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Comparative Study of Lips in Three Hill-Stream Fishes (*Botia almorhae, Homaloptera brucei and Schizothorax richardsonii*) of Kumaun Region: A SEM Investigation

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Abstract: ***The lips and associated structures, in different groups of fishes are greatly modified in relation to the characteristic mode of feeding, food preference and the mode of life exhibited by the fish. The successful maintenance of fish populations in challenging environments requires responsive adjustments in their behaviour, morphology and physiology and these have been reflected by modifications at the level of their organ systems, organs and tissues. The lips are no exception to this. A number of the multifunctional roles of the fish lips and associated structures that are discussed incorporate distinctive morphological feature that will be highlight in this study.***

Keywords: Hill-stream fishes, morphology, Kumaun Himalaya and lips.

1. Introduction

B. almorhae, H. brucei and *S. richardsonii* (Hora, 1930) belongs to the family Cyprinidae and order Cypriniformes. These are hill-stream fishes and are predominantly adapted to life in swift flowing waters. These three fishes are very well adapted to the fast flowing or torrential streams having heterogeneous substrate. The functional aspects of the lips and associated structures in family: Cobitidae, Balitoridae, Cyprinidae and few species of Sisoridae show considerable variation and exhibit unique morphological modifications associated with their lips and other structures around the mouth regarding information on the level of surface architecture as seen under SEM in relation to various food and feeding habits and ecological niches. The gross and fine structure of the lips, the rostral caps and the horny jaw sheath is externally varied. This involves, among other things, formation of papillae or tubercles, unculi, ridges and grooves of variable height and distribution on the lips and the rostral cap and sharp cutting edge, cone shaped structure or unculi on the horny jaw sheaths.

2. 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. [1] 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 ± 2^0C. The fishes were cold anesthetized following [2], for SEM preparation. Skin fragments of about 10×10 mm were cut from lips. 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.

3. Results

The epidermis, the most superficial and external layer of the skin, is composed of a stratified squamous epithelium. The present investigation is concerned with the occurrence of keratinization and non-keratinization in the structures associated with lips of a hill-stream fishes; *B. almorhae*, *H. brucei* and *S. richardsonii*. The anterior and posterior lips are associated with a greatly enlarged rostral cap and an adhesive pad, respectively.

3.1 Rostral cap

In the *B. almorhae*, the rostral cap is divided into three lobes. The median lobe is larger than the lateral lobes and its tip sharply triangular while tips of lateral lobes are gently rounded (Figure 1), but these lobes are not shown in *H. brucei* and *S. richardsonii*.

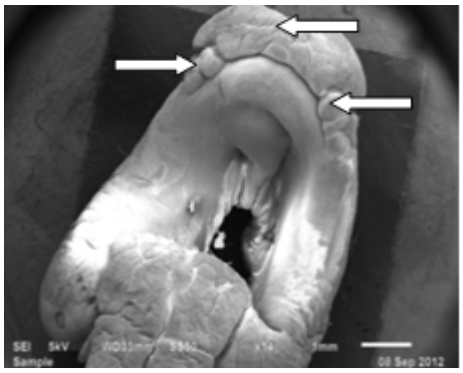


Figure 1: SEMPH of the *B. almorhae* showing rostral caps and anterior lip (Scale bar- 500µm).

The surface of the rostral cap a large number of elevations are observed as tubercles. Each epithelial tubercle is characteristically associated with a taste bud (Figure 2).

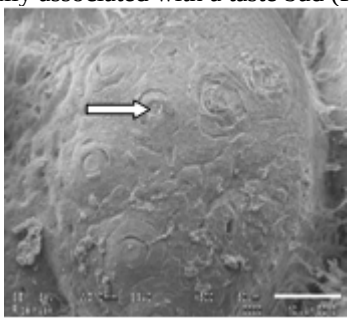


Figure 2: SEMPH showing the taste buds on tubercle of rostral cap in *B. almorhae* (Scale bar- 50 µm).

Each taste bud is situated on a small epithelial papilla/tubercle projection at the surface. At the apical surface of each tubercle, closely packed microvilli are observed. These microvilli represent the taste hairs originating from taste cells of the buds and are projected through the rounded taste pores at the summit of these tubercles (Figure 3).

