm).
Figure 3. SEMPH of the GBE of *Schizothorax richardsonii* showing polygonal epithelial cells at high magnification (marked by arrow) (Scale bar =5 μm).

The collected fishes were transferred to the laboratory in well-ventilated plastic containers and kept for 5-6 days in glass aquaria having an artificially prepared rocky bed with aquatic vegetation grown therein. The aquaria were cleaned and supplied with fresh spring water on alternate days. The fishes were fed on aqua feed (tropical fish food).

The following procedure was adopted for the preparation of the specimen for SEM. The maintained specimen in laboratory at 25±2°C were cold anesthetized based on Mittal and Whitear (1978), for SEM preparation. Skin fragments of about 10×10 mm were cut from their dorsal sides just behind their heads. Tissue were excised and rinsed in 70% ethanol with one change of saline solution to remove debris and then fixed in 3% Glutaraldehyde in 0.1M phosphate buffer at pH 7.4 overnight at 4°C in a refrigerator. The tissues were washed with 2-3 changes in phosphate buffer and dehydrated in ascending series of ice cold Acetone (30%, 50%, 70%, 90% and 100% approximate 20-30 mins.) and dried at critical point using a 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). The samples were coated with gold using a sputters coater (JFC 1600) and examined under (JEOL, JSM- 6610 LV) scanning electron microscope and the images were observed on the screen.

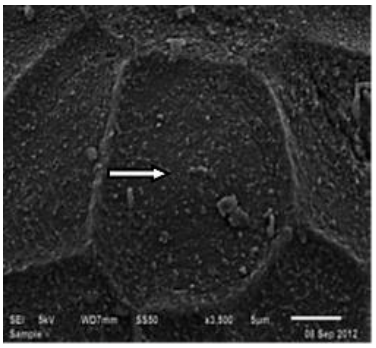
Results

The skin covering the general body surface of *B. almorhae*, *H. brucei* and *S. richardsonii* are rough and covered with a large number of scales. In *B. almorhae*, the entire external body surface is covered by minute scales; however those of in *H. brucei* and *S. richardsonii* have large number and small scales, respectively. Each scale is covered externally by the epidermis which reaches the posterior free margins transversing a short distance on its inner surface and then continue to the outer surface of the underlying scale.

The polygonal epithelial cells are shown in the GBE of *B. almorhae, H. brucei* and *S. richardsonii* (Figs. 1, 2, 3); the free surface of the epithelial cells is differentiated into microridges, forming characteristic patterns.

In *B. almorhae,* the epithelial cells bear numerous short, sinus and branched interwoven microridges (Fig. 4). However the finger print-like patterns of microridges are often shown on the surface of the epithelial cells of *H. brucei* and *S. richardsonii* (Figs. 5, 6). These type microridges are often interconnected with fine transverse connections, the microbridges (Fig. 7), these microbridges shown only in GBE of *H. brucei* and *S. richardsonii*.

In *B. almorhae,*the epithelial cells bear microridges and are commonly associated with mucus secreting cells, the mucous cells, which are scant in number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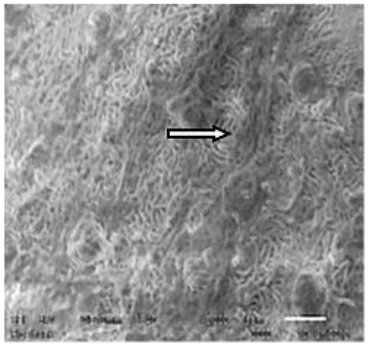
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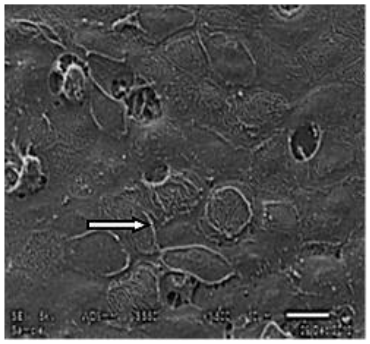
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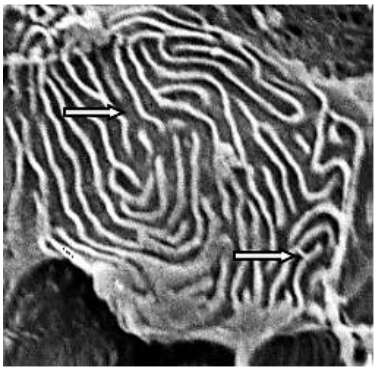
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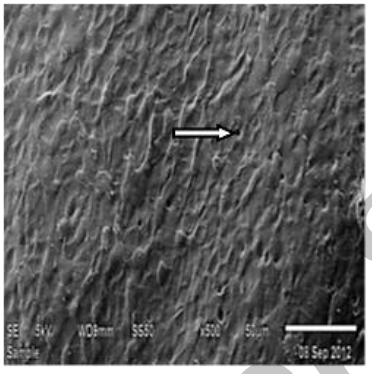
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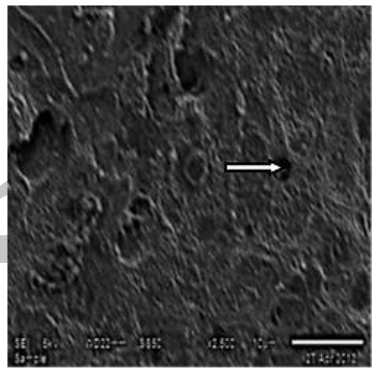
Fig. 9

