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Research Article

SCANNING ELECTRON MICROSCOPIC STUDY OF TUBERCLES IN CERTAIN HILL-STREAM FISHES OF KUMAUN HIMALAYA: SEM STUDY

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ABSTRACT
The epidermis covering the skin of hill-stream fishes; *B. almorhae, H. brucei and S. richardsonii* is smooth and rough, rough epidermis provides large number of tubercles. Tubercles are keratin based epidermal outgrowth. Which are found in at least 15 families of fishes in four orders. The tubercles are found mostly on male and are induced by several hormones. They are usually expressed during the breeding season when they help males maintain contact with females during spawning. Best known are probably the breeding tubercles of cyprinid fishes in which high numbers of tubercles have been shown to increase male reproductive success. Tubercles may be used for conspecific recognition, or for protection against mechanical injuries. Cumulative action of tubercles and mucus enables the fish to make firm hold on the substratum. The aim of our study is the structural and functional organization of the tubercles in some hill-stream fishes.

Keywords: *Hill-Stream Fishes, Kumaun Himalaya and Tubercles*

INTRODUCTION
The skin of fish is divided into three layers; the epidermis (outer) layer, the dermis and hypodermis. The epidermis is made up of epithelial cells, arranged one above the other. These cells are constantly shed and replaced with new ones. The epidermis divided into smooth and rough, rough epidermis provides large number of tubercles. The present study show the structural architecture of the tubercles of the hill-stream fishes; b. Almorhae, h. Brucei and s. Richardsonii developed during breeding season. During their developmental stages they showed several type of structures e.g. Knob-like, wart like and conical shaped. The study also showed that tubercles in the fishes; b. Almorhae, h. Brucei and s. Richardsonii are widely distributed among the epithelium of the gbe, snout epidermis and paired fin. Thus the highly developed tubercles in fishes, their early differentiation and wide distribution in the body indicate the great importance of these organs to the fishes.

MATERIALS AND METHODS
The live fishes e.g. *Botia almorhae* (Teleostei: Cobitidae), (Approximately 5-7 inches in length) were collected from river Kosi at Kakrighat Distt. Nainital (elevation- 1200m above mean sea level), *Homaloptera brucei* (Teleostei: Balitoridae), (Approximately 3-4 inches in length) were collected from west Ramganga at Chaukhutia Distt. Almora (elevation-1200m above mean sea level) and *Schizothorax richardsonii* (Teleostei: Cyprinidae), (Approximately 6-8 inches in length) were collected from river Kosi at Hawalbagh Distt. Almora (elevation- 1194m above sea level) Uttarakhand. The water current is very fast having the velocity 0.5 to 2.0 m/sec. Bhatt and Pathak (1991) and the bed is rocky.
The fishes were transferred from the site of collection to laboratory in well ventilated plastic containers and were kept for a period of about 5-6 days in glass aquaria having an artificially made rocky bed and 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).
To study the details of the morphological adaptations in some fishes, SEM were done. The following procedure was adopted for the preparation of specimen for SEM.
Specimen was maintained in laboratory at 25 ± 2^{0}C. The fishes were cold anesthetized following Mittal and Whitear (1978), for SEM preparation. Skin fragments of about 10×10 mm were cut from different

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parts (GBE, snout, lips and fins). Tissue were excised and rinsed in 70% ethanol and one change saline solution to remove debris and fixed in 3% Glutaraldehyde in 0.1M phosphate buffer at pH 7.4 over night at 4^0C at refrigerator. The tissues were washed 2-3 changes in phosphate buffer and dehydrated in ascending series of ice cold Acetone (30%, 50%, 70%, 90% and 100% approximate 20-30min.) 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). The samples were coated with gold using a sputter coater (JFC 1600), examined under (JEOL, JSM- 6610 LV) scanning electron microscope and the images were observed on the screen.