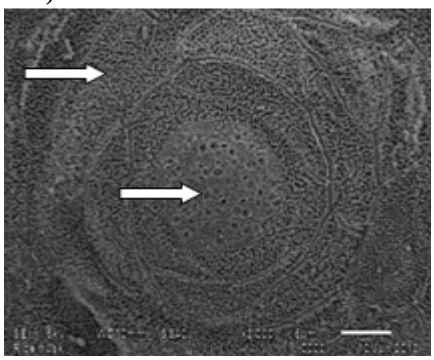
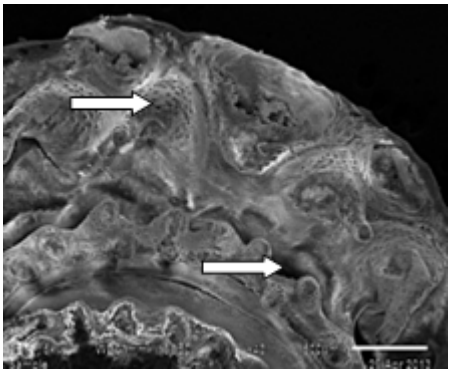


Figure 3: SEMPH showing the labyrinthine pattern microridges and rounded taste pores at the summit of these elevations (Scale bar- 5 µm).

In *H. brucei*, the anterior lip is associated with the rostral cap through a thin and extensive fold of skin, which lies in a deep groove; the rostral cap is greatly enlarged, well developed tubercles are present on rostral cap with the taste buds (Figure 4 and 5).



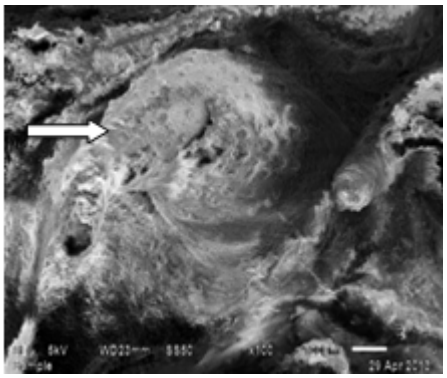


Figure 4 and 5: SEMPH showing the deep groove between the anterior lip and rostral cap; marked by arrow and the elongated papillae with the taste buds on rostral cap of *H. brucei* (Scale bar- 500µm and 100µm).

In *S. richardsonii*, the surface of the rostral cap is differentiated into both mucogenic and keratinized areas, the mucous cells in the epithelium of the lips and associated structures in *S. richardsonii*, in general, appear rounded or typically goblet-shaped. These cells open on the surface by small pores through which they void their secretions on the surface of the epithelium; the mucous cells are in general, the most common unicellular glands and the epithelial cells is characterized by well developed microridges and the taste buds (Figure 6, 7 and 8).

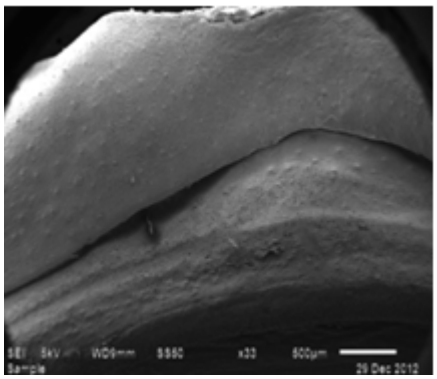


Figure 6: SEMPH of the anterior lip is associated with the rostral cap of *S. richardsonii* (Scale bar- 500 µm).

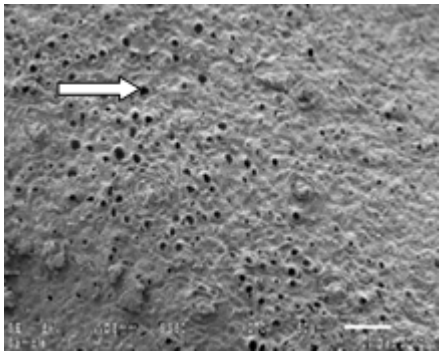


Figure 7: SEMPH of the rostral cap, bordering the mouth opening, is differentiated into both mucogenic and keratinized areas (Scale bar- 50µm).

