Structure and Histochemistry of the Epithelia of Lips and Associated Structures of a Catfish *Rita rita*

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Abstract In Rita rita the upper lip is associated with the rostral cap and the lower lip with a skin fold. The epithelia of the lips, which are modified with respect to the peculiar feeding behaviour of the fish, are mucogenic. The superficial layer of epithelial cells, in addition to the mucous cells, are involved in active secretion of mucopolysaccharides. Mucus, apart from its diverse functions, as in fish epidermis, lubricates the epithelia giving protection against possible mechanical injury during the searching and catching of prey from bottom debris. Furthermore, the epithelia are thick, an adaptation to their mucogenic nature, so as to provide additional protection. The contents of the club cells are proteinaceous, which correlates with the helical filaments reported in similar cells in fish epidermis. Characteristic ridges on the surface of the lips are considered to be an adaptation to assist in manipulation during the procuring of food. Mucus secreted in the grooves between them reduces friction and prevents the deposition of foreign matter. The skin fold facilitates an increase in gape. The taste buds and ampullary organs, which on the lips and the rostral cap are associated with the localization of prey and triggering of a pick-up reflex, are not located on the skin fold, as the latter often remains concealed between the lower lip and the ventral head skin. The importance of histochemical analysis as a supplement to histological studies is emphasised.

Studies on the epithelia of the lips and associated structures in fishes, that show characteristic modifications with respect to their food and feeding habits, are limited and have not attracted the attention of researchers from the standpoint of their structural organization and histochemistry. Agrawal and Mittal (1991) reviewed the literature and described the structural organization of the epithelia of the lips and associated structures of a surface plankton and detritus feeder, the Indian major carp, *Catla catla*.

The present study is concerned with the structural organization and histochemical characterization of carbohydrates and proteins in the cellular components of the epithelia of the lips and associated structures of *Rita rita* (Hamilton), a sluggish, bottom dwelling, carnivorous catfish, belonging to the siluriform family Bagridae (Misra, 1962).

Materials and methods

Live specimens of *Rita rita* (approx. 160 mm in length) collected from the River Ganga at Varanasi, India, were maintained under laboratory conditions and fed with fresh goat liver on alternate days.

The upper and lower lips along with their associated structures were excised and fixed in 10% neu-

tral formalin or aqueous Bouin's fluid. Paraffin sections were cut at $6 \mu m$ and stained with Ehrlich's hematoxylin-eosin (HE), Verhoeff's hematoxylineosin (VHE), Papanicolaou's stain (PS), Feulgen reaction for DNA and by further methods for the location and differentiation of carbohydrate and protein fractions in the cellular components, following Lillie (1954), Gurr (1958), Bancroft and Stevens (1982) and Pearse (1985) (Table 1).

A stage micrometer and ocular graticule (Carl Zeiss) was used to measure the thickness of the epithelia at different sites in the cross sections. For each measurement random sampling of a series of locations (5) on each section were made, 10 sections each of the lips and associated structures being utilized, again after being randomly selected from the total preparations from each specimen. Data thus obtained for 5 individuals were pooled. Results are expressed as mean value \pm SD throughout.

Observations

In Rita rita the lips are thick and the jaws, unlike those of Catla catla (Agrawal and Mittal, 1991), are not protrusible. The upper lip is associated on its dorsal side with the rostral cap (Fig. 1, 2). The



Fig. 1. Photograph of the head region of *Rita rita* with open mouth. LL, lower lip; RC, rostral cap; UL, upper lip. Scale line, 10 mm.

lower lip is associated on its ventro-lateral sides with a fold of skin between it and the ventral head skin (Fig. 3). The narrow mid-ventral portion of the lower lip, however, is continuous with the ventral head skin without any differentiation into a skin fold. The skin fold otherwise occurring behind the lower lip is contained in a groove when the mouth is closed, but is stretched flat when the mouth is opened.

The epithelia of the lips and associated structures show significant differences in their thickness, which is summarized in Table 2.

Upper lip

The free surface of the upper lip is thrown into characteristic ridges, separated by grooves. The ridges are, in general, broad and elongated somewhat irregular in outline, and in cross section give a papillate appearance to the lip. The upper lip epithelium is stratified and may be divided into three

Table 1. A summary of histochemical techniques used to locate and differentiate chemical moieties in the epithelia of the lips and associated structures of Rita rita. —, negative.

