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Diversity of Lips and Associated Structures in Fishes by SEM

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1. Introduction

Lips are specialized structures that cover the jawbones, and border the anterior orifice of alimentary canal, the mouth. In general, lips and structures associated with them in different fish species may be considered as mainly concerned with the selection, capture, deglutition and pre digestive preparation of food. The effectiveness of these structures is dependent on modifications in relation to food and feeding habits of the fishes and environmental niches inhabited by them.

Morphological data are also key to understanding fish nutrition in ecology and aquaculture, and during development as well as mechanisms for physiological adaptations to a changing environment. A number of the multifunctional roles of the fish lips and associated structures that are discussed incorporate distinctive morphological features that will be highlighted in this chapter. The lips and associated structures represent a significant vertebrate innovation and are highly diversified.

Therefore, present work was undertaken to investigate diversity of the epithelia of lips and associated structures in different fresh-water fish species with the aim to elucidate the surface architecture using Scanning Electron Microscope. The functional aspects of the lips and associated structures in family Gobiidae, Cobitidae, Belontiidae and few species of Cyprinidae 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.

When we started to survey, collect and organize the current knowledge on lips and associated structures for the invited chapter on SEM, we soon realized that such a study would lead to a greater understanding only if the lips were discussed as incorporate distinctive morphological features.

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. The importance of food in daily life of a fish is obvious, and is reflected in the form of the mouth, lips, jaws and so on. These structures present more diverse modifications than any other organ of the body.

Lips and the structures intimately associated with them in different fish species are in direct contact with a complex ever-changing aquatic environment and ecological conditions in which fish inhabits. They are highly sensitive, serve a variety of functions and are characteristically modified in different groups of fishes. These modifications may be associated in some way either with the diet or the method of feeding.

In general, the upper and the lower jaws bear relatively simple and thin lips. These may be thick, fleshy and fimbriated or even unculiferous. In some cases one or both of the lips may regress or fail entirely to develop. 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 upper lip and plays no direct role in feeding, while in others it is greatly enlarged, partially or completely overlies the upper lip and plays a major role in gathering food from the substrate. In the older literature the rostral cap and horny jaw sheaths frequently are confused with the lips.

The gross and fine structure of the lips, the rostral caps and the horny jaw sheaths is extremely varied. This involves, among other things, formation of unculiferous fimbriae, tubercles, unculti, papillae, or ridges and grooves of variable height and distribution on the lips and the rostral cap, and sharp cutting edge, cone shaped structure or unculti on the horny jaw sheaths.

Literatures pertaining to the morpho-anatomical structures of the lip in freshwater teleosts are fragmentary and many authors while studying the alimentary canal, briefly described the morphology and structural organisation of the lips of different fish species (Vanajakshi, 1938; Kapoor, 1958; Khanna, 1961, 1962; Pasha, 1964 a, b, c; Saxena & Bakhshi, 1964; Lal *et al.*, 1964; Chitray, 1965; Sehgal, 1966; Moitra & Bhowmik, 1967; Lal, 1968; Sehgal & Salaria, 1970; Moitra & Sinha, 1971; Sinha, 1975; Sinha & Moitra, 1975, 1976, 1978; Kapoor *et al.*, 1975). Suzuki (1956) described the histological organisation of lips of bottom feeding scythe fish *Pseudogobio esocinus*. At the surface of lips, he reported the presence of characteristic processes, with numerous taste buds, playing an important role as food finders. Miller & Evans (1965) studied relationship between the external morphology of brain and lips with emphasis on the distribution of taste buds. Branson & Hake (1972) described morphological organisation of the lips of *Piaractus nigripinnis* and reported that the lips in this fish are adapted for accessory respiratory function. Kiyohara *et al.* (1980) gave an account of the distribution of taste buds on the lips of a minnow *Pseudorasbora parva*. Ono (1980) reported epidermal projections associated with taste buds on the lips in some loricariid catfishes.

Agrawal & Mittal (1991, 1992 a, b, c) reviewed the literature and described the structural organisation of the epithelia of lips and associated structures of three Indian major carps – a surface plankton and detritus feeder, *Catla catla*; a herbivorous column feeder, *Labeo rohita* and an omnivorous bottom feeder, *Cirrhina mrigala*; and a sluggish, bottom dwelling, carnivorous catfish, *Rita rita*. Mittal & Agrawal (1994) reported the structural organisation of the epithelia of lips and associated structures of an active predatory fish *Channa striata*.

Scanning Electron microscope (SEM) reveals the details of surface architecture of tissues to an extent not possible by other procedures. In spite of this fact, the review of literature reveals that not much attention has hitherto been paid to study the surface architecture of fish lips and structures associated with them using SEM. In view of this, Roberts (1982) who

examined a variety of fish species using scanning electron microscope, reported that differences in morphology of the lips and associated structures include:

1. Degree of development and specialisation of the lips,
2. Degree of development and specialisation of the rostral cap,
3. Presence or absence of horny jaw sheaths on the jaws in addition to, or in place of the normal lips, and
4. Form and distribution of uncini on the rostral cap, lips and horny jaw sheaths.

Recently, Ojha & Singh (1992), using SEM described functional morphology of the anchorage system and food scrapers of *G. lamta*. Again, Pinky et al. (2002) made a detailed report on lips and associated structures of the same fish *G. lamta*. Yashpal et al. (2009) briefly reported the presence of uncini on the upper jaw epithelium of *Cirrhinus mrigala*. More recently, Tripathi & Mittal (2010) made a detailed report on lips and associated structures of the fish *Puntius sophore*.

Our current knowledge on aspects of modifications in lips and associated structures in fish arises from too little data to arrive at general trends without running the risk of confusing variability, both real and experimental with adaptive phenomenon. What will be required to remedy this situation is a more extensive examination of a larger variety of species, particularly species adapted to different living conditions. This is an area of research for which the extreme biodiversity of fishes will be a powerful tool. Recently, the lips and associated structures of the Labeonini cyprinids have traditionally been identified as important characters in their classification (Yang & Mayden 2010).

The present work has, therefore, been undertaken with the aim to make a comparative study of the organisational pattern of lips and structures associated with them, at scanning electron microscopic levels, in certain fresh-water fish species having different feeding habits and inhabiting varied ecological niches, to address following specific questions:

Do the lips and the structures associated with them in fish species with different feeding habits and inhabiting varied ecological niches show modifications in their organisational pattern?

Does the surface architecture of epidermis of lips and associated structures of the fish species show adaptive modifications in relation to their ecophysiological status and varied feeding habits?

This chapter treats the morphology of lips and associated structures of the family Gobiidae, Cobitidae, Belontiidae and few species of Cyprinidae. The fresh-water fish species inhabiting different ecological niches and having different feeding habits selected for this study are *Glossogobius giuris*, *Noemacheilus botia*, *Colisa fasciata*, *Garra lamta*, *Puntius sophore* and *Cyprinus carpio*.

Glossogobius giuris (Hamilton, 1822), belongs to the Family Gobiidae, suborder Gobioidi and Order Perciformes. It is predominantly a fresh water fish found throughout the plains of India and sometimes also found in brackish waters. The members of this genus, in general, are bottom fishes and food chiefly consists of small bottom living animals (Günther, 1989). *G. giuris* is a carnivorous surface feeder and feeds on small crustaceans, insects, molluscs, smaller fishes and tadpoles (Hora & Mukerji, 1953; Khanna, 1993).

Noemacheilus botia (Hamilton, 1822) belongs to the family Cobitidae, sub-order Cyprinoidei, and order Cypriniformes. It is a bottom dweller fish, which usually live under stones, and in currents swim from stone to stone (Nikolsky, 1963). It is an omnivorous very undemanding species, which accepts any kind of food and feeds on algal films as well.

Colisa fasciata (Bloch & Schneider, 1801) belongs to the family Belontiidae, suborder Anabantoidei and order Perciformes (Graham, 1997). It is an air breathing fish and the natural habitats of the members of this genus include very weedy rivers, streams and ponds, irrigation ditches, flooded rice fields and also very dirty accumulations of water such as drains (Günther, 1989). It is an omnivorous fish and feeds on nearly equal amount of plant and animal material (Khanna, 1993). It has been regarded a good larvicidal form and is a very efficacious fish (Hora & Mukerji, 1953).

Garra lamta (Hamilton, 1822) belongs to the family Cyprinidae, sub-order Cyprinoidei and order Cypriniformes. It is a hill stream fish and is predominantly adapted to life in swift-flowing waters. Behind the ventral mouth there is a sucking disc which enables the fish to hold fast in strong currents, mountain streams and rapids. Riverbeds comprising mainly rocks, boulders, stones and gravel form a useful hiding and anchoring substratum for the fish. These fishes have “stone clinging” and “stone licking” habit. Food mainly consists of algal felts, mats and periphyton that they scrape off stones.

Puntius sophore (Hamilton, 1822) belongs to the family Cyprinidae, sub-order Cyprinoidei and order Cypriniformes. It inhabits in shallow ponds and streams and is often found in large numbers in the polluted waters where large drains enter the main river (Hora & Mukerji, 1953). It is an omnivorous column feeder (Khanna, 1993) and likes to grub about the bottom (Günther, 1989). Food of the fish consists of much larger amount of plant material than that of animal.

Cyprinus carpio var. communis (Linnaeus, 1758) belongs to the family Cyprinidae, sub-order Cyprinoidei and order Cypriniformes. It is a cold water fish, but being very hardy, easily adapts to warm water. It is an omnivorous feeder. It browses on the shallow bottom and margins, takes in vegetable debris, insects, worms, crustaceans and also planktonic algae (Alikunhi, 1957). The species has been implicated in degradation of the aquatic environment mainly through its habit of rooting in the bottom that causes turbidity and deoxygenated conditions (Welcomme, 1988). In searching for worms and insect larvae, it burrows hole into the embankments (Hora & Pillay, 1962).

2. Materials and methods

Live specimens of *Glossogobius giuris* (approximately 60 ± 10 mm in length), *Noemacheilus botia* (approximately 35 ± 5 mm in length), *Colisa fasciata* (approximately 55 ± 5 mm in length), *Puntius sophore* (approximately 50 ± 10 mm in length), *Cyprinus carpio var communis* (approximately 40 ± 5 mm in length) were collected from river Ganges and ponds at Varanasi, Uttar Pradesh, India. *Garra lamta* (approximately 60 ± 10 mm in length) were collected from hill streams, Jonha Falls at Ranchi, Bihar, India. The fishes were maintained in the laboratory conditions at controlled room temperature ($25 \pm 2^\circ\text{C}$). The fishes were cold anaesthetised following (Mittal & Whitear, 1978). Pieces of lips and associated structures were excised and rinsed in physiological saline, dipped briefly in 0.1% solution of S-carboxymethyl- L-cysteine to remove mucus (Whitear and Moate, 1994) and fixed in 3%

glutaraldehyde in 0.1M sodium cacodylate buffer, at pH 7.4 for 4h at 4°C. Following fixation, the tissues were washed in 0.1M sodium cacodylate buffer (pH 7.4) and dehydrated at 4°C with graded ethyl alcohol in ascending concentrations. The tissues were then treated with ethyl alcohol and acetone in the ratios 3:1, 1:1 and 1:3, anhydrous acetone and critical point dried 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), coated with gold using a sputter coater (AGAR, B7340, England) and examined with a Scanning Electron Microscope (Leo, 435 VP, England).

3. Results

In all the fish species investigated the upper jaws, in general, show a variable degree of protrusion. The upper lip (UL), borne on upper jaw, is associated with the rostral cap (RC) (rostralkappe = Minzenmay, 1933), through a fold of skin (FSUR), that in turn continues with the dorsal head skin. The FSUR, in general, is thin and membranous, and shows remarkable capacity of extendibility. The FSUR, when mouth is closed, lies in a deep groove between the UL and the RC.