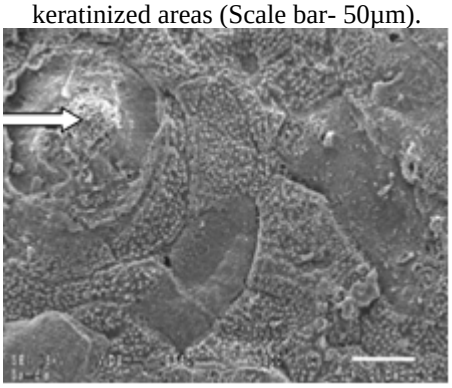


Figure 8: SEMPH of the rostral cap the surface of the taste bud and epithelial cells; characterized by well-developed microridges (Scale bar- 10µm).

3.2 Anterior and posterior lip

3.2- .1 Epithelial cells and microridges

The anterior lip is divided into two regions; anterior and posterior in these fishes, the anterior region surrounds the mouth opening. The epithelium of the lip and its associated structures is stratified in nature and is composed of the polygonal epithelial cells. Epithelial cells are characterized with microridges. In *B. almorhae*, these microridges are interwoven forming web-like or mesh-like patterns. The boundaries between adjacent epithelial cells are demarcated by smooth well-defined uninterrupted double row of closely approximated microridges (Figure 9),

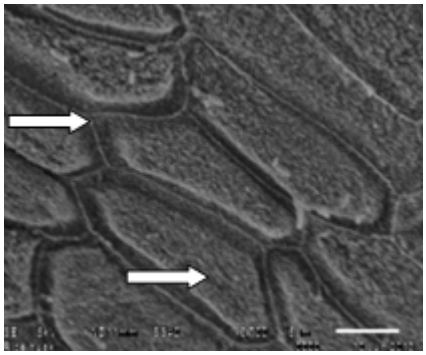


Figure 9: SEMPH of the anterior lip of *B. almorhae* polygonal epithelial cells in anterior region towards the mouth showing web-like or mesh-like patterns microridges. (Marked by arrow) (Scale bar- 10µm and 5µm).

in the posterior region, which are demarcated by well developed boundary. The boundaries between adjacent epithelial cells are very prominent and appear slightly raised from the general surface of the epithelia (Figure 10).

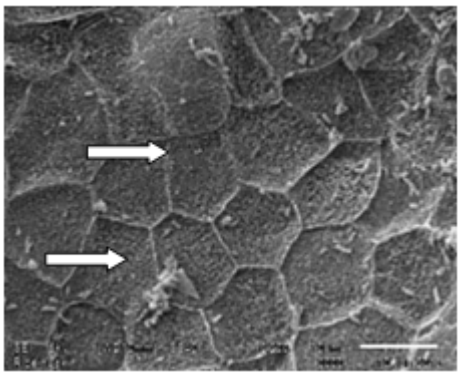


Figure 10: SEMPH showing the boundaries between adjacent epithelial cells are demarcated by smooth well-defined uninterrupted double rows of microridges (marked by arrow) (Scale bar- 10µm).

These type of cells are not shown in anterior lip of *H. brucei* and *S. richardsonii*. In *B. almorhae*, the posterior lip is divided into two regions anterior and posterior marked by arrow. The surface of the epithelia at the anterior region of the posterior lip is covered by irregularly polygonal epithelial cells of varied dimensions.The microridges are small, low, irregular and well defined. The microridges, in general, appear sinuous, having smooth surface, short with abrupt ends and irregularly interwoven to form maze-like patterns. The boundary between the adjacent epithelial cells is delineated either by a double row of microridges (Figure 11).