Figure 4. SEMPH of the GBE of *Botia almorhae* epidermis showing microridges at the surface epithelium (Scale bar=5 μm).
Figure 5. SEMPH of the GBE of *Homaloptera brucei* showing that the microridges are generally; finger print- like, and are often arranged in the form of small groups (Marked by arrows) (Scale bar =5 μm).
Figure 6. SEMPH of the GBE of *Schizothorax richardsonii* showing finger print-like patterns of microridges (Marked by arrows) (Scale bar=10 μm).
Figure 7. SEMPH of the GBE of *Schizothorax richardsonii* showing finger print-like patterns of microridges that have canaliculi and microbridges (Marked by arrows) (Scale bar =5 μm).
Figure 8. SEMPH of the GBE of *Botia almorhae* showing the opening of mucous cells (marked by arrows) (Scale bar=50 μm).
Figure 9. SEMPH of the GBE of *Homaloptera brucei* showing the openings of mucous cells (marked by arrows) (Scale bar=10 μm).

(Fig. 8) while the mucous cell apertures are rare comparatively and occur at the border of three or four epithelial cells in *H. brucei* (Fig. 9) but the mucous cells, though distributed throughout the epidermis are, in general, concentrated mainly on the outer layer of the epidermis, often releasing their secretory contents profusely at the surface through small pores in the *S. richardsonii,* (Fig. 10).

A large number of tubercles are found on the epidermal surface of *H. brucei*, these tubercles exist in a well-designed pattern. The unculi are equidistantly placed and supported by epithelial cells. Polygonal outlining of the epidermal cells is seen at the base of the unculi, indicating unculi to be modified epithelial cells (Figs. 11, 12), all these structures are not shown in the GBE of *B. almorhae* and *S. richardsonii.*

Discussion

The epidermis is ectodermal in origin and consists of several layers of simple cells, of which the outer are being constantly worn away by wear and tear and replaced by newer ones which develop at their base. These layers of cells are composed of flattened cells, known as epithelium cells, of which the deepest layers are made up of columnar cells forming the stratum germinativum in which cells are always multiplying by mitotic division to replace the outer worn out cells. A superficial layer of dead horny cells, forming the stratum corneum is not present in fishes as an adaptation to life in water (Khanna, 1993).

The epidermis of the GBE of *B. almorhae* and the structures associated with them show considerable structural modifications. These may be considered as