The lower jaws in the fish species, in contrast, are only slightly protrusive. The lower lip (LL) is borne on lower jaw. Generally, it continues with the ventral head skin directly at the narrow middle region and through a fold of skin (FSLS) at the lateral sides of the lower jaw. In *G. lamta*, however, a specialised structure - the adhesive pad (AP) is located between the LL and the ventral head skin. The AP is separated from the LL by a narrow groove lined by a thin fold of skin (FSLA) and from the ventral head skin by a deep cleft lined by a thin and extensive fold of skin (FSAV).

In *N. botia*, *C. fasciata*, *G. lamta*, *P. sophore* and *C. carpio* the UL and the LL, on the side facing the mouth opening, are associated with the horny upper jaw sheath (HUJS) and the horny lower jaw sheath (HLJS) respectively. The horny jaw sheaths are absent in *G. giuris*. Several papilliform teeth are also observed in the jaws of *G. giuris* and *C. fasciata*.

In the fish species investigated, in general, the epithelia of the UL, the RC, the FSUR, the LL, the FSLS, the AP, the FSLA and the FSAV are mucogenic. The epithelia of the HUJS and the HLJS, in contrast, are keratinized. In the epithelia of the RC and the AP in *G. lamta* and those of the HUJS and the HLJS in *C. fasciata*, however, both mucogenic and keratinized regions are observed.

In the present account uncini or keratinization are recorded in a total of 5 fishes, and SEM observations are recorded for all of the above mentioned 6 fishes. These observations may be resulted as follows:

4. *Glossogobius giuris*

In *G. giuris* the mouth is terminal (Fig 1 a). The lower jaw remains slightly projected beyond the upper jaw. The upper jaw, as compared to the lower jaw, is highly protrusive (Fig. 1 b, c). The UL and the LL covering the upper jaw and the lower jaw respectively are thick and prominent.

The epithelia of the UL, the LL, the RC, the FSUR and the FSLS are mucogenic and are covered by a mosaic of irregularly polygonal epithelial cells of varied dimensions (Fig. 2 a,

b, c). The surface architecture of the epithelial cells is characterised by the presence of a series of micro-ridges. The boundaries between adjacent epithelial cells are demarcated by smooth well-defined uninterrupted double row of closely approximated micro-ridges.

The oral cavity of *G. giuris* is prominent in the strong dentition of their jaws, armored by several papilliform teeth (Fig.1c).

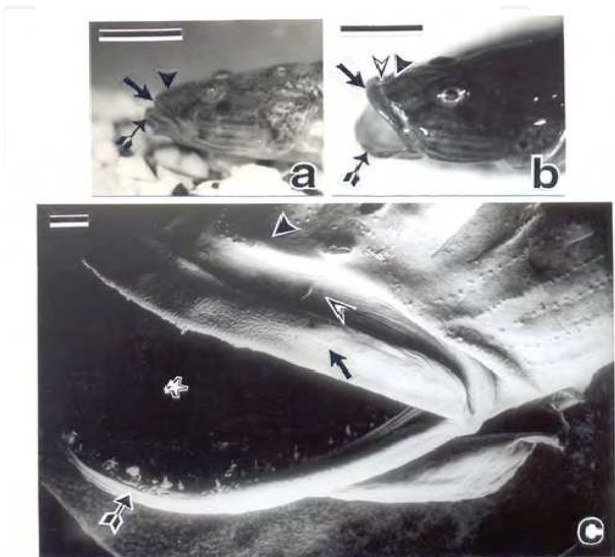


Fig. 1.

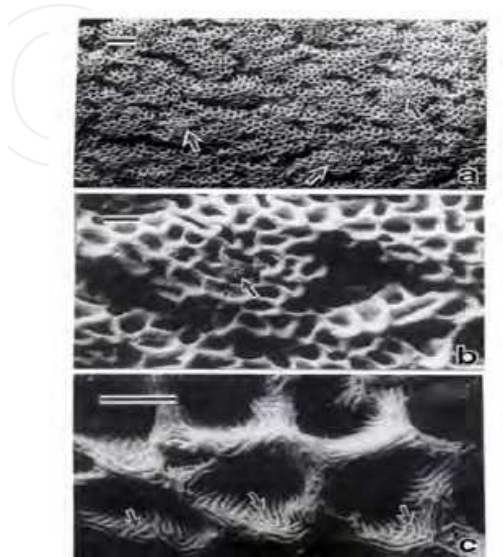


Fig. 2.

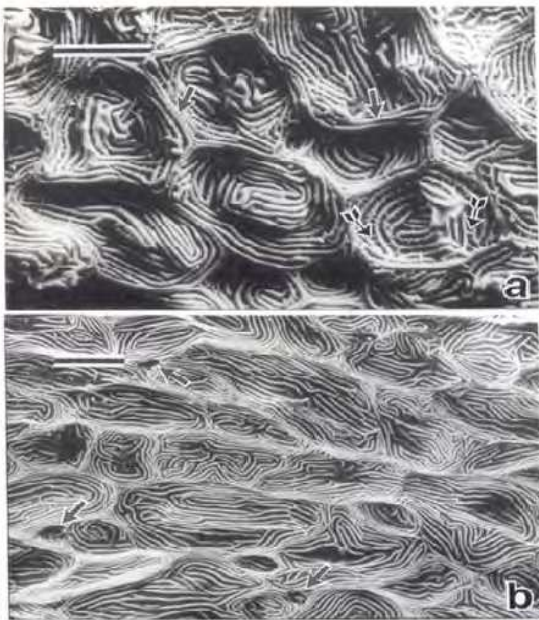


Fig. 3.

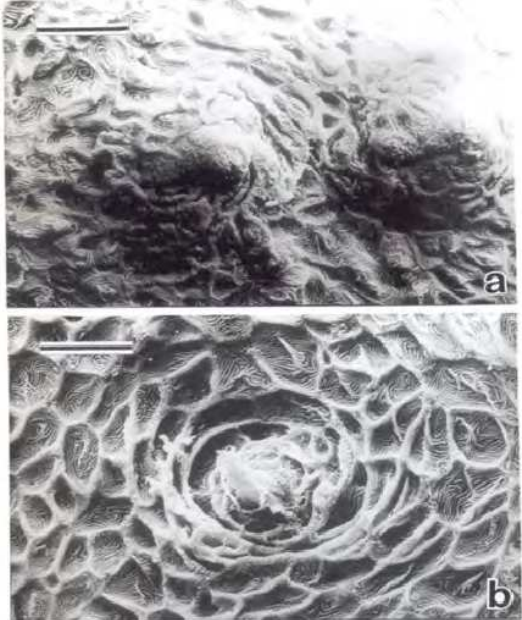


Fig. 4.

4.1 Epithelial cells

The surface of the epithelia of the UL and the LL characteristically appears like that of honeycomb. The epithelia appear folded and differentiated in to wide ridges separated by shallow and narrow furrows. The epithelial cells show a variable degree of invagination and

thus their surfaces characteristically appear as concave depressions of varied depth (Fig. 2 a, b, c).

The micro-ridges on the surface of the epithelial cells in the epithelia of the UL and the LL appear smooth, extensive, uninterrupted, and are separated by wide furrows. In general, these appear systematically arranged parallel to each other often traversing towards the deeper regions of concave depressions in each cell (Fig. 2 b, c). The micro-bridges interconnecting the adjacent micro-ridges are prominent and are often located close to each other (Fig. 2 c).

The surfaces of the epithelial cells in the epithelium of the RC, in contrast, show only a slight concavity and appear as shallow depressions. The micro-ridges appear smooth, extensive, uninterrupted, at times branched and are separated by wide furrows. In general these are arranged systematically in a concentric manner, traversing almost parallel to the boundary of the cell forming intricate patterns. The micro-bridges interconnecting the adjacent micro-ridges are prominent similar to those in the epithelia of the UL and the LL (Fig. 3 a).

The epithelial cells in the epithelia of the FSUR and the FSLS, unlike those in the epithelia of the UL, the LL and the RC, appear flattened and do not show depressions at their surfaces. The micro-ridges on the surface of the epithelial cells in these regions though are extensive and often traverse parallel to each other are not interconnected by micro-bridges (Fig. 3 b).

4.2 Mucous cells

Interspersed between the epithelial cells in the epithelia of the FSUR and the FSLS mucous cell apertures of varied dimensions are observed similar to those in *P. sophore*, *C. carpio*, *N. botia* and *C. fasciata* (Fig. 3 b). In the epithelia of the UL, the LL and the RC, however, the mucous cell openings could not be clearly distinguished.

4.3 Taste buds

There are two types of TBs in *G. giuris*, type I-minute taste buds could be located on careful search in the epithelia of the UL and the LL. The epithelial cells around each taste bud are concentrically arranged (Fig. 2 a, b).

On the other hand type II-big taste buds on the surface of the epithelium of the RC are observed. At intervals, rounded mounds of epithelial cells bearing this type of comparatively big taste buds (Fig. 4 a). In the central region, the microvilli are arranged in the form of characteristic rosettes (Fig. 4 a). The microvilli of each rosette probably represent sensory hairs originating from sensory cells of the taste buds (Fig. 4 a).

4.4 Superficial neuromasts

Superficial neuromasts sunk slightly in the epithelium are observed. The central region of each such superficial neuromast is characterised by the presence of a characteristic structure consisting of tall-elongated closely approximated projections. This could represent the cupula of the superficial neuromast. The epithelial cells surrounding the superficial neuromasts are concentrically arranged to form a characteristic ring like pattern. (Fig. 4 b).

5. *Noemacheilus botia*

In *Noemacheilus botia* the mouth is small, inferior or sub-terminal, transverse and semicircular (Fig. 5 a). The upper jaw is highly protrusive (Fig. 5 b, c) and the lower jaw is only slightly protrusive. The HUJS and HLJS in this fish are prominent and are characteristically modified. Further, the UL and the LL in this fish are thick and plicate (Fig. 5 a, b, c).

5.1 Mucogenic epithelia

The epithelia of the UL, the LL, the RC, the FSUR and the FSLs are mucogenic. The epithelia of the UL and the LL are thrown in to distinctive protuberances of variable dimensions delineated by narrow furrows (Fig. 6 a, b). Further, the epithelia of the FSUR and the FSLs are characteristically pleated (Fig. 6 a, b, c).

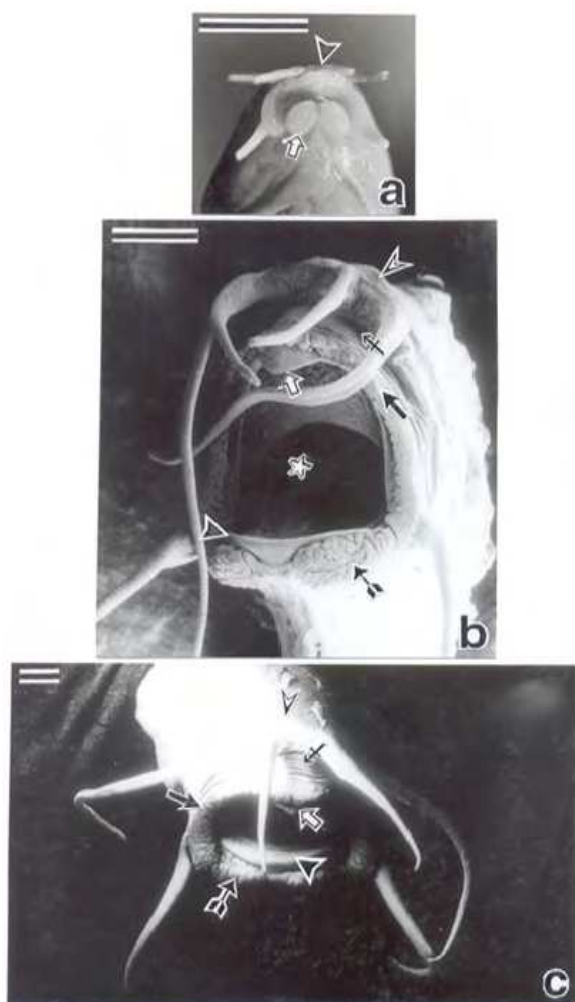


Fig. 5.