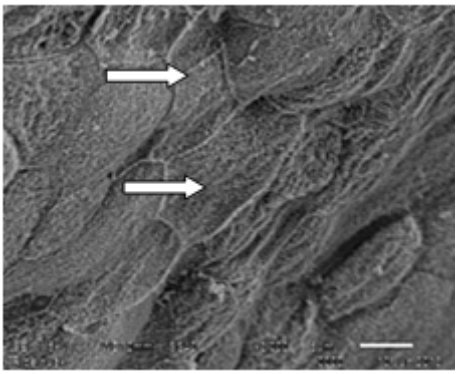


Figure 11: SEMPH showing the microridges, maize like patterns. The boundary between the adjacent epithelial cells is delineated either by shallow separating clefts or by a double row of microridges separated by distinct spaces on the anterior regions of the posterior lip (marked by arrow) (Scale bar- 5µm).

Posterior region covered by a mosaic of irregularly polygonal epithelial cells of varied dimensions (Figure 12).

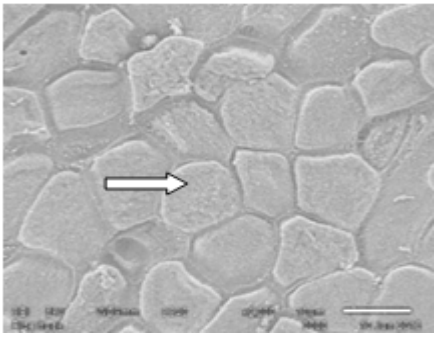


Figure12: SEMPH showing the mosaic of irregularly polygonal epithelial cells on the posterior region of the posterior lip (marked by arrow) (Scale bar- 10µm).

The surface architecture of the epithelial cells is characterized by the presence of a series of microridges. The boundary between the adjacent epithelial cells is delineated either by shallow separating clefts or by a double row of microridges separated by distinct spaces (Figure 13).

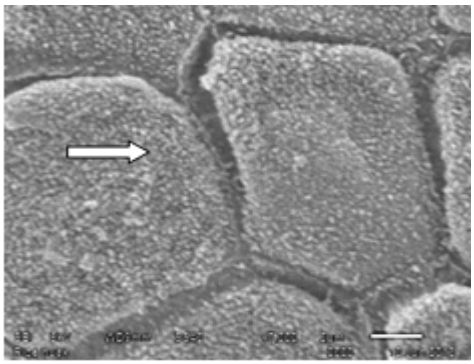


Figure13: SEMPH of the posterior region of the posterior lip of *B. almorhae* showing microridges and the boundary between the adjacent epithelial cells is delineated either by shallow separating clefts or by a double row of microridges separated by distinct spaces. (Marked by arrows) (Scale bar- 2µm).

All these type cells are not shown in the *H. brucei* and *S. richardsonii*.

3.2 -.2 Tubercles and taste bud

In *H. brucei*, the anterior region of the anterior lip has large number of tubercles or papillae, these tubercles characterized by their disc shape (Figure 14),

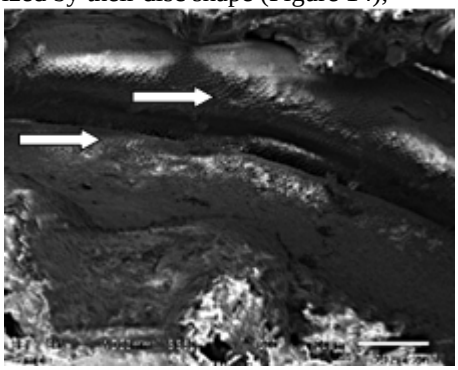


Figure14: SEMPH of the anterior portion of the anterior lip and posterior lip showing the papillae or tubercle of *H. brucei* (Scale bar- 100µm).

each tubercle bears numerous glandular secretive device, which are well developed taste buds (Figure 15), on the other hand the posterior region of the anterior lip encircled with two rows of uneven tubercles with the large number of taste buds (Figure 16).

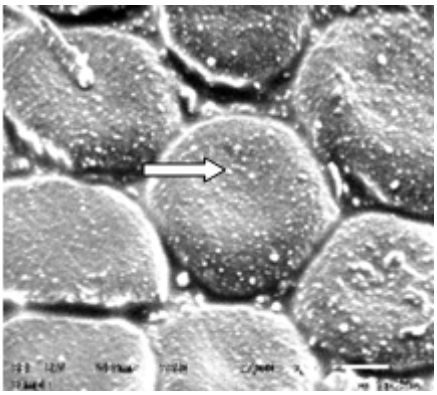


Figure15: SEMPH showing the taste buds on the papillae or tubercle of the anterior portion of anterior lip in *H. brucei* (Scale bar- 2µm).