Histochemical techniques	Reaction	Chemical constituents
No treatment/Schiff	Magenta	Free aldehydes
Periodic acid/Schiff (PAS)	Magenta	Polysaccharides
Acetylation/PAS	_	
Deacetylation/PAS	Magenta	
Diastase/PAS	-	Glycogen
	Magenta	Mucopolysaccharides
Toluidine blue	Metachromasia	Acid mucopolysaccharides
Alcian blue (AB) pH 2.5	Greenish blue	Acid mucopolysaccharides
High temperature methylation/AB pH 2.5		
High temperature methylation/ saponification/AB pH 2.5	Greenish blue	Non-sulphated acid mucopolysaccharides
Mild methylation/AB pH 2.5	Greenish blue	Sulphated acid mucopolysaccharides
Alcian blue pH 1.0	Greenish blue	Sulphated acid mucopolysaccharides
Alcian blue pH 2.5/PAS	Magenta	Neutral mucopolysaccharides
	Greenish blue	Acid mucopolysaccharides
	Purple	Mixed acid and neutral mucopolysaccharides
Mercury-bromphenol blue	Blue	General proteins
Acid solochrome cyanine	Red	Basic proteins
	Blue	Nuclear proteins
Ninhydrin/Schiff	Purple	Protein bound -NH ₂ groups
Deamination/ninhydrin/Schiff		
p-dimethylamino benzaldehyde (DMAB)-nitrite Iodination/DMAB-nitrite	Blue	Tryptophan
Millon reaction	Red	Tyrosine
Iodination/Millon reaction	rcu	Tyrosine
Dihydroxy dinaphthyl disulphide (DDD)	Blue or Red	Cysteine bound sulphydryl (-SH) groups
N-ethyl maleimide/DDD	— Mide of Red	Cysteine bound surphydryr (-311) groups
Iodine oxidation/DDD	_	
Performic acid/alcian blue (PFA/AB)	Blue	Cystine bound disulphide (-SS) groups
Thioglycollate reduction/PFA/AB	Diuc —	Cystille bound disaiplinde (-55) groups
Sakaguchi reaction	— Red	Argining
Phenylglyoxal/Sakaguchi reaction		Arginine



Fig. 2. Photomontage of cross sections showing the disposition of the upper lip (UL) and associated structures, i.e. rostral cap (RC) in *Rita rita* (HE Stain). Scale line, 150 μ m.

principal layers, viz. the superficial layer, the middle layer and the basal layer. It consists mainly of epithelial cells, mucous cells, club cells and lymphocytes. In addition, sensory structures—taste buds and ampullary organs—are present.

Epithelial cells. The superficial layer epithelial cells appear polygonal, often being flattened or somewhat rectangular (Fig. 4). At intervals, these cells exfoliate at the surface in sheets (Fig. 5). The middle layer epithelial cells are arranged in several layers. They often appear vertically elongated in the

deeper layers and gradually acquire a polygonal, somewhat vertically compressed shape towards the surface (Fig. 4). The basal layer epithelial cells are columnar and are arranged in a single layer on a thin non-cellular basement membrane (Fig. 6).

The nuclei of the epithelial cells, in general, are centrally positioned, being elongated or flattened and have distinct chromatin material and nucleoli. They stain moderately blue with HE, PS and acid solochrome cyanine R methods, blue black with VHE, and light magenta with Feulgen reaction for DNA.

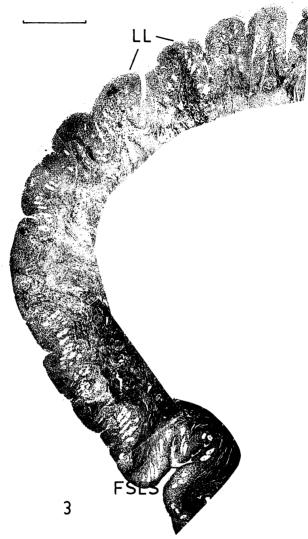


Fig. 3. Photomontage of cross sections showing the disposition of the lower lip (LL) and associated structures, the skin fold between LL and the ventral head skin (FSLS) in *Rita rita* (HE stain). Scale line, $150 \,\mu\text{m}$.