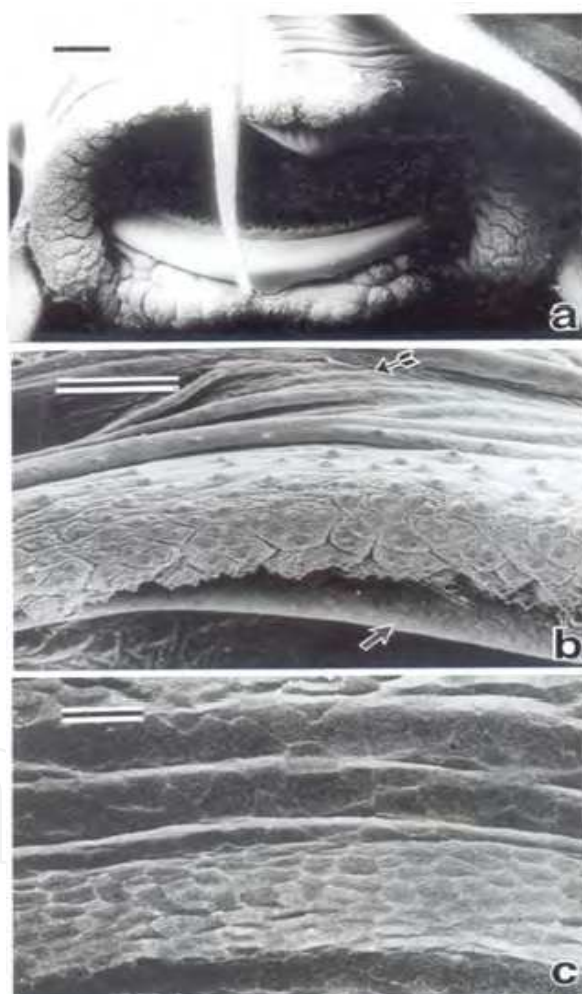


Fig. 6.

5.1.1 Epithelial cells

The epithelia of the UL, the LL, the RC, the FSUR and the FSLs in *N. botia* like those of *G. lamta*, *P. sophore* and *C. carpio* are covered by a mosaic of irregularly polygonal epithelial cells of varied dimensions (Fig. 6 c). The surface architecture of the epithelial cells is

characterised by the presence of a series of micro-ridges separated by prominent irregular spaces. The micro-ridges in general appear sinuous having roughed surface, short with abrupt ends and irregularly interwoven to form web like patterns (Fig. 7 a, b). The boundaries between adjacent epithelial cells are demarcated by a well-defined double row of micro-ridges, which are often connected by transverse strands to give a braided appearance (Fig. 7 a, b).

5.1.2 Mucous cells

Mucous cell openings, seen as wide, rounded apertures or crypts, often containing blobs of mucus, are interspersed between the epithelial cells. Generally, such apertures occur where the boundaries of 3 or more epithelial cells meet (Fig. 7 a, b).

5.1.3 Taste buds

The most conspicuous surface feature of the epithelia of the UL and the LL in *N. botia* is the presence of distinct protuberances each studded with a large number of small, protrusions or elevations (Fig. 6 a, b; Fig. 8 a, b, c). These elevation appear conical and extend beyond the general epithelial surface (Fig. 8 a). The slopes of each elevation or papilla are covered by a mosaic pavement of concentrically arranged epithelial cells. The epithelial cells covering the surface of these papillae and at their vicinity, in general, are arranged concentrically (Fig. 8 b, c). The apex of each elevation is characterised by the presence of numerous closely packed microvilli (Fig. 8 b, c). These microvilli represent the taste hairs originating from taste cells of the buds and are projected through the rounded taste pore at the summit of these elevations.

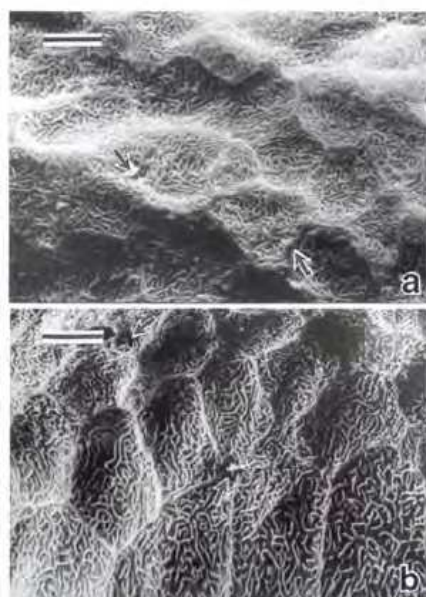


Fig. 7.

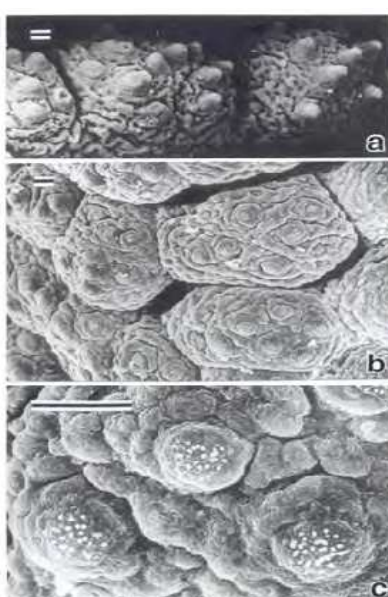


Fig. 8.

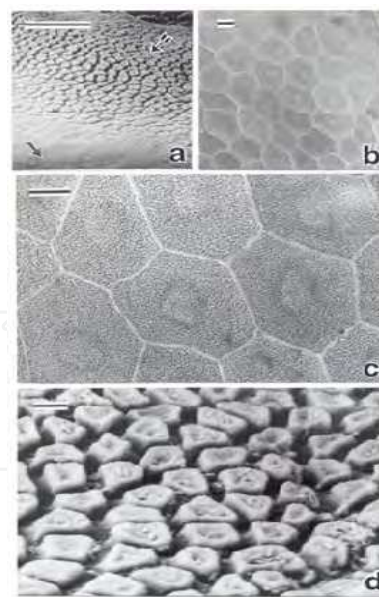


Fig. 9.

5.2 Keratinized epithelia

The epithelia of the HUJS and the HLJS are keratinized and resemble with each other in their surface architecture. The surface epithelial cells of the HUJS and the HLJS, in general,

towards the proximal regions gradually get transformed in to truncated specialised structures - the unculi. Thus the surface architecture of the horny jaw sheaths at their distal regions i.e. the regions boarding the mouth is strikingly distinct from those at the proximal regions i.e. towards the buccal cavity (Fig. 9 a).

The surface of the epithelia at the distal regions of the HUJS and the HLJS are covered by a mosaic pavement of irregularly polygonal epithelial cells of varied dimensions (Fig. 9 b). The free surface of these cells is characterised by the presence of compactly arranged micro-ridges separated by narrow irregular spaces. The micro-ridges, in general, are short, sinuous, beaded, branched with abrupt ends and are irregularly interwoven to form maze like pattern (Fig. 9 c). The boundaries between adjacent epithelial cells are very prominent and appear slightly raised from the general surface of the epithelia (Fig. 9 c). These are demarcated by well-defined double rows of micro-ridges, which appear either lying very close to each other or fused. The central regions of these epithelial cells, in general, show rounded bulge at the surface. Each bulge is further demarcated by a narrow depression around them in the form of a ring (Fig. 9 b, c). These bulges could represent the nuclei of these epithelial cells, which appear greatly flattened in cross sections.

The surface of the epithelia at the proximal regions of the HUJS and the HLJS are studded with characteristic truncated, polygonal unculi (Fig. 9 d). The unculi, in general, appear uniform in dimensions and remain projected at the free surface. Each uncus represents modified surface relief of fine projections of a superficial layer epithelial cell. The unculi appear irregularly arranged and the central part of each uncus is wide and often irregularly distributed projections. The boundaries of the adjacent epithelial cells bearing the unculi are distinctly demarcated.

6. *Colisa fasciata*

In *C. fasciata* the mouth is oblique or slightly upturned (Fig. 10 a). The upper jaw is highly protrusive and extends forward to a great extent (Fig. 10 b, c). The lower jaw, however, is only slightly protrusive. Further, the UL and the LL, in general, are thin. The UL is, however, relatively less conspicuous than the LL. The lips are relatively thick and appear lobular at the lateral sides of the mouth. The lobes are separated by shallow grooves. The HUJS and the HLJS are very conspicuous and their distal regions are modified into prominent sharp cutting edges. At intervals papilliform teeth like structures are observed protruding on the surface of the jaw sheaths (Fig. 10 d).

The epithelia of the UL, the LL, the RC, the FSUR and the FSLs are mucogenic. In contrast, the epithelia of the HUJS and HLJS are keratinized and the dead keratinized epithelial cells at the surface are commonly visualised to be lifted up from the underlying tissues. They are probably in the process of being exfoliated. Further, the jaw sheaths are characterised by the presence of papilliform teeth like structures, which protrude at intervals from their surfaces facing the mouth opening (Fig. 11 a, b, c). The surface of the UL epithelium shows slight infoldings, which are visible even in stretched conditions. The folds on the surface of the LL, in contrast, are more distinct and are distinguished in to prominent ridges separated by shallow gutter-like depressions. Generally, these ridges run parallel to each other along the surface bordering the mouth. (Fig. 11 a, c).

6.1 Mucogenic epithelia

6.1.1 Epithelial cells

The epithelia of the UL, the LL, the RC, the FSUR and the FSLs in *C. fasciata* like those of *G. lamta*, *P. sophore*, *C. carpio* and *N. botia* are covered by a mosaic of irregularly polygonal epithelial cells of varied dimensions (Fig. 12 a). The surface architecture of the epithelial cells is characterised by the presence of a series of micro-ridges. The boundaries between adjacent epithelial cells are demarcated by smooth well-defined uninterrupted double row of closely approximated micro-ridges (Fig. 12 a, b, c).

The micro-ridges on the surface of the epithelial cells in the epithelia of the UL and the LL are generally short, straight or sinuous and smooth often arranged in the form of small groups (Fig. 12 c; Fig. 13 b). Several such groups of micro-ridges may be observed on the surface of each cell. Adjacent groups of micro-ridges are delineated from each other by extensive micro-ridges, which are often branched and encircle each group. The micro-ridges within a group are generally arranged parallel to each other either linearly or concentrically. The adjacent micro-ridges are interconnected with each other by fine transverse connections, the micro-bridges (Fig. 12 b).

The micro-ridges on the surface of the epithelial cells in the epithelium of the RC, in contrast, appear smooth, extensive, uninterrupted, at times branched and are separated by wide furrows. In general these are arranged systematically in a concentric manner, traversing almost parallel to the boundary of the cell forming intricate patterns. In the narrow central region of these cells, the micro-ridges are often either indistinct or fragmented (Fig. 12 a, b). The micro-ridges on the surface of the epithelial cells in the epithelia of the FSUR and the FSLs are relatively few, extensive and are located parallel to each other at long intervals (Fig. 13 a). Further micro-bridges could not be located.