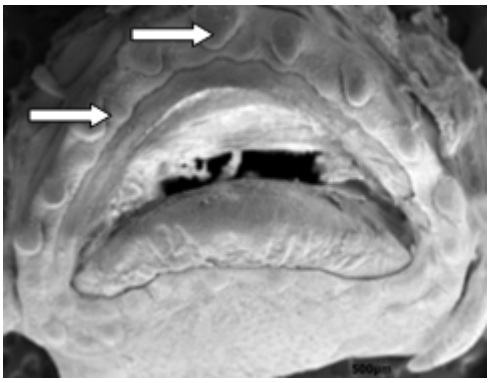


Figure16: SEMPH of the posterior region of the anterior lip showing two rows of tubercles of *H. bucei* (Scale bar- 500µm).

The outer row with eight to ten bigger tubercle /papillae, while inner row with ten smaller ones. The outer-most tubercles of outer row are slightly elongated and bear taste buds (Figure 17).

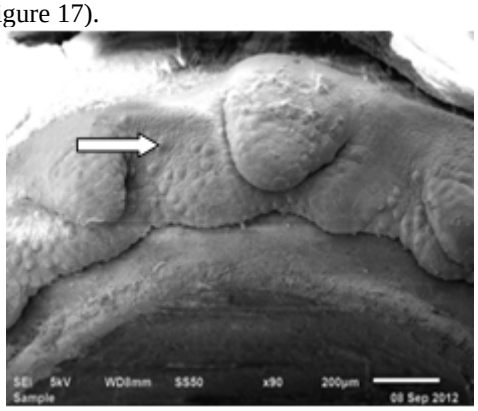


Figure17: SEMPH of the posterior region of the anterior lip showing the tubercles with taste buds of *H. bucei* (Scale bar- 200µm).

In *S. richardsonii* the anterior region of the anterior lip has much small and large number of tubercles or papillae; different shape and size such as spherical, cylindrical and elongated (Figure 18).

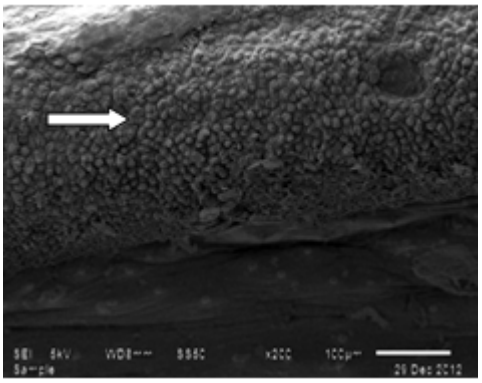
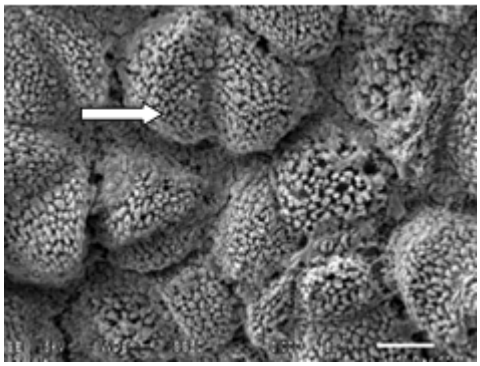


Figure18: SEMPH of the anterior region of anterior lip has much small and large number of tubercles with different shapes and sizes (Scale bar- 100µm).

In variably projected significantly on the surface and each supported by a tubercles/ papillae from the epithelium are located in large number of the taste buds are located in a good number in the anterior lip on the characteristic epithelial papillae-like projections and are projected at the surface (Figure 19 and 20).