RESULTS AND DISCUSSION

The skin also provides an individual identity to an organism's appearance. The skin of hill-stream fishes; *B. almorhae, H. brucei* and *S. richardsonii* (Gray 1830) is composed of three distinctive layers; epidermis, dermis and hypodermis. Epidermis or top covering of body is the outermost defense organ against the surrounding aquatic environment comes into direct contact with the mechanical hazard. The epidermis is both types smooth or non-keratinized and rough or keratinized. The smooth epidermis possesses epithelial cells and mucous cell apertures intersperse between the epithelial cells in these fishes.

The general body epidermis of *H. brucei* both type smooth and rough (Figure 1A), smooth epidermis possesses epithelial cells and mucous cells. Large number of tubercles is found on the epidermal surface of *H. brucei*, these tubercles are in well designed pattern (Figure 1B, C and D). The breeding tubercles are keratin-based epidermal nodules. The epithelial cells of each tubercle develop into unculi (Figure 1E), on the other hand tubercles are absent on the general body epidermis of *B. almorhae* and *S. richardsonii*. Tubercles are of different sort, although also horny, occur on the soft mouthparts. These, sometimes referred to as papillae, are particularly prominent on the rostral cap, lower lip and mental disc in many *Garra spp.* Tyson and Roberts (1990).

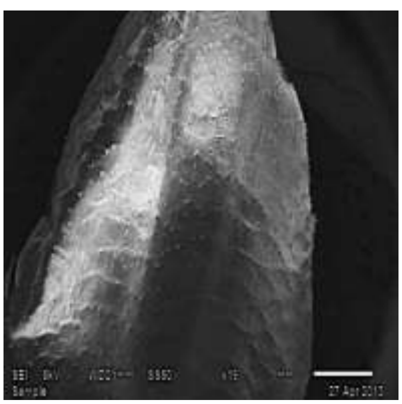
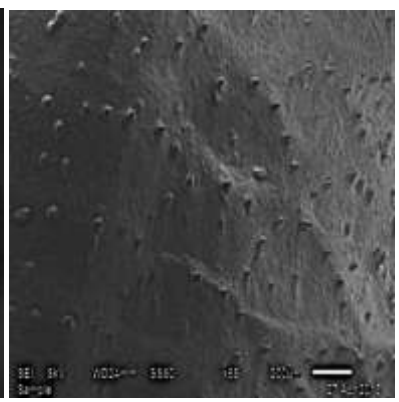


Figure 1A



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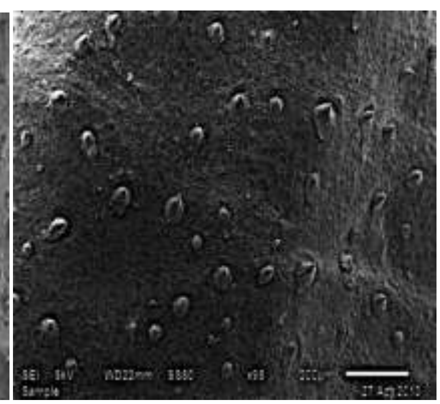