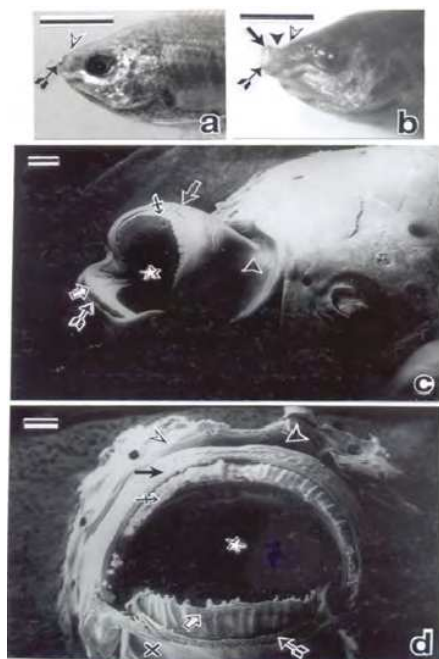


Fig 10.

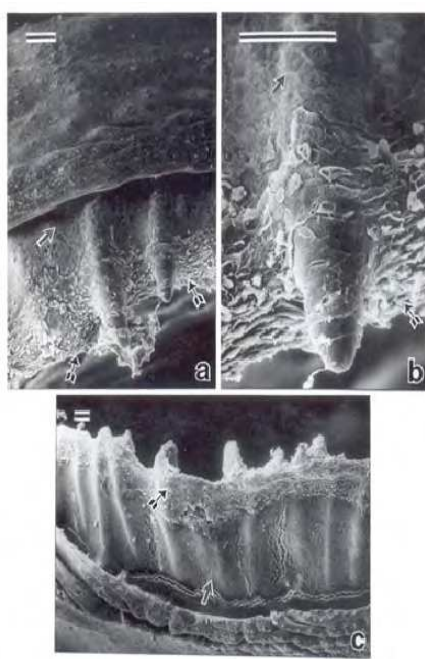


Fig. 11.

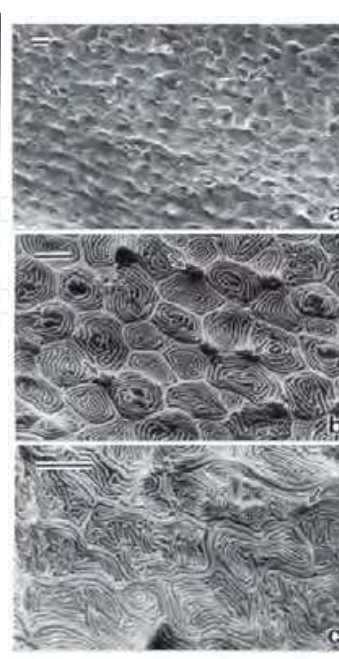


Fig. 12.

6.1.2 Mucous cells

Interspersed between the epithelial cells in the epithelia of the UL, the LL, the RC, the FSUR and the FSLs mucous cell apertures of varied dimensions are observed similar to those in *G. lamta*, *P. sophore*, *C. carpio* and *N. botia* (Fig. 12 a, b, c).

6.1.3 Taste buds

Taste buds are located on the ridges at the surface of the UL and the LL. Further, the epithelial surface in the regions where taste buds are located is thrown into papillae like projections protruding beyond the general surface of the epithelia. At the summit of each such papilla several microvilli representing the taste hairs of the taste buds are located. The arrangement of the epithelial cells at and around each papilla bearing a taste bud is concentric and the appearance of the taste buds at the summit of these papillae are similar to those in *G. lamta*, *P. sophore*, *C. carpio* and *N. botia* (Fig. 13 a, b).

6.2 Keratinized epithelia

The HUJS and HLJS are covered with a pavement of epithelia of close packed polygonal cells of irregular shape and size. The epithelial cells, however, show regional variations in their surface architecture.

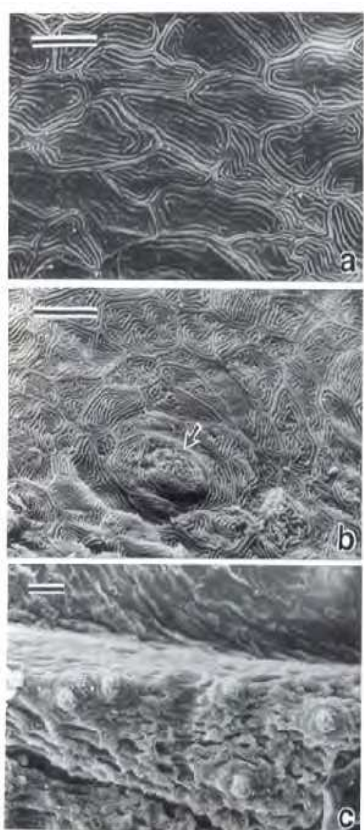


Fig. 13.

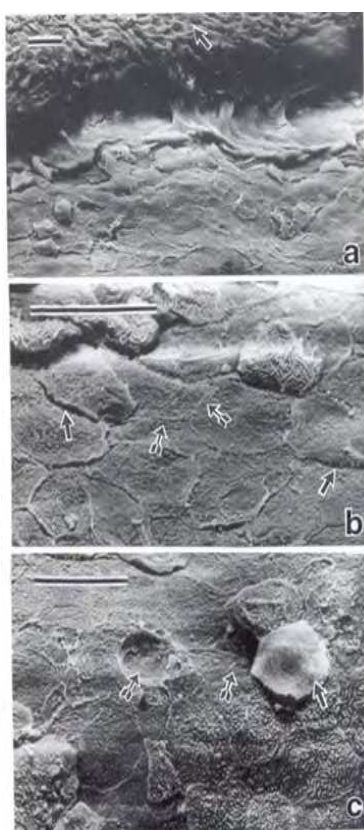


Fig. 14.



Fig.15.

At the proximal regions of the jaw sheaths, the surface of the epithelial cells appears scrawly. The micro-ridges are small, low, irregular and ill defined. Further, the pattern

formed by these at the cell surfaces seems indistinctive. The boundary between the adjacent epithelial cells is delineated either by shallow separating clefts or by a double row of micro-ridges separated by distinct spaces (Fig. 14 a, b).

At the distal regions of the jaw sheaths the micro-ridges at the surface of the epithelial cells are frequently punctated (Fig. 14 c) and are separated by wide spaces. In addition short, sinuous, branched micro-ridges interwoven to form characteristic patterns are also observed (Fig. 15 a). The boundaries between adjacent epithelial cells are demarcated by double row of closely approximated micro-ridges.

At and near the apical margins of the horny jaw sheaths the epithelial cells generally, exhibited a surface relief of fine closely approximated micro-ridges such being often prominent in the central parts of the cells. The micro-ridges at the narrow peripheral portions of the cells were relatively short being more widely spaced and irregularly located. The boundaries of the epithelial cells were demarcated by prominent continuous marginal elevations of adjacent cells, sometimes with an inconspicuous gap between them.

The epithelial cells at the distal regions including the apical margins are frequently observed to be lifted up from the underlying tissues. They were probably in the process of being sloughed (Fig. 14 c; Fig. 15 a, b).

7. *Garra lamta*

In *Garra lamta* mouth is sub-terminal and is situated on the ventral side of the head. In this fish the upper jaw and the lower jaw are only slightly protrusive. The UL and the LL are rudimentary and are represented by slight thickening of the epithelia, at narrow regions, covering the upper jaw and the lower jaw respectively. In the regions bordering the lateral margins of the mouth, however, the lips are distinguished as small, stumpy, papillae like structures (Pinky et al., 2002, 2008).

7.1 Mucogenic epithelia

The RC is very prominent and greatly enlarged. Its epithelium may be distinguished in to a keratinized belt towards the mouth opening and a major mucogenic region towards dorsal head skin. At the apical margins of the RC mucogenic islands are observed between the keratinized regions. These non-keratinized and keratinized regions show characteristic alternate arrangements.

The epithelium of the AP like that of the RC is distinguished in to mucogenic and keratinized regions. The epithelium of the major central region of the AP is mucogenic. The narrow peripheral regions of the AP are, however, keratinized.

7.1.1 Epithelial cells

The surface of the mucogenic epithelium of the RC is covered by a mosaic pavement of irregularly polygonal epithelial cells of varied dimensions. The free surface of each epithelial cell is characterised by the presence of a series of compactly arranged micro-ridges separated by narrow irregular spaces. The micro- ridges, in general, appear sinuous, having smoothed surface, short with abrupt ends and irregularly interwoven to form maze like

patterns. The boundaries between adjacent epithelial cells are demarcated by well-defined double row of micro- ridges, which are often interwoven to give a braided appearance.

7.1.2 Mucous cells

Interspersed between the epithelial cells rounded or irregular shaped crypts could be observed. These crypts often contain blobs of mucus and represent mucous cell openings. Generally, these apertures are located at the points where the boundaries of three or more epithelial cells meet.

7.1.3 Taste buds

A large number of epithelial protrusion or elevations that extends beyond the epithelial surface are located at irregular intervals. Each epithelial elevation is characteristically associated with a taste bud. The epithelial cells covering the surface of the elevations and at their vicinity, in general, are arranged concentrically. At the apical surface of each epithelial elevation, closely packed microvilli are observed. These microvilli appear to represent the taste hairs originating from the sensory cells of the taste buds.

The mucogenic islands in between the keratinized regions at the apical margin of the RC are characterised by the presence of several stumpy epithelial protrusions lying close to each other. Each epithelial protrusion is associated with a taste bud.

The surface relief of the epithelial cells in the mucogenic region of the AP, in general, is similar to that of the epithelial cells in the mucogenic epithelium of the RC. Further, the mucogenic epithelium of the AP resembles with that of the RC in the distribution of a large number of taste buds and in the presence of mucous cell openings.

7.2 Keratinized epithelia

7.2.1 Rostral cap

The surface of the epithelium of the RC in the keratinized regions, in contrast, to that in mucogenic regions appears shaggy. In general, it is matted with rounded projections or excrescencies in an organised array that are separated by shallow grooves.

The surface of each excrescence is represented by a cluster of several (15-25 or even more) prominent somewhat curved spine like unculi each having a broad base and a narrow apical end. Each unculus is projected at the free surface and represents modified surface relief of fine projections of a superficial layer epithelial cell.

These projections show a gradual increase in their height from the peripheral margin to the centre of the cell and in general appear compactly arranged or fused. The apical end of an unculus is either blunt or conical and the surface is rough with vertically oriented micro-villous projections. Between the unculi, the boundaries of the adjacent epithelial cells demarcated by distinct rows of micro-ridges may be observed.

The epithelial cells in shallow grooves between the excrescencies also show modified surface relief of fine projections. These are, however, less prominent and are not differentiated in to unculi like structures.

7.2.2 Adhesive pad

The keratinized epithelium at the posterior and lateral margins of the AP is characterized by the presence of rounded projections or excrescencies similar to those in the keratinized epithelium of the RC. In the keratinized epithelium at the anterior margin of the AP, in contrast, these excrescencies appear relatively prominent and tall. In general, these appear inverted cone shaped or basket like, each with a narrow proximal base, which gradually becomes relatively wide at the distal region. Like in the keratinized epithelium of the RC, the distal surfaces of these projections are characterised with the presence of a cluster of uncili, which represent modified surface relief of fine projections of superficial layer epithelial cells. The boundaries of the adjacent epithelial cells are often clearly demarcated by well-defined uninterrupted rows of micro-ridges.

7.2.3 Horny upper jaw sheath & Horny lower jaw sheath

The surface sculpture of the epithelia of the HUJS and the HLJS is similar to each other and are characteristically studded with tall, truncated, polygonal uncili. These uncili, in general, appear uniform in dimensions and shape and remain projected at the free surface. The uncili are arranged diagonally in parallel rows in an organised manner to form a characteristic pattern on the surface of the horny jaw sheaths. Each uncilus, like that of the RC and the AP, represents modified surface relief of fine projections of a superficial layer epithelial cell. In contrast, these projections appear more developed smooth and prominent at the margins of the cells and show a gradual decline in their height towards the central part of the cell. This results in the formation of a characteristic sharp edge at the margin and a deep depression at the central region of each uncilus. Each uncilus thus appear very much like a tooth.