Table 2. Thickness (mean ±SD) of the epithelia of the lips and associated structures in *Rita rita* (approx. 160 mm in length)

Lips and associated structures	Epithelial thickness (µm)
Upper lip	151.04±7.65
Rostral cap	210.45 ± 8.52
Lower lip	165.26 ± 7.39
Fold of skin between the lower li and the ventral head skin	ip 92.24±7.85

The nuclei of the exfoliating epithelial cells, in contrast, appear small and rounded, being pyknotic with indistinct, compact chromatin material and nucleoli (Fig. 5). They stain strongly.

The cytoplasm of the epithelial cells throughout the epithelium, appears homogeneous and slightly eosinophilic, stains moderately for general proteins and shows weak reactions for basic proteins, that is, for protein bound $-NH_2$ group, tyrosine and tryptophan. The epithelial cells in the superficial layer, and in the underlying one or two layers show mod-

erate reactions for neutral mucopolysaccharides (Fig. 7). The reactions are either weak or absent in the basal and middle layer epithelial cells. At the narrow, free margins of the superficial layer epithelial cells, the intensity of the reactions, both for protein end groups and neutral mucopolysaccharides, is relatively strong (Fig. 7).

Mucous cells. The mucous cells are located mainly between the epithelial cells towards the surface of the epithelium and void their secretions at the surface through narrow pores. They are of two types-elongate mucous cells and goblet mucous cells. The elongate mucous cells appear very long and slender, often with swollen apical parts and in the epithelium are confined mainly to the ridges of the lip (Fig. 7). In HE, PS and VHE, although the swollen apical parts of these cells may be located with some difficulty, the slender proximal parts, in general, can not be distinguished. In PAS and AB/ PAS, however, these cells are easily located. The goblet mucous cells appear goblet-shaped or rounded and often lie close to each other mainly in the epithelium lining the grooves. The nuclei of the mucous cells are basal and stain similarly to those of the epithelial cells.

The secretory contents of the two types of mucous cells appear slightly basophilic, do not stain for protein end groups, and in general, show strong reactions for a mixture of acidic (both sulphated and non-sulphated) and neutral mucopolysaccharides. In addition, a few elongate mucous cells secrete only neutral mucopolysaccharides.

Club cells. The club cells are generally few in number and are often located in the middle layer. They are spherical, oval or vertically elongated and bi-nucleated. The nuclei are central, often shrunken, with compact chromatin material and indistinct nucleoli (Fig. 4), and stain similarly to those of the epithelial cells.

The contents of these cells appear homogeneous and exhibit variable degree of shrinkage and vacuolization in different fixatives. They are slightly eosinophilic, do not stain for carbohydrates, stain strongly for general proteins, moderately for basic proteins and protein bound $-NH_2$ groups and weakly for tyrosine. The boundaries of these cells, in addition, show weak reactions for tryptophan.

Lymphocytes. Lymphocytes enclosed within characteristic lymphatic spaces are observed in large numbers mainly between the basal layer and lower middle layer epithelial cells (Fig. 6). A few such

cells may be observed between the outer middle layer epithelial cells.

The lymphocytes are rounded, oval or irregular in outline, each having an off-centered nucleus with dense, compact chromatin material, a nucleolus and a thin rim of cytoplasm which may be thrown into pseudopodia-like processes. The cytoplasm stains light blue in HE and PS. The nuclei stain deep blue in HE and PS, acid solochrome cyanine R, black in VHE and show strong reactions with Feulgen reaction for DNA. In addition, both the cytoplasm and the nuclei stain strongly for general proteins.

Taste buds. The taste buds are pear-shaped and are distributed in large numbers, being located either singly or in groups of 2 or 3, mainly in the outer layers of the epithelium, often projecting slightly at the surface (Fig. 8). They are supported by prominent papillae from the sub-epithelial tissue through which they get their nerve and blood supply. The papillae often traverse the entire thickness of the epithelium to reach the taste bud situated near the surface of the latter. The neuroepithelial cells and surrounding supporting cells are not differentiated from each other. They are slightly eosinophilic and show moderate reactions for neutral mucopolysaccharides and general proteins, and weak reactions for basic proteins, protein bound -NH₂ groups, tyrosine and tryptophan.