Figure 1C

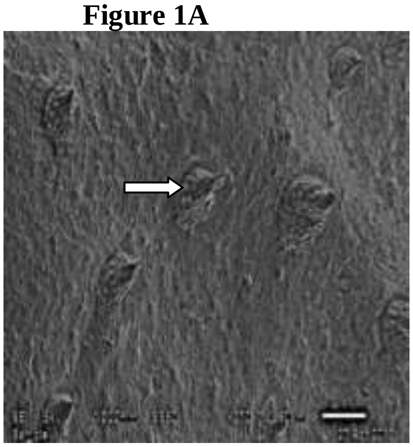

Figure 1D

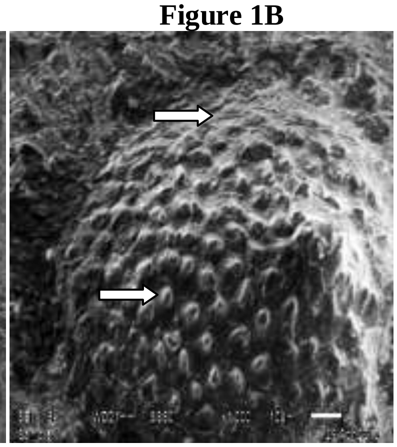

Figure 1E

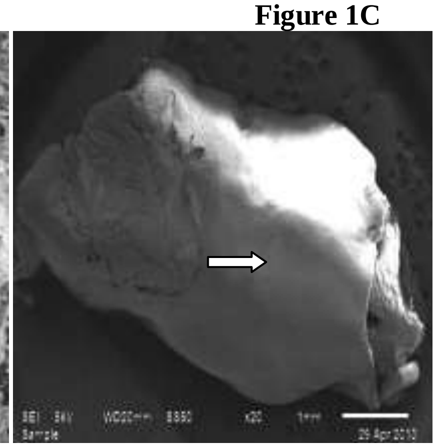

Figure 1F

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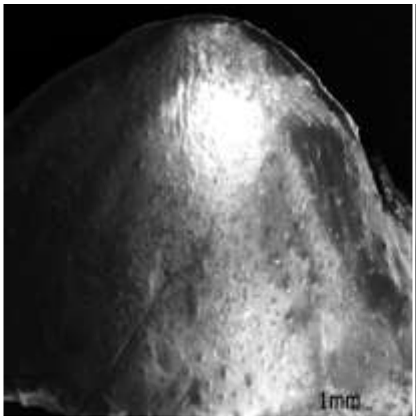

Figure 1G

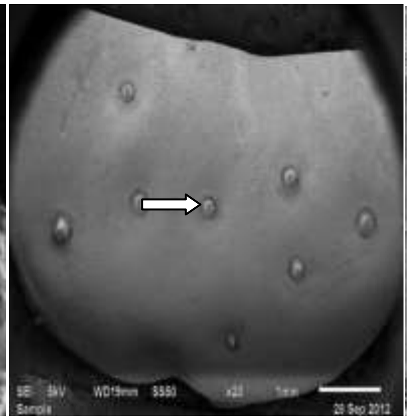

Figure 1H

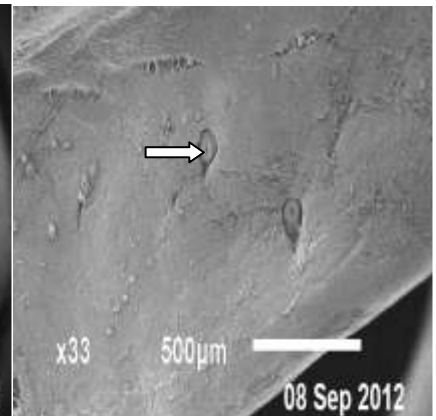

Figure 1I

Figure 1A: SEMPH of the GBE of *H. brucei* showing epidermis with small tubercles (Scale bar-5µm).
Figure 1B and 1C: SEMPH of the GBE of *H. brucei* showing the well-developed tubercles (marked by arrows) (Scale bar-200µm and 100µm).
Figure 1D: SEMPH of the GBE of *H. brucei* showing the well-developed tubercles at high magnification (marked by arrows) (Scale bar-50µm).
Figure 1E: SEMPH of the GBE of *H. brucei* of single tubercle showing polygonal epithelial cells and unculi (Marked by arrow) (Scale bar- 10µm).
Figure 1F, G and H: SEMPH of the scale less snout epidermis of *B. almorhae, H. brucei and S. richardsonii* (Scale bar- 1 mm)
Figure 1I: SEMPH of the snout epidermis of *B. almorhae* showing knob-like epidermal tubercles (Marked by arrows) (Scale bar- 500µm)