The rudimentary UL and the LL, and the delicate FSUR, the FSLA, the FSACAP and the FSAV remain concealed and thus the surface architecture could not be visualized because these regions are deeper in position.

8. *Puntius sophore*

In *Puntius sophore* the mouth is terminal (Fig. 16 a, b, c). The upper jaw is highly protrusive. In contrast, the lower jaw is only slightly protrusive. Further, the UL and the LL are prominent. The UL is however, thin and the LL, in contrast, is very thick (special permission for figures 16-19 have been taken from Tissue and Cell).

8.1 Mucogenic epithelia

8.1.1 Epithelial cells

Surface architecture of the mucogenic epithelia of the UL, the LL, the RC, the FSUR and the FSLs, in general, resembles with each other. The surface of the epithelia is covered by a mosaic pavement of irregularly polygonal epithelial cells of varied dimensions. The free surface of each epithelial cell is characteristically thrown in to a series of micro-ridges having smooth surface. The micro-ridges, in contrast to those of *G. lamta*, are separated by wide furrows and are extensive, at times branched and traverse almost parallel to the boundary of the cell forming intricate patterns (Fig. 17 a, b). Further, the adjacent micro-ridges are interconnected with each other by fine transverse connections, the micro-bridges.

The boundaries between adjacent epithelial cells are demarcated by smooth well-defined double row of closely approximated micro-ridges (Fig. 17 a, b). In addition, epithelial cells with compactly arranged sinuous, short micro-ridges with abrupt ends to form maze like pattern or with micro-ridges giving a punctated appearance to the surface are frequent in the epithelia on the apical side of the UL and the LL (Fig. 17 c).

8.1.2 Mucous cells

Crypts representing the mucous cell openings, often containing blobs of mucus, are frequently observed at the borders of 3 or 4 epithelial cells (Fig. 17 a). The crypts are relatively conspicuous, large, rounded and frequent in the epithelia of the UL, the LL, and the RC, than those of the FSUR and the FSLs.

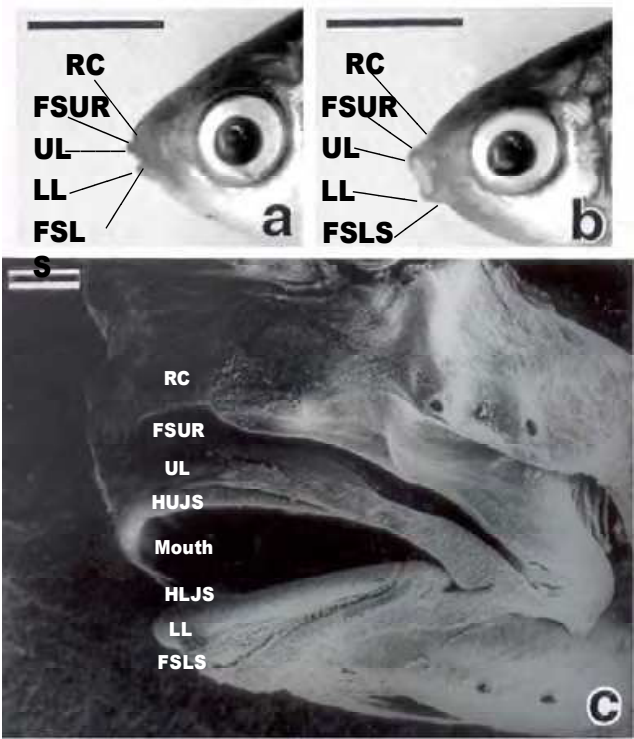


Fig. 16.

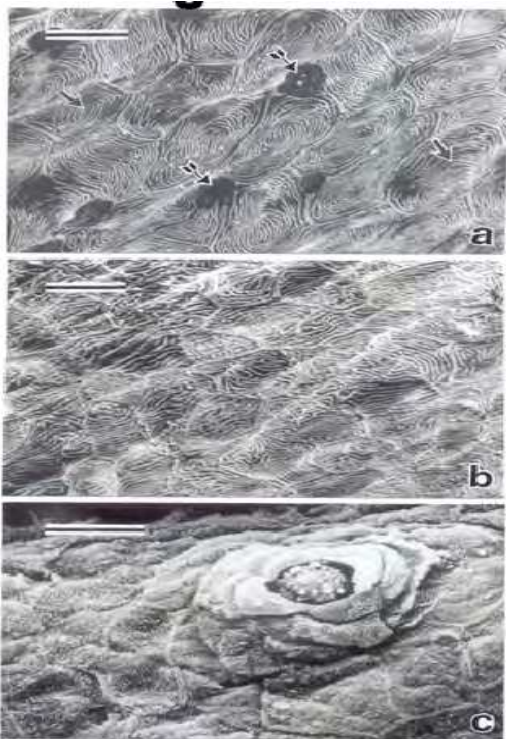


Fig. 17.

8.1.3 Taste buds

In the epithelia of the UL and the LL a large number of taste buds are observed (Fig. 18 a, b). Each taste bud is situated on a small epithelial papilla projecting at the surface. The epithelial cells covering the surface of these papillae and at their vicinity, in general, are arranged concentrically (Fig. 17 c; Fig. 18 a, b). At the summit of each papilla closely packed microvilli are observed (Fig. 17 c). These microvilli appear to represent the taste hairs originating from the sensory cells of the taste buds.

8.2 Keratinized epithelia

The epithelial surface of the HUJS and the HLJS at their distal regions are studded with characteristic truncated, polygonal unculti (Fig. 18 b). The unculti, in general, appear uniform

in dimensions and remain projected at the free surface (Fig. 18 c; Fig. 19 a). Each uncus represents modified surface relief of fine projections of a superficial layer epithelial cell and resemble in their shape and organisation to those in the epithelia at the proximal regions of the HUJS and the HLJS of *N. botia*. The uncus in this fish appear relatively less orderly arranged. Further, the central part of each uncus is much wide and often shows the presence of characteristic irregularly distributed projections (Fig. 19 a, b).

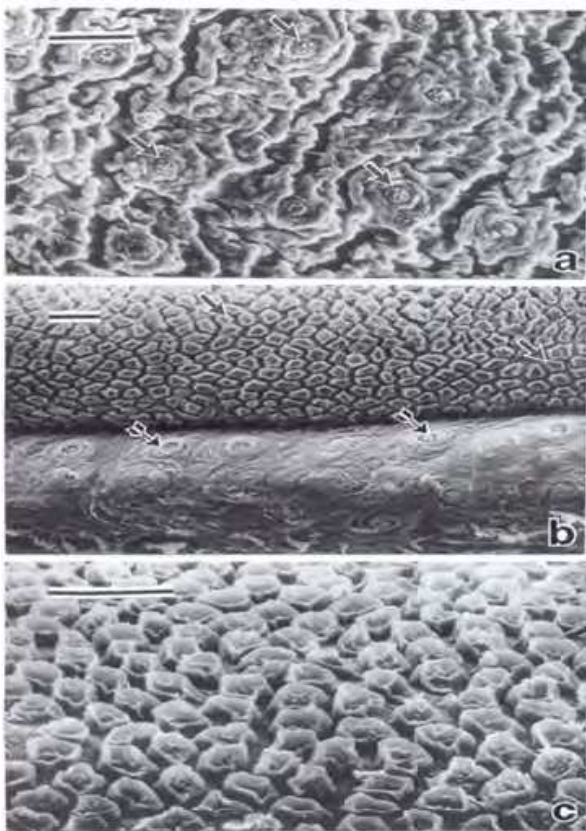


Fig. 18.

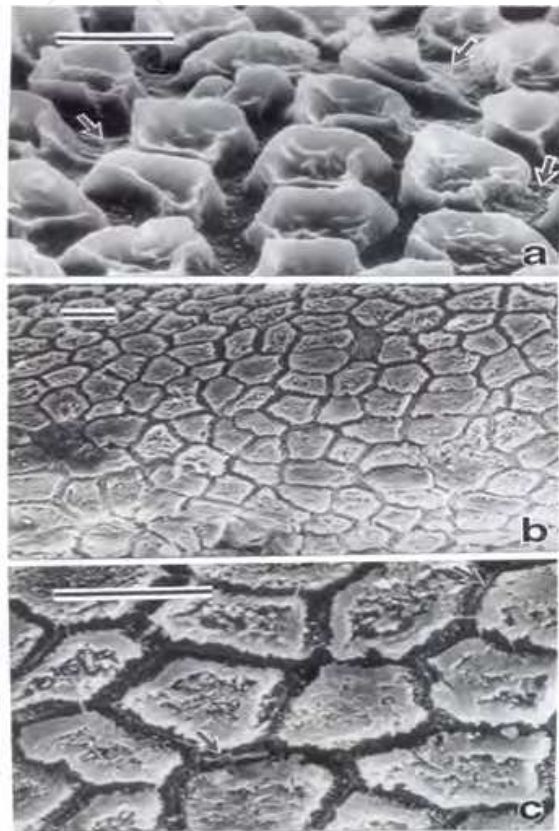


Fig. 19.

The uncus show a gradual decrease in their height towards the proximal regions of the HUJS and the HLJS. At these regions the surface relief of each uncus in contrast to those at the distal regions appears scraggy (Fig. 19 b, c). The micro-villous projections at the peripheral region on the surface of each cell appear fused to form the outer boundary of each uncus with scrawly surface (Fig. 19 c). The major central part of each uncus is occupied with micro-villous projections, which often appear fused awkwardly to give a scrambled or frothy appearance to the surface (Fig. 19 c).

Between the uncus both at the distal and proximal regions of the HUJS and the HLJS the boundaries of the adjacent epithelial cells demarcated by distinct rows of micro-ridges are observed (Fig. 19 a, c).

9. *Cyprinus carpio*

In *C. carpio* the mouth is terminal (Fig. 20 a, b). The upper jaw is highly protrusive (Fig. 20 c, d) and the lower jaw is slightly protrusive. The UL and the LL covering the upper jaw and

the lower jaw respectively, in contrast, are thick and prominent and are associated with relatively inconspicuous HUJS and the HLJS.

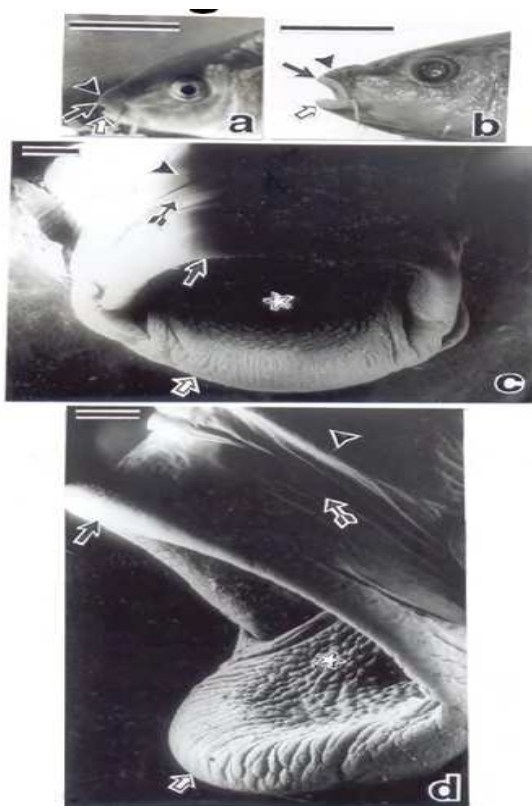


Fig. 20.