Ampullary organs. Ampullary organs are few, being restricted mainly to the outer layers of the epithelium. Like the taste buds they are supported by prominent papillae from the sub-epithelial tissue, often traversing the entire thickness of the epithelium, through which they get their nerve and blood supply. Ampullary organs consist of a swollen receptor cavity, the ampulla and a narrow canal, which opens at the surface by a pore (Figs. 9, 10). The basal part of the ampulla is lined by pear-shaped, neuro-epithelial cells surrounded by supporting cells. The ampullary canal is filled with a mucus secretion showing strong reactions for neutral mucopolysaccharides (Fig. 10). The neuro-epithelial cells and supporting cells stain similarly to the taste buds.

Rostral cap, lower lip and skin fold

The epithelia of the rostral cap, lower lip and skin fold between the lower lip and the ventral head skin, in general, resemble the upper lip epithelium in their structural organization and the histochemical nature of their cellular components.

In contrast, the rostral cap epithelium is not thrown into characteristic ridges and grooves, and its free surface appears smooth. It is equipped with a large number of voluminous club cells, arranged in 2 or 3 rows (Fig. 2) and only one type of mucous cells—the goblet mucous cells—are discernible. They are small, relatively few and are located mainly in the outer layers (Fig. 11). Relatively speaking, taste buds are few, whereas ampullary organs are in large numbers. In the lower lip, the ridges at the surface are more prominent and separated by deeper clefts than those of the upper lip. The clefts are often branched in the deeper layers of the epithelium and are invariably lined by a high number of profusely secreting mucous goblet cells (Fig. 12). In the epithelium of the skin fold between the lower lip and the ventral head skin, elongated mucous cells are absent, goblet mucous cells only being present. Club cells are relatively few and small, and both taste buds and ampullary organs are absent.

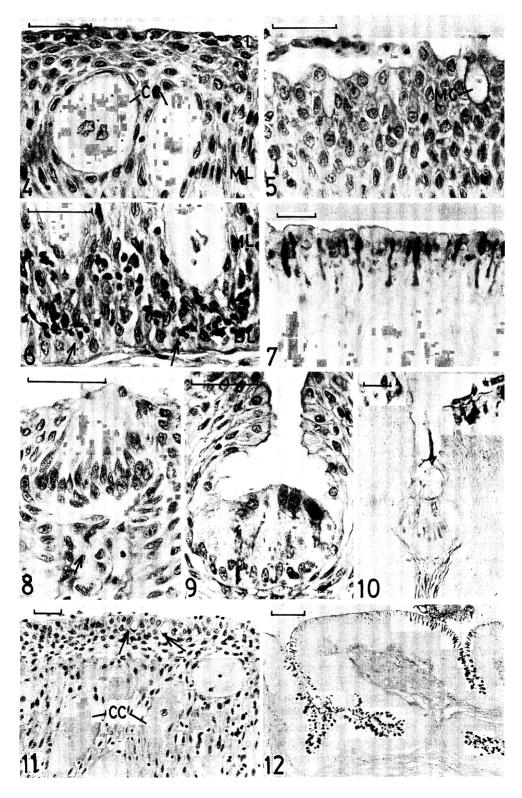
Discussion

The epithelia of the lips, rostral cap and skin fold of the Rita rita reflect adaptive features which are primarily significant with respect to food preference and feeding behaviour. The species feeds mainly on water insects, crustaceans, blood worms and limpets (Khanna, 1962) and has the habit of searching for food amongst bottom debris.

In general, the superficial layer epithelial cells in the epithelia of the lips and associated structures are involved in the secretion of mucopolysaccharides, which may result in the formation of an extracellular, cuticular coat, glycocalyx or fuzz at the surface, as in fish epidermis (Schliwa, 1975; Schwerdtfeger and Bereiter-Hahn, 1977; Whitear and Mittal, 1986; Whitear, 1986). At intervals, exfoliation of the epithelial cells occurs as the cells age and die. Agrawal and Mittal (1991, in press) also observed a similar condition in the lip epithelia of *Catla catla* and *Cirrhina mrigala*. Previous workers have made no reference to such an exfoliation of superficial layer epithelial cells.