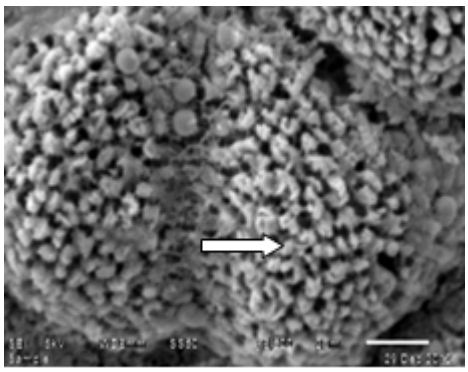


Figure19 and 20: SEMPH of the anterior region of the anterior lip has papillae or tubercles, which bear numerous glandular secretive devices and possibly a keratinized spine (Scale bar- 5µm and 2µm).

These have been associated with the acute gustatory sense of the fish. The posterior region of the anterior lip has also taste buds (Figure 21); the taste bud bears a group of cilia that are mechano-sensory in function (Figure 22).

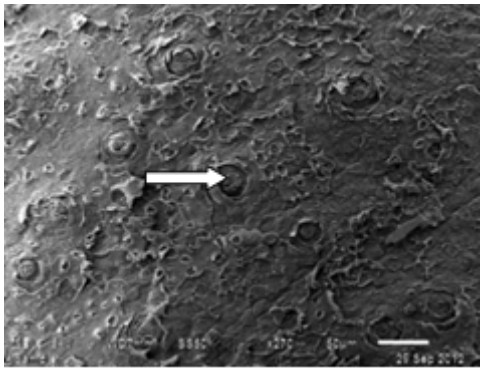


Figure 21: SEMPH of the anterior lip showing taste buds (Honey comb-like feature) (Scale bar- 50µm).

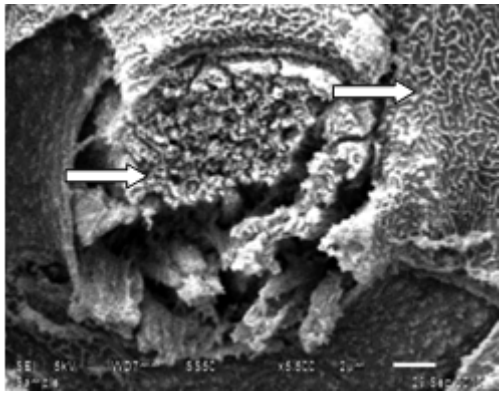


Figure 22: SEMPH of the anterior lip showing a bunch of mechano-sensory cilia-stereocilia and mesh like or web-like patterned microridges around them (Scale bar- 2µm).

[3] suggested the taste buds; in the anterior lip numerous continuous pores or pits are present forming honeycomb-like structure with scattered round shaped sensory vesicles having slight projection. The bunch of mechano-sensory cilia covered in gelatinous sheath come out from these taste buds. The posterior lip attached with the callus part and the adheshive organ (Figure 23).

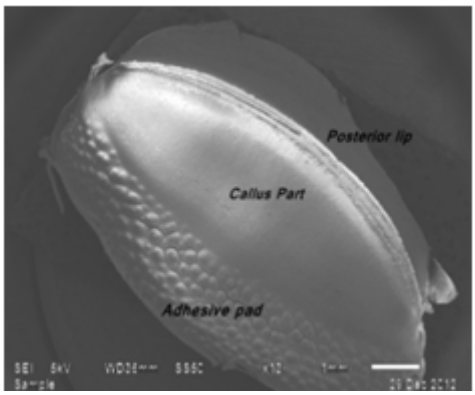


Figure 23: SEMPH showing the posterior lip with the callus part and the adhesive organ (Scale bar- 1mm).

Callus part possess epithelial cells which are appear overlapping to each other and the boundary between the adjacent epithelial cells is delineated either by shallow separating, clefts or by a double row of microridges (Figure 24). Epithelial cells exhibit a close meshed reticular pattern microridges (Figure 24).