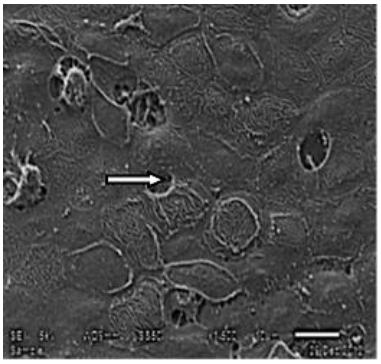
Fig.10

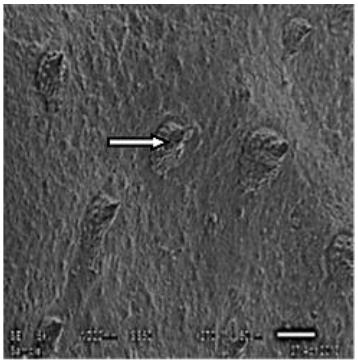
Fig.11

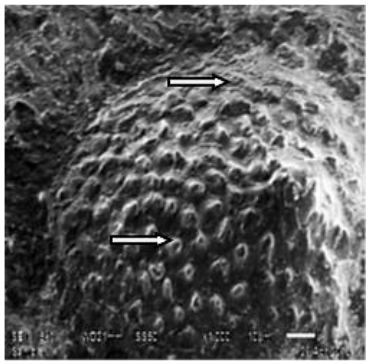
Fig.12

Figure 10. SEMPH of the GBE of *Schizothorax richardsonii* showing the mucous openings and their secretory contents profusely at the surface through a small pore. (Marked by arrows) (Scale bar=10 μm).

Figure 11. SEMPH of the GBE of *Homaloptera brucei* showing the well-developed tubercles at high magnification (marked by arrows) (Scale bar =200 μm).

Figure 12. SEMPH of the GBE of *Homaloptera brucei* of showing polygonal epithelial cells and unculi on the tubercles (Marked by arrow) (Scale bar=10 μm).

adaptations in relation to its peculiar habit and habitat. *Homaloptera brucei* is adapted to live in hill-streams characterized by fast flowing current under boulders. It is found in mountain streams (high gradient streams). The general body epidermis of *H. brucei,* exhibits compactly arranged microridges forming intricate mesh-like patterns, which are characteristic of the habitat under the boulders and stones. Furthermore, these microridges may gain a firm base and support from a dense network of fine filaments. The free surface of each epithelial cell is characterized by the presence of a series of microridges. The microridges of the cells appear smooth and uniform in width. Frictional force is less under boulder and stones; therefore, the requirement of lubrication is minimum in *H. brucei*. The epidermis of *H. brucei* possesses a large number of elevations distributed at irregular intervals. The epidermis with elevations alternates with that of the non-elevated surface. The average thickness of the epidermis varies in the two regions of *H. brucei* (Non-elevated region: 61.7 μm, at elevated region: 85.9 μm) (Bisht, 1999). Breeding tubercles are keratin based epidermal nodules, which are found in at least fifteen families of fishes in four orders. Breeding tubercles might offer a workable tool for examination of sexual selection among Cyprinids. The large number of tubercles in males indicates increasing reproductive power of the fishes. The primary function of the epidermis is to provide protection against environmental hazards. In fish, this function is mainly attributed to the gland cells which secrete their contents on the surface (Singh, 2014).

In the general body epidermis of *H. brucei* and *S. richardsonii* finger print-like microridges, may in addition impart firm consistency or rigidity to the free surface of the epithelial cells. This could be considered as an adaptation to withstand mechanical stress and protect the surface of the fish, which has the characteristic habit of bottom dwelling. This specific pattern of microridges helps in the spreading of mucus from mucous cells over a wide area. The sudden spread of mucus is facilitated by numerous canaliculi formed by epidermal microridges. The abundance of mucus on the skin of *S. richardsonii* exhibits its habitat in open water or bottom dwellings, where frictional force is very high. This study indicates that the presence of mucus secretion is performing multi-functional activities, assisting the fish to adapt to their characteristic mode of life for their maintenance against adverse environmental conditions, to which these are exposed. On the other hand open water surfaces have more pathogenic agents, which affect the epidermis; therefore, *S. richardsonii* has a greater more requirement of mucus. It also renders the skin less permeable and prevents the entry of pollutant materials and micro-organisms, which would otherwise infect the fish. Fish skin is a multipurpose tissue that serves numerous vital functions including

chemical and physical protection, sensory activity, behavioural purposes or hormone metabolism. Further, it is an important first line defence system against pathogens, as fish are continuously exposed to multiple microbial challenges in their aquatic habitat (Rakers et al., 2010). Studies of fish skin indicated that epidermal cells follow separate pathways of differentiation in different fishes. In most of the fishes, the epidermis is related more to the deposition of slime over its surface and undergoes the process of mucogenesis and in some the epidermal cells undergo the process of keratinization forming a layer at the surface (Singh and Bisht, 2014).

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