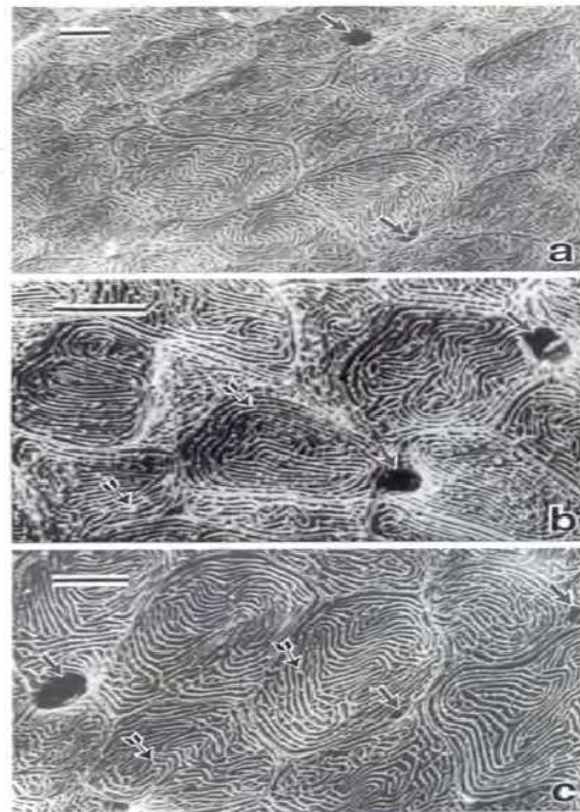


Fig. 21.

9.1 Mucogenic epithelia

9.1.1 Epithelial cells

The surface of the mucogenic epithelia of the UL, the LL, the RC, the FSUR and the FSLs, like those of *P. sophore*, is covered by a mosaic pavement of epithelial cells. The micro-ridges on the surface of the epithelial cells of these epithelia are separated by wide furrows and are extensive. In contrast, the micro-ridges in this fish often appear beaded, branched and interlocked to form intricate patterns (Fig. 21 a, b, c). Further, the micro-bridges interconnecting the adjacent micro-ridges are more prominent and frequent. The boundaries between adjacent epithelial cells are demarcated by well-defined double row of closely approximated micro-ridges often interconnected by transverse connections (Fig. 21 b, c).

9.1.2 Mucous cells

Interspersed between the epithelial cells in the epithelia of the UL, the LL, the RC, the FSUR and the FSLs are observed wide, rounded crypts or pores of varied dimensions, like in *P. sophore*. Generally these crypts or pores occur where the boundaries of three or four epithelial cells meet. These could represent the openings of the mucous cells. This is further confirmed by the presence of blobs of mucus in most of these crypts (Fig. 21 a, b, c).

9.1.3 Taste buds

In the RC epithelium, small taste buds are located individually at long intervals (Fig. 22 a). In the epithelia of the UL and the LL, however, a large number of taste buds are observed, each located on a small epithelial papilla projecting at the surface (Fig. 22 a, b). The taste buds at the major distal portion of the lips are characteristically located in-groups arranged in parallel rows (Fig. 22 b, c). The arrangement of the epithelial cells at and around each papilla bearing a taste bud and the appearance of the taste buds at the summit of these papillae are similar to those in *P. sophore* and *G. lamta*.

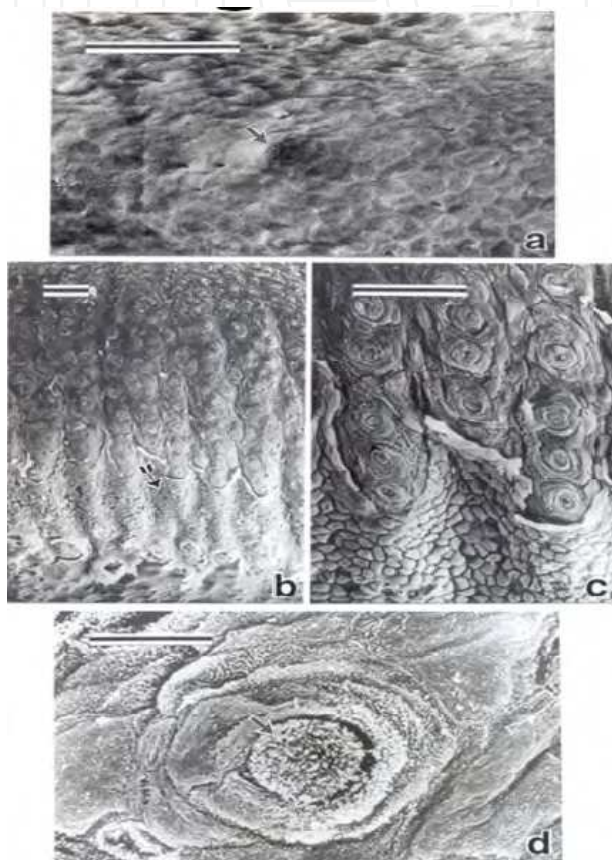


Fig. 22.

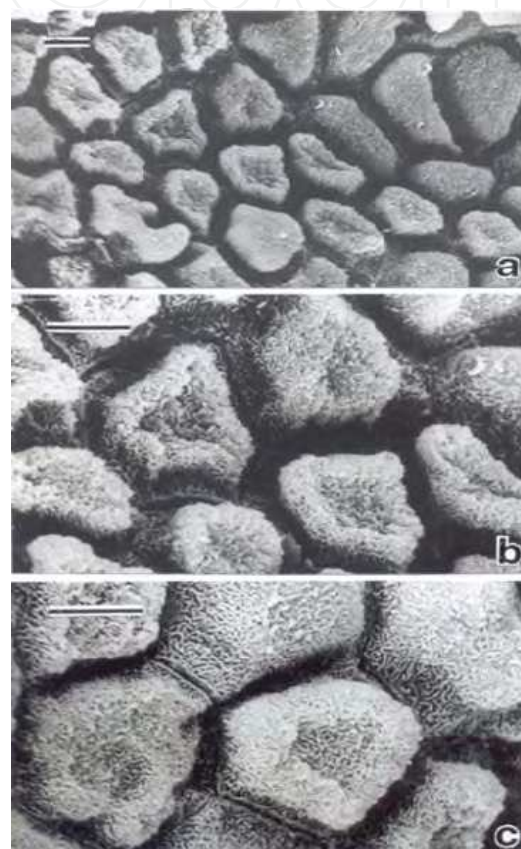


Fig. 23.

9.2 Keratinized epithelia

The epithelial cells at the surface of the HUJS and the HLJS are studded with characteristic polygonal uncini (Fig. 23 a, b, c). The surface relief of each uncinus appears truncated. Each uncinus is raised significantly from the general surface as a narrow bend at the periphery and has a shallow depression at its major central region (Fig. 23 a, b, c). Further, the surface relief appears scraggy similar to those at the proximal regions of the HUJS and the HLJS of *P. sophore*. In contrast, the micro-ridges at the surface of each uncinus are prominent, separated by wide spaces and do not fuse with each other. These micro-ridges are interwoven forming web-like patterns (Fig. 23 b, c). The boundaries of the adjacent epithelial cells, modified as uncini, are demarcated by distinct double rows of micro-ridges (Fig. 23 c). The space between these micro-ridges is relatively prominent and wide.

10. Explanation of figures

Figure 1 (a - b) Photographs of a part of the dorso-lateral side of the head of *G. giuris*, with closed mouth (a) and with open mouth (b) showing the RC (arrowhead), the UL (arrow), the FSUR (white arrowhead) and the LL (winged arrow) (Scale bar = 5 mm) **(c)** Scanning electron photomicrograph of a part of the dorso-lateral side of the head of *G. giuris*, showing the RC (arrowhead), the FSUR (white arrowhead), the UL (arrow), the LL (winged arrow) and the mouth (white star). Several papilliform teeth are also observed inside the mouth. (Scale bar = 400 μ m)

Figure 2 (a - c) Scanning electron photomicrographs showing surface architecture of the epithelia of the UL of *G. giuris*. (Scale bar = (a) 30 μ m, (b, c) 10 μ m). **(a)** Showing the epithelial cells having a variable degree of invagination and small type I taste buds (arrows). **(b)** Same as (a) in higher magnification. **(c)** Same as (b) in higher magnification, showing the surface of the epithelial cells characterised with smooth, extensive, uninterrupted micro-ridges traversing towards the deeper regions of concave depressions and are separated by wide furrows. Note fine micro-bridges interconnecting the adjacent micro ridges (arrows).

Figure 3 (a - b) Scanning electron photomicrographs showing surface architecture of the epithelia of the RC (a), the FSUR (b) of *G. giuris*. (Scale bar = 10 μ m). **(a)** Showing the surface of the epithelial cells is characterised with smooth, extensive, uninterrupted sometimes branched micro-ridges separated by wide furrows. Note boundary is demarcated by double row of micro-ridges (arrows) and fine micro-bridges interconnecting the adjacent micro-ridges (winged arrows). Note each epithelial cell shows slight concavity, compare with plate 51 (b, c). **(b)** Showing micro-ridges forming characteristic pattern on the epithelial cell surface and small mucous cell apertures (arrows).

Figure 4 (a - b) Scanning electron photomicrographs showing surface architecture of the epithelia of the RC of *G. giuris*. (Scale bar = 20 μ m) **(a)** Showing big type II taste buds located on the mounds of epithelial cells. Note the epithelial cells at each mound are characteristically arranged concentrically. **(b)** Showing superficial neuromast sunk slightly in the epithelium with characteristic cupula like structure.

Figure 5 (a) Photograph of the head region of *N. botia* showing the UL (arrow), the LL (white arrow) and the RC (arrowhead). (Scale bar = 5 mm). **(b - c)** Scanning electron photomicrograph of a part of the head of *N. botia* showing the RC (white arrowhead), the FSUR (barred arrow), the UL (arrow), the HUJS (white arrow), the HLJS (arrowhead), the LL (winged arrow) and mouth (white star) (Scale bar = 400 μ m).

Figure 6 (a) Scanning electron photomicrograph of a part of the head of *N. botia* showing the RC, the FSUR, the UL, the HUJS, the HLJS and the LL. On the UL and the LL protuberances of various dimensions separated by furrows are discernible. (Scale bar = 200 μ m). **(b)** Scanning electron photomicrograph of the epithelium of the UL showing protuberances of variable dimensions delineated by narrow furrows. A part of the HUJS (arrow) and the FSUR (winged arrow) is also discernible. (Scale bar = 200 μ m). **(c)** Scanning electron photomicrograph of the epithelium of the FSUR showing characteristic pleats and a mosaic pavement of surface epithelial cells. (Scale bar = 20 μ m).

Figure 7 (a, b) Scanning electron photomicrographs of the epithelium of the RC (a) and the FSUR (b) of *N. botia*. (Scale bar = 5 μ m). Showing intricate patterns of micro-ridges at the surface of the epithelial cells. Note the presence of mucous cell apertures (arrows).

Figure 8 (a - c) Scanning electron photomicrographs of LL of *N. botia*. (Scale bar = 10 μ m). (a) Showing distinct protuberances separated by furrows. Each protuberance is studded with a large number of epithelial elevations. (b) Same as (a) in higher magnification. Showing taste buds at the apex of each epithelial elevation. (c) Same as (b) in still higher magnification. Showing the apex of each epithelial elevation characterised by the presence of numerous closely packed microvilli, which represent the taste hairs originating from taste cells of the taste buds. Note the epithelial cells at the epithelial elevations are concentrically arranged.

Figure 9 (a - d). Scanning electron photomicrographs of HLJS of *N. botia*. (Scale bar = (a) 50 μ m, (b, c, d) 5 μ m). (a) Showing the superficial layer epithelial cells at the distal region (arrow) that gradually get transformed into truncated uncini towards the proximal region on the buccal cavity side of the HLJS (winged arrow). (b) Showing a mosaic pavement of irregularly polygonal epithelial cells at the distal region of the HLJS. Note distinct boundaries between the epithelial cells and characteristic rounded bulge in the central region of the epithelial cells. (c) Same as (b) in higher magnification, showing double row of micro-ridges demarcating the boundaries of the adjacent cells. Note the micro-ridges on the surface of the epithelial cells are short, sinuous and compactly arranged. (d) Proximal region of the HLJS, towards buccal cavity showing the epithelial cells modified into truncated uncini.