The epidermis of snout of all these three fishes is scale less (Figure 1F, G and H). In the rough epidermis of *B. almorhae* small knob-like structures, the tubercles are present (Figure 1I). Unculi are absent on the tubercles. At the apical end of each tubercle closely packed microvilli are observed (Figure 2J).
The dorsal surface of snout epidermis of *H. brucei* is also tuberculated, these tubercles are small, wart-like or conical shaped tubercles, these are discrete (Figure 2K and 2L), generally are conical and multicellular. This may play a role in breeding behavior in Cyprinids, they are especially large and numerous on the snout. The epithelial cells of each tubercle develop into unculi. These unculi are equidistantly placed and supported by epithelial cells, polygonal outlining of the epidermal cells are seen at the base of the unculi, indicating that unculi are modified epithelial cells (Figure 2M). The snout 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 epidermis varies in the two regions of *H. brucei* (Non-elevated region: 61.7 µm, at elevated region: 85.9 µm) Bisht (1999).
The snout epidermis of *S. richardsonii* is both types smooth or non-keratinized and rough or keratinized. The smooth epidermis of snout possesses epithelial cells and mucous cell apertures intersperse between the epithelial cells and rough epidermis possesses tubercles (Figure 2N and 2O). The snout is tuberculated and blunt in the mature male while non-tuberculated and pointed in female Singh (2003). The length of epidermal tubercles of *S. richardsonii* is approximately 128.88µm. The base of each tubercle is broad, rounded and approximate width is 282.06µm (Figure 2P and Q).
The paired fin epidermis of *H. brucei* has tubercles; it is interesting to note that a large number of well protruded tubercles appearing to be cleaved in the centre are present in between the epithelial cells (Figure2R). The density of these tubercles is however much greater in dorsal surface than that of ventral surface. Roberts (1982) also described such tubercles in a large number of Ostariophysan fishes. They are

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well developed in the dorsal surface of the paired fins in sexually mature males and poorly developed or absent in females.