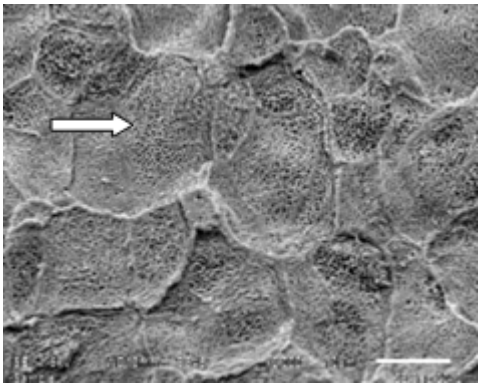


Figure 24: SEMPH of the callus part showing epithelial cells exhibiting a close meshed reticular pattern (Scale bar- 10µm).

The posterior lip on its ventral side is associated with a specialized structure the tuberculated adhesive pad through a thin fold of skin. The adhesive pad is characteristically large and arched structure that appears like a shallow plate divided into a central region and a relatively wide peripheral region (Figure 25).

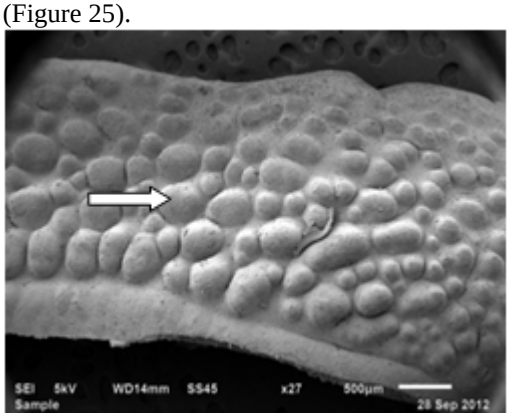


Figure 25: SEMPH of the posterior lip showing specialized structure the adhesive pad (Scale bar- 500µm).

Adhesion results due to friction between tubercles and surface of substratum

4. Discussion

The structure of the lip epidermis covering the skin was analysed by scanning electron microscopy in the Hill stream fishes; *B. almorhae, H. brucei* and *S richardsonii* of Almora District Rivers in Kumaun region. The mouth is inferior with fleshy lips, cone-shaped, horse shoe shaped and blunt. The epithelia of the lips, rostral cap and adhesive pad of the *B. almorhae, H. brucei* and *S. richardsonii* reflect adaptive features which are primarily significant with respect to food preference and feeding behaviour.

The surface architecture of the superficial layer of the epithelial cells in the lips and associated structures in characterized by specialized structure, the microridges forming different patterns in different fish species investigated in this study. These structures in the other fishes have been described as cytoplasmic folds[4], microvilli[5], microfolds[6] or ridges[7] . In so far as these structures appeared as microridges under SEM and microvilli under TEM. This term “microridges” is used in this study following[8], [9] [10] and seems appropriate.

The mucous cells in the epithelium of the folds of between the anterior lip and rostral cap and between the posterior lip and the ventral head skin are voluminous in dimensions and secrete profusely at the surface. In addition to their primary role to protect and to keep the surface of the epithelium clean by preventing the deposition of foreign matters in the groove, profuse mucous secretions in these regions may play a significant role in providing extra lubrication to the surface of the folds of skin.

The presence of a large number of taste buds increases the probability of detecting and locating accurately prey concealed by darkness or turbidity and may also permit the accurate location of small food particles, which would be missed otherwise. The projected taste buds are the first to come in contact with the surface water and enable the fish to sense the nature of food available in a particular feeding zone before it opens its mouth to form a temporary feeding tube to gulf in large quantities of water along with available food particles[11].

The rostral cap is probably present in most fish species, although it may be so reduced as to be overlooked easily. In many forms it lies well above the anterior lip and plays not direct role in feeding, while in others it is greatly enlarged, partially or completely overlies the anterior lip and plays a major role in gathering food from the substrate.

The physical features of fish “lips” determine what kind of food they can eat. Because fish have different adaptations they are able to feed on different kinds of food. This allows many fish to survive in one habitat without competing for food. This suggests that lips also prevent the fish from skidding, thus playing an important role in adhesion. The mouth of *H. brucei* along with the lip is well protected by surrounding structures i.e. three pairs of short stumpy barbels and a well developed rostral hood [12].

5. Acknowledgement

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