Figure 10 (a - b) Photographs of the head region of *C. fasciata* with closed mouth (a) and with open mouth (b) showing the RC (white arrowhead), the FSUR (arrowhead), the UL (arrow) and the LL (winged arrow). (Scale bar = 5 mm). (c - d) Scanning electron photomicrograph of a part of head region of *C. fasciata* in dorso-lateral view (c) and in front view (d), showing the RC (white arrowhead), the FSUR (arrowhead), the UL (arrow), the HUJS (barred arrow), the HLJS (white arrow), the LL (winged arrow), the FSLs (cross) and mouth (white star). (Scale bar = 400 μ m).

Figure 11 (a - c) Scanning electron photomicrograph showing the surface architecture of the HUJS (a, b) and the HLJS (c) of *C. fasciata*. (Scale bar = 50 μ m) (a) Note the difference in the surface architecture of the epithelia at the proximal region (arrow) and the distal region (winged arrows) of the HUJS. The epithelial cells in the distal region exfoliate at the surface. Note the presence of papilliform teeth like structures protruding at the surface. (b) Same as (a) in higher magnification. (c) Note the difference in the surface architecture of the epithelium at the proximal region (arrow) and the distal region (winged arrow) of the HLJS. The epithelial cells in the distal region exfoliate at the surface. Note the presence of papilliform teeth like structures protruding at the surface.

Figure 12 (a - c) Scanning electron photomicrograph showing the surface architecture of the RC (a, b), the UL (c) of *C. fasciata*. (Scale bar = 5 μ m). (a) Showing mosaic pavement of irregularly polygonal epithelial cells interspersed with mucous cell apertures (arrows). Epithelial cells with a series of micro-ridges and the boundaries demarcated by smooth well-defined un-interrupted double row of micro-ridges. (b) Same as (a) in higher magnification. (c) The micro-ridges are short, straight or sinuous and smooth often arranged in the form of small groups.

Figure 13 (a - c) Scanning electron photomicrograph showing surface architecture of the FSUR (a), the LL (b, c) of *C. fasciata*. (Scale bar = 10 μm). **(a)** Showing mosaic pavement of irregularly polygonal epithelial cells. **(b)** The micro-ridges are short, straight or sinuous and smooth often arranged in the form of small groups. Note a taste bud at the summit of a papilla like projection (arrow). The epithelial cells are arranged concentrically around the taste bud. **(c)** Showing taste buds at the summit of papilla like projections on the surface of the LL thrown in to papillae like projections protruding beyond the general surface of the epithelia. At the summit of each such papilla several microvilli representing the taste hairs of the taste buds are located.

Figure 14 (a - c) Scanning electron photomicrographs showing surface architecture of the epithelia of the HLJS of *C. fasciata*. (Scale bar = 20 μm) **(a)** Showing a mosaic pavement of irregularly polygonal epithelial cells. A part of the lower lip is also visible (arrow). **(b)** Showing ill-defined micro-ridges on the surface of the epithelial cells at the proximal region of the HLJS. Note the boundaries between the adjacent epithelial cells are delineated either by shallow clefts (arrows) or by double row of micro-ridges (winged arrows). **(c)** Showing the epithelium at the transitional zone between the proximal region and the distal region of the HLJS. Note an epithelial cell separated from the underlying epithelial cell before its exfoliation (arrow). Impressions left on the surface (winged arrows) of the epithelium is due to the exfoliated epithelial cells. The surface of the epithelial cells at the distal region is characterised with punctated micro-ridges.

Figure 15 (a - b) Scanning electron photomicrographs showing surface architecture of the epithelia of the distal region of the HUJS of *C. fasciata*. (Scale bar = 5 μm) **(a)** Micro-ridges at the surface are either punctated or are short and sinuous. Note an exfoliated epithelial cell (arrow). **(b)** Showing prominent and raised micro-ridges in the central region of the epithelial cells (arrows).

Figure 16 (a - b) Photographs of the head region of *P. sophore* with closed mouth (a) and with open mouth (b), showing the upper lip (UL), the lower lip (LL), the rostral cap (RC), the fold of skin between UL and RC (FSUR) and the fold of skin between LL and ventral head skin (FSLs). (Scale bar = 5 mm). **(c)** Scanning electron photomicrograph of a part of the lateral side of mouth of *P. sophore* (Scale bar = 400 μm).

Figure 17 Scanning electron photomicrographs showing surface architecture of the epithelia of the UL **(a)**, the FSUR **(b)** and the LL **(c)** of *P. sophore*. (Scale bar = 10 μm). **(a)** Showing micro-ridges arranged characteristically (arrows) at the surface of the epithelial cells. Note wide mucous cell apertures (winged arrows). **(b)** Showing characteristic pattern of micro-ridges at the surface epithelial cells. **(c)** Showing a taste bud at the apex of a mound of epithelial cells. Note the epithelial cells surrounding the taste bud are arranged concentrically.

Figure 18 Scanning electron photomicrographs showing surface architecture of the epithelia of the UL **(a)**, distal region of HLJS and the LL **(b)** and distal region of the HLJS **(c)** of *P. sophore*. (Scale bar = 10 μm). **(a)** Showing a large number of taste buds (arrows). **(b)** Showing truncated uncini at the HLJS (arrows) and taste buds at the LL (winged arrows). **(c)** Showing truncated uncini with central concavity and raised margins.

Figure 19 Scanning electron photomicrographs showing surface architecture of the epithelia at the distal region of the HLJS (a) and at the proximal region of the HLJS (b, c) of *P. sophore*. (Scale bar = 5 μ m). (a) Same as 21 c, in higher magnification, showing the superficial layer epithelial cells modified in to truncated uncini. Note the boundaries of the adjacent epithelial cells are demarcated by distinct micro-ridges (arrows). (b) Showing scraggy surface relief of the uncini. (c) Same as (b) in higher magnification, showing the scrawly surface of the uncini. The microvillar projections occupying the central part of each uncus give a frothy appearance to the surface. Note, between the uncini the boundaries of the adjacent epithelial cells are demarcated by distinct micro-ridges (arrows).

Figure 20 (a - b) Photographs of the head region of *C. carpio* showing the UL (arrow), the LL (white arrow), the RC (arrowhead). (Scale bar = 5 mm). (c - d) Scanning electron photomicrograph of a part of the head with open mouth showing the UL (arrow), the LL (white arrow), the RC (arrowhead) and the FSUR (winged arrow) and mouth (white star) of *C. carpio*. (Scale bar = 400 μ m). (c) front view (d) lateral view.

Figure 21 (a - c). Scanning electron photomicrographs showing surface architecture of the epithelia of the UL (a, c) and the RC (b) of *C. carpio*. (Scale bar = 5 μ m). (a) Showing mosaic pavement of irregularly polygonal epithelial cells with micro-ridges forming intricate patterns. Note mucous cell apertures (arrows) at the boundary of the epithelial cells. (b) Showing micro-ridges forming intricate patterns at the surface of the epithelial cells. Note fine transverse connections in between the micro-ridges (winged arrows) and mucous cell apertures (arrows). (c) Showing micro-ridges forming intricate patterns at the surface of the epithelial cells. Note fine transverse connections in between the micro-ridges (winged arrows) and mucous cell apertures (arrows).

Figure 22 (a - d) Scanning electron photomicrographs showing surface architecture of the epithelia of the RC (a) and the LL (b, c, d) of *C. carpio*. (Scale bar = (a, b, c) 50 μ m and (d) 10 μ m). (a) Showing a mosaic pavement of the epithelial cells and a taste bud at the apex of a mound of epithelial cells (arrow). (b) Showing major mucogenic region characterised with the presence of a large number of taste buds arranged in parallel rows. Note a narrow keratinized region, the surface of which appears rough and studded with pebble like structures (winged arrows). (c) Same as (b) in higher magnification. (d) Showing a taste bud (arrow) at the apex of a mound of epithelial cells.

Figure 23 (a - c) Scanning electron photomicrographs showing surface architecture of the epithelium of HLJS of *C. carpio*. (Scale bar = 5 μ m). (a) Showing truncated polygonal uncini with concavity in the central region of each uncus. (b) Same as (a) in higher magnification. Note prominent micro-ridges at the surface of the uncini, separated by wide spaces. (c) Same as (b) in higher magnification. Note the boundaries of the adjacent uncini are demarcated by double row of micro-ridges.

11. Discussion

Research on lips and associated structures began about 200 years ago, as described by Anson, 1929 in his manuscript "The comparative anatomy of the lips and labial villi of vertebrates". He made an attempt to define lips and on Danforth's interpretation of homology, homologous lips are found at certain stages of development in some representatives of all classes of vertebrates. The primary lips characteristic of selachians,

after the maxillary and premaxillary bones have developed within the territory of the upper lip (toadfish, cod), may disappear (trout, *Spelerpes*), accompanied by a forward migration of the lower jaw. The secondary lips of higher forms are first indicated in certain teleosts and amphibians. Lips vary in structure to accord with their physiological functions, whether sensory, prehensile, or adhesive (Anson, 1929). By precise comparative morphology and gene expression analyses, a possibility was inferred that ammocoete lips may not be identical to gnathostome jaws (Kuratani, 2003).

The surface architecture of the superficial layer of epithelial cells in the lips and associated structures is characterised by specialised structures, the micro-ridges forming different patterns in different fish species investigated in this study. These structures in other fishes, have been described as cytoplasmic folds (Merrilees, 1974), microvilli (Harris & Hunt, 1975), microfolds (Hunter & Nayudu, 1978) or ridges (Iger *et al.*, 1988). Insofar as these structures appeared as micro-ridges under SEM and microvilli under TEM. The term “microridges” is used in this study following Whitear & Mittal (1986), Whitear (1990), Suzuki (1992) and Whitear & Moate (1998) and seems appropriate.

The micro-ridges on the surfaces of the mucogenic epithelia form characteristic maze like patterns in different fish species. Fishelson, (1984) correlated the variations in micro-ridge pattern with the locomotory activity of the fish. He suggested that in faster swimming fishes, micro-ridges are most developed and serve to trap mucus on the epithelial surface. The present study, however, is not in support of this since micro-ridges are well developed and conspicuous on the free surface of the lips and associated structures in all the six fish species investigated showing significant difference in their habits and habitats.

The retention of secretion has been the most popular hypothesis of micro-ridge function (Hughes & Wright, 1970; Hughes, 1979; Tillman *et al.*, 1977; Meyer-Rochow, 1981; Fishelson, 1984). Modifications in the pattern of micro-ridges can also be caused by various intrinsic, e.g. hormonal (Schwerdtfeger, 1979 a, b), or extrinsic factors e.g. temperature (Ferri, 1982), salinity (Ferri, 1983), mercury salts (Pereira, 1988), organic pollutants, (Iger *et al.*, 1988), handling and ectoparasites (Whitear, 1990). Some speculations about the function of micro-ridges have centred on mechanical considerations (Lanzing & Higginbotham, 1974; Hawkes, 1974; Sibbling & Uribe, 1985). The provision of reserve apical membrane to allow for distortion was postulated by Zeiske *et al.*, (1976) but Sperry & Wassersug (1976) found no change of pattern after stretching fish oesophageal epithelium and suggested that spread of mucus from goblet (mucous) cells might be guided by the direction of ridges.