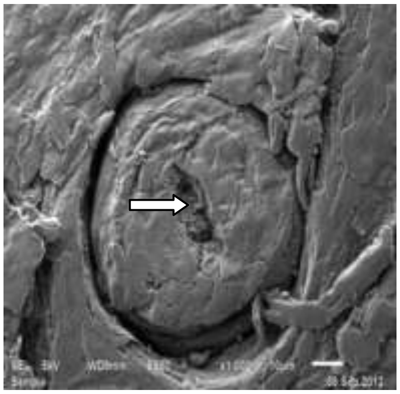

Figure 2J

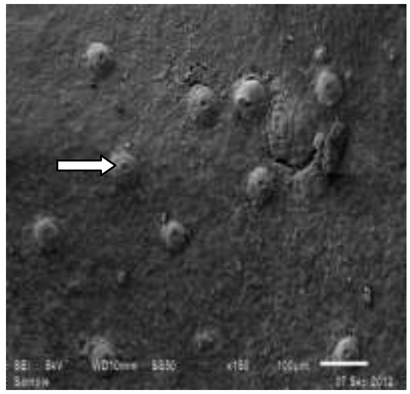

Figure 2K

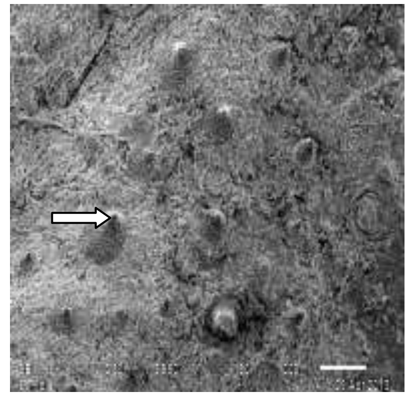

Figure 2L

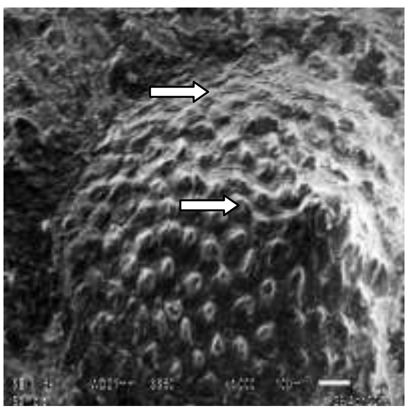

Figure 2M

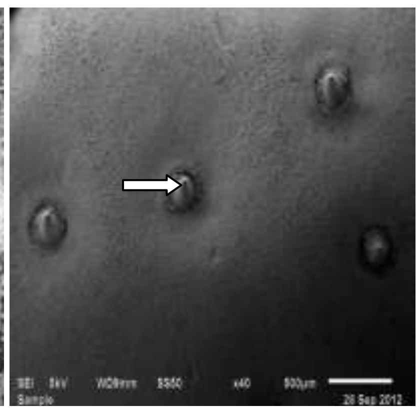

Figure 2N

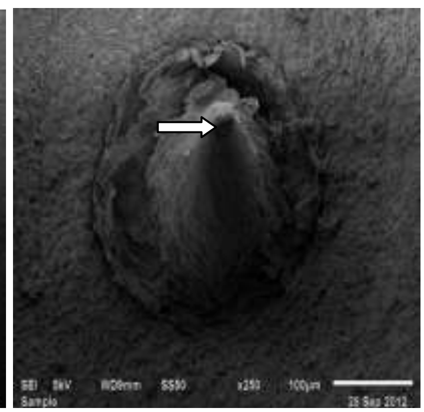

Figure 2O

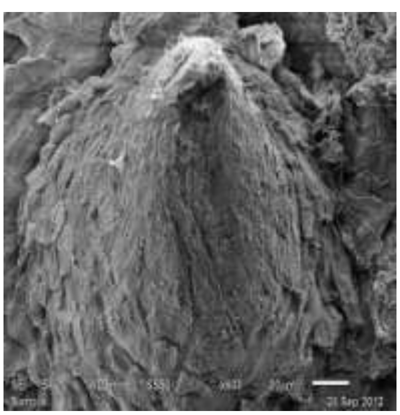

Figure 2P

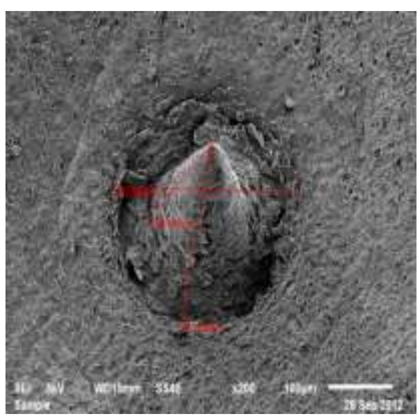

Figure 2Q

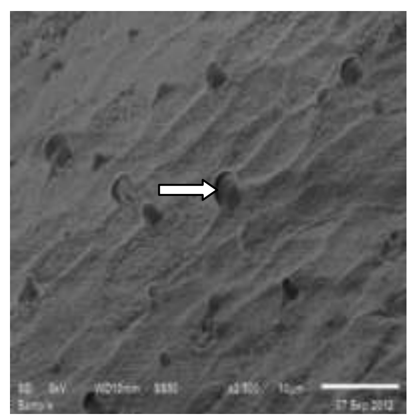

Figure 2R

Figure 2J: SEMPH of the snout epidermis of *B. almorhae* showing closely packed microvilli at the apical surface of the tubercles at high magnification (Scale bar- 10µm)
Figure 2K and 2L: SEMPH of the snout epidermis of *H. brucei* of showing wart-like or conical tubercles (Marked by arrow) (Scale bar- 100µm)
Figure 2M: SEMPH of *H. brucei* of showing polygonal epithelial cells and unculi on the tubercles of snout epidermis (Marked by arrow) (Scale bar- 10µm)

Figure 2N: SEMPH of snout epidermis of *S. richardsonii* showing epidermal tubercles (Marked by arrows) (Scale bar- 500 µm)

Figure 2O: SEMPH of snout epidermis of *S. richardsonii* showing single epidermal tubercles (Scale bar- 100 µm)

Figure 2P: SEMPH of snout epidermis of *S. richardsonii* showing single epidermal tubercles at high magnification (Scale bar- 20µm)

Figure 2Q: SEMPH of snout epidermis of *S. richardsonii* showing broad base tubercles at high magnification (Scale bar- 20µm)

Figure 2R: SEMPH of dorsal surface of pectoral fins of *H. brucei* epidermis showing well protruded tubercles in between epithelial cells (Scale bar-10µm)

SEMPH: Scanning Electron Micro Photograph

The rough epidermis possesses tubercles. Large number of tubercles is found in *H. brucei* and *S. richardsonii* in comparison to *B. almorhae*. The tubercles are found most often on males. The large number of tubercles in male indicates increasing reproductive power of the fishes. The primary function of the epidermis is protection against environmental hazards. The epidermal tubercles generally found in the general body epidermis, snout, lips, rostral cap and paired fins of these three hill-stream fishes.

Breeding tubercles may be used for conspecific recognition Vladykov *et al.*, (1985), or for protection against mechanical injuries, Ahnelt and Keckeis (1994). Muller and Ward, 1995 suggested that breeding tubercles may be used as weapons in intense pre-spawning male behaviour (defence of nests and territories). However, Wiley and Collette, (1970) proposed that tubercles originally evolved to allow breeding individuals to maintain close contact during spawning as a means to ensure fertilization of the eggs. Breeding tubercles may also act as hydrodynamic or tactile stimulators of females during courtship. In roach, breeding tubercles are presumed to give females detailed information about a male's parasite load, Wedekind, (1992) and Kortet and Taskinen, (2004) and parasite resistance Taskinen and Kortet, (2002) and Kortet and Taskinen, (2004) and to act as a sexual ornament indicating his quality. They are predominantly found in males and usually expressed during the breeding season when they help males maintain contact with females during spawning. Best known are probably breeding tubercles of Cyprinid fishes in which high numbers of tubercles have been shown to increase male reproductive success.

Cumulative action of tubercles and mucus enables the fish to make firm hold on the substratum. Das and Nag, (2006, 2008) suggested the bases of the available morphological evidence, it is possible to suggest the mechanism of attachment in *G. gotyla.* It is likely that mechanical interlocking and suction and perhaps viscous forces are involved in this mechanism already mentioned, the epidermal tubercles are equipped with spines.

Kortet and Taskinen, (2004) reported breeding tubercles might offer a workable tool for examination of sexual selection among Cyprinids. Appearance of tubercles in different areas of the body during breeding season has been observed in seven families of bony fishes under five orders; namely Salmoniformes, Gonorhynchiformes, Cypriniformes, Scorpaeniformes and Perciformes, Jyoti and Sharma, (2006). These breeding tubercles are local keratinizations distinctly different from normal epidermis and they facilitate contact between the individual fishes during breeding, Jyoti and Sharma, (2006). The development of breeding tubercles may take weeks rather than days and they are shed shortly spawning Wiley and Collette, (1970).

There are some reports on the unusual evolutionary adaptation of the integument in teleosts. For example, the breeding tubercles of the male Cyprinids, Wiley and Collette, (1970), the hooks of the male nursery fish; *Kurtus gulliveri*, Berra and Humphrey, (2002) and the cotylephores of the ghost pipefish (*Solenostomus spp.*) and the Catfish; *Platystacus spp*., Wetzel *et al.*, (1997) represent the various types of epidermal outgrowths that are used for courtship and/or for attachment of eggs, whereas these structures are the result of evolutionary adaptations to different reproductive strategies.

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