Presence of conspicuous micro-ridges on the surfaces of the mucogenic epithelia in the fish species investigated could be considered to reflect high secretory activity of the surface epithelial cells in these regions. Secretion of glycoproteins (GPs), shown histochemically (Pinky *et al.*, 2008, Tripathi & Mittal, 2010), in the surface epithelial cells in the mucogenic regions is in support of this. Further, Whitear (1990) proposed that the form of micro-ridges correlate with the type and rate of secretion at the cell apex. The development of micro-ridges would then be a consequence of arrival of new membranes as vesicles carrying the secretion fuse with the apical plasmalemma and high ridges would indicate a rapid sequence of arrival of secretory vesicles at the surface.

In the epithelia of lips and associated structures of all fish species studied, the adjacent micro-ridges are often interconnected with each other by fine cross connections i.e. micro-

bridges. It should be pointed out that such specific structures connecting micro-ridges have also been reported previously in fish epidermis (Whitaker, 1990) and have been variously named as interconnections (Reutter *et al.*, 1974), micro-bridges (Karlsson, 1983), cross-bridges (Avella & Ehrenfeld, 1997). Previous workers have not commented on the functional significance of these structural peculiarities. It is possible that these structures may provide mechanical strength to the micro-ridges. However, it is open to other interpretations.

Secretions elaborated by the epithelial cells and the mucous cells in the mucogenic epithelia could be regarded as an adaptation to lubricate and protect the epithelia from abrasion (Pinky *et al.*, 2002). The role of mucus was likewise postulated previously to inhibit the invasion and proliferation of pathogenic micro-organisms and to prevent their colonisation in fish epidermis (Nigrelli, 1937; Nigrelli *et al.*, 1955; Hildemann, 1962; Liguori *et al.*, 1963; Lewis, 1970).

In the oral cavity the lining mucous membrane becomes keratinized to varying degrees in different animals and also in different areas of the mouth (Adams, 1976). Some of the most dramatic advances made over the past 2-3 decades in epidermal research have come about through the utilization of newly developed biochemical investigative techniques, examples of which include the use of gene cloning to study the organization of the keratin gene family, and the use of immuno-fluorescence with monoclonal antibodies to discern when various keratin proteins appear during differentiation. In SEM studies, the Keratinized surfaces of the fishes studied are shaggy and are matted with an organised array of horny projections separated by shallow grooves. The boundaries of the adjacent epithelial cells (the surfaces of which are modified into uncini) are demarcated by well-defined and distinct rows of microridges.

Horny projections from single cells of lips and associated structures have been reported in a wide variety of fishes by various workers (Leydig, 1895; Rauther, 1911; 1928; Hora, 1922; Minzenmay, 1933; Saxena, 1959; Thys, 1961; Kaiser, 1962; Lal *et al.*, 1966; Saxena & Chandy, 1966; Roberts, 1982). Girgis (1952), in a herbivorous bottom feeder *Labeo horie*, observed horny protuberances on lips and two sharp horny cutting edges lying in the upper and lower borders of mouth immediately inside the lips. Mester (1971) made a morpho-histological analysis of the buccopharyngeal cavity in *Noemacheilus barbatulus*, and reported horny plates on the inner surface of the lips that are used by the fish for trituration of nutrients. Verighina (1971) while describing the structure of the digestive tract, reported the presence of horny cutting edge on the lower lip of periphyton eater fish *Chondrostoma nasus variable*. Agrawal & Mittal (1992 a) reported the presence of keratinized uncini on the ventral side of the upper lip and keratinized cone like structure with sharp cutting edge on the horny lower jaw sheath associated with the lower lip of an omnivorous bottom feeder, *Cirrhitina mrigala*. Agrawal & Mittal (1992 b) observed keratinized uncini on the ventral side of the upper lip and on the dorsal side of the lower lip facing the mouth opening. In addition, they observed keratinized cone like structure on the horny upper jaw sheath and on the horny lower jaw sheath associated with the lips of a herbivorous column feeder, *Labeo rohita*.

The present study shows that in *N. botia*, *C. fasciata*, *G. lamta*, *P. sophore* and *C. carpio* the UL and the LL, on the side facing the mouth opening, are associated with the horny upper jaw sheath (HUIS) and the horny lower jaw sheath (HLJS) respectively. The horny jaw sheaths are absent in *G. giuris* having few villiform teeth.

Keratinization occurs in the structures associated with lips of fish *Garra lamta* by histochemical investigations (Pinky et al., 2004). The uncini also observed in the upper jaw of an herbivorous fish, *Cirrhinus mrigala* by SEM studies. More recently, in *Puntius sophore*, the HUJS and the HLJS are keratinized. The surface epithelial cells in these regions are modified to form single cell modification—the uncini, give positive results for keratin with histochemical reactions. The uncini appear functionally significant on the lips and associated structure in this fish species lacking jaw teeth (Tripathi & Mittal, 2010).

In the epithelia of the RC and the AP in *G. lamta* and those of the HUJS and the HLJS in *C. fasciata*, however, both mucogenic and keratinized regions are observed. In *C. fasciata* the apical regions of the horny jaw sheaths are modified into cone like structures each consisting of several superimposed keratinized epithelial cells. The spine like or conical uncini in the keratinized regions of the RC and AP of *G. lamta* may be considered to facilitate clinging or adherence of the fish to the substratum by engaging irregularities on the surface of rocks or stones. Truncated uncini on the surface of HUJS and HLJS in 4 fish species investigated except *G. giuris* and apical cone like structure of the horny jaw sheaths in *C. fasciata* may have a function to assist the fish in scooping mud from the bottom in search of food or to act as sharp cutting edge assisting the fish to browse upon or scrape the food attached with the substratum for feeding.

The keratinized epithelia are mainly composed of the epithelial cells only. The mucous cells and the taste buds are not observed. The absence of the gland cells in the keratinized epithelia suggests an inverse relationship between the degree of keratinization and slime secretion.

In most vertebrates the sense of taste is used as a close range receptor for food item discrimination. Fish are unique among vertebrates in having taste buds widely distributed over various regions. The present study shows that, the taste buds in the epithelia of the lips and structures associated with them are conspicuous in *C. fasciata* and are small and inconspicuous in *G. giuris*. In both these fish species, however, the taste buds are few and could be located at long intervals. This indicates that the cutaneous gustatory function in these fish species is probably of less importance. Low density of taste buds, in *G. giuris* having active predatory habit could be associated with the presence of superficial neuromasts and canal neuromasts in the localisation of its prey. This is supported by reports that the taste buds are absent on the lips of the ox-eye herring *Megalops cyprinoides* (Pasha, 1964 c), are poorly developed on lips of *Channa striata* (Agarwal & Mittal, 1994) and are relatively few on the outer surface of the body than those on the lips and palatal organs and gills in *Pseudorasbora parva* (Kiyohara et al., 1980) all these fishes are active predators.

In the lips and associated structures of *G. lamta*, *P. sophore*, *C. carpio* and *N. botia* taste buds, as compared to those in *C. fasciata* and *G. giuris*, are very prominent and are distributed in large numbers. This indicates the development of acute gustatory function, an adaptation to the peculiar mode of life of these fishes. A similar correlation between the distribution of taste buds and gustatory feeding has been shown in two races of the Mexican characin, *Astyanax mexicanus*; one lives in caves and is sightless, and the other lives in river and is visually normal. Schemmel (1967) has shown that the cave-dwelling race has a larger number and a more extensive distribution of external buds than the river-dwelling race, corresponding to acute gustatory feeding behaviour.

Presence of large number of taste buds in *C. carpio* and *N. botia* could be due to its habit to live at the muddy bottom of water bodies. *C. carpio* browses on the shallow bottom and margins, takes in vegetable debris, insects, worms, crustaceans and also planktonic algae. *N. botia* is a bottom dwelling fish, which accepts any kind of food and feeds on algal films as well. At muddy bottoms the visibility, in general, is poor owing to (i) depth and (ii) increase in turbidity caused by the disturbance of the bottom mud due to the movements of the fish, which is in habit of suddenly burrowing into the mud or sand on the bottom to protect itself from predators. 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 taste buds in the lips and associated structures of all the six fish species investigated remain encircled by characteristic concentric whorls of epidermal cells. Harvey & Batty (1998), suggested that it was sometimes possible to locate and count taste buds by the presence of the characteristic ring of epidermal cells surrounding the sensory apex, even when the apex itself was damaged or missing.

Earlier SEM studies reported that fish taste buds fall into three categories based on their external surface morphology (Reutter *et al.*, 1974; Ezeasor, 1982). In addition to the three types of taste buds previously described from various teleost fish, a fourth type comprising very small buds, was found in some cardinal fish (Fishelson, 2004). The taste buds in the mouth cavity of *Rita rita* are of three types which are elevated from the epithelium at different levels, which may be useful for ensuring full utilization of the gustatory ability of the fish, detection and analysing of taste substances, as well as for assessing the quality and palatability of food, during its retention in the mouth cavity (Yashpal *et al.*, 2006). In *C. mrigala*, there is only one type of taste buds observed in mouth cavity (Yashpal *et al.*, 2009). In the present study only one type of taste buds are observed in the 5 fish species whereas in *G. giuris* there are two types of taste buds are observed in different regions of lips and associated epithelia.

In *G. giuris*, in addition to the taste buds, specialised sensory structures the superficial neuromasts are also observed in the epithelium of the RC and in the epithelia of the FSLs close to the ventral head skin. Neuromasts can be found on the entire body surface including the tail with the cupulae extending into the water (Schellart & Wubbels, 1998; Eastman & Lannoo, 2003; Tarby and Webb, 2003; Sane *et al.*, 2009).

The fishes studied are characterised by the peculiar trophic niche they occupy: many scrape epilithic or epiphytic algae and other food items from submerged substrates. This specialized feeding type is possible thanks to the remarkably formed, ventrally placed suckermouth of *G. lamta* that allows itself to attach to a surface while scraping and eating the food attached to it. In spite of this highly specialized feeding apparatus, diversity in both thickness of the different regions of lips and in shape of lips exists, and these fishes actually feed on a broad range of food. As such, Cyprinidae are the most specialized and successful fish family within the order Cypriniformes. More basal families within the teleosts like Gobiidae, Cobitidae and Belontiidae mostly display a more general (non-specialized) feeding mode, with a typical feeding apparatus suitable for finding and processing insects and other food items that abound in the water column or in the bottom. Some knowledge exists on the trophic, evolutionary trend in the group, but detailed studies dealing with the morphology of lips and associated structures or the feeding apparatus are few and often fragmentary.

12. References

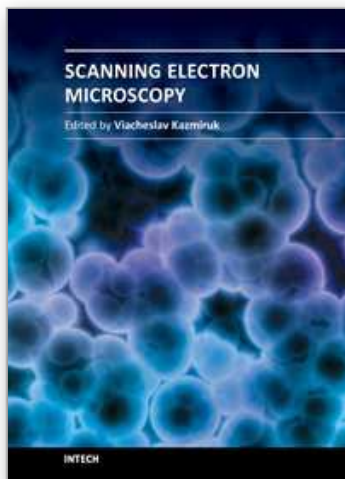
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Today, an individual would be hard-pressed to find any science field that does not employ methods and instruments based on the use of fine focused electron and ion beams. Well instrumented and supplemented with advanced methods and techniques, SEMs provide possibilities not only of surface imaging but quantitative measurement of object topologies, local electrophysical characteristics of semiconductor structures and performing elemental analysis. Moreover, a fine focused e-beam is widely used for the creation of micro and nanostructures. The book's approach covers both theoretical and practical issues related to scanning electron microscopy. The book has 41 chapters, divided into six sections: Instrumentation, Methodology, Biology, Medicine, Material Science, Nanostructured Materials for Electronic Industry, Thin Films, Membranes, Ceramic, Geoscience, and Mineralogy. Each chapter, written by different authors, is a complete work which presupposes that readers have some background knowledge on the subject.

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