Characteristic ridges on the surface of the lips in *Rita rita* may be regarded as assisting the fish in its characteristic food procuring movements. The papillae, developed from the subepithelial tissues, in

- Fig. 4. Cross section of the upper lip epithelium showing the elongated epithelial cells in deeper middle layers (ML), that become polygonal or flattened in the outer layers. The surface epithelial cells (SL) are flattened and voluminous club cells (CC) can be seen between the epithelial cells. Note a club cell with two nuclei (HE stain). Scale line, $10 \, \mu \text{m}$.
- Fig. 5. Cross section of the upper lip epithelium showing epithelial cells with pyknotic nuclei exfoliating at the surface in the form of a sheet. Note lightly stained mucous cells (MC) between the superficial layer epithelial cells (HE stain). Scale line, $10 \,\mu m$.
- Fig. 6. Cross section of the upper lip epithelium, showing a large number of lymphocytes (arrows) between the basal cells (BL) (HE stain). Scale line, $10 \mu m$.
- Fig. 7. Cross section of the upper epithelium showing a mixture of neutral and acidic mucopolysaccharides (deep purple in original) in the mucous cells. The epithelial cells in the outer regions of the epithelium show strong reactions for neutral mucopolysaccharides (magenta in original). Note the underlying epithelial cells and the club cells which remain unstained (AB/PAS). Scale line, $10 \,\mu m$.
- Fig. 8. Cross section of the upper lip epithelium showing a pear-shaped taste bud in the epithelium supported by a papilla (arrow) from the underlying tissues (HE stain). Scale line, 10 µm.
- Fig. 9. Cross section of the upper lip epithelium, showing an ampullary organ. Note the presence of neuro-epithelial sensory cells, supporting cells at the base of the ampulla and a narrow lumen leading to the surface (HE stain). Scale line, $10 \, \mu \text{m}$.
- Fig. 10. Cross section of the upper lip epithelium, showing the lumen of the ampullary organ filled with mucous secretion stained strongly for neutral mucopolysaccharides (magenta in original). Note a moderate reaction (deep pink in original) in the neuroepithelial sensory cells and supporting cells (AB/PAS stain). Scale line, 10 μm.
- Fig. 11. Cross section of the rostral cap epithelium, showing a part of the epithelium. Note a few, small mucous cells (arrows) in the superficial layer and voluminous club cells (CC) in the deeper layers (HE stain). Scale line, $10 \,\mu\text{m}$.
- Fig. 12. Cross section of the lower lip epithelium, showing deep clefts in the epithelium. The mucous cells lining the grooves secrete profusely and stain strongly for a mixture of neutral and acidic mucopolysaccharides (deep purple in original) (AB/PAS stain). Scale line, 10 µm.



association with such ridges may furnish a definite shape and firmness to the latter. Profuse mucus secretion in the grooves between the ridges, that has remarkable ability to precipitate mud held in suspension (Hora, 1934), may prevent the grooves from being choked by mud and bottom debris, thus enabling the free movement of the ridges. In addition, lubrication provided by mucous, which has been reported to reduce water friction around the body of the fish (Rosen and Cornford, 1971), may also reduce friction which is likely to be generated during movement of the ridges.

The mucus secreted by the epithelia of the lips and associated structures in Rita rita may have diverse functions, as in fish skin (Mittal and Banerjee, 1980; Imaki and Chavin, 1984). Lubrication provided by mucus may protect the lip epithelia from mechanical injury, to which these are highly vulnerable during searching for and catching of prey from bottom debris, a characteristic feeding behaviour of the fish. The role of mucus in protection against physical abrasion has also been suggested in relation to the general body surface of fishes specially during burrowing (Liem, 1967; Mittal and Munshi, 1971). In Catla catla, a surface plankton feeder, in which the epithelia of the lips are not often prone to abrasion during feeding, mucous cells have been reported to be few or even absent at some locations (Agrawal and Mittal, 1991).

The epithelia of the lips and rostral cap in *Rita rita* are thick, which may be an adaptation to their mucogenic nature, and provide additional protection when feeding. In *Cirrhina mrigala*, an omnivorous fish which also has the habit of procuring food from bottom debris, the epithelia of the horny lower jaw sheath and on the ventral surface of the upper lip are contrastingly thin where these are keratinized as an adaptation to withstand various traumatic conditions. In this fish the epithelia of the rostral cap, the dorsal side of the upper lip and the lower lip protuberances are, however, thick and mucogenic (Agrawal and Mittal, in press).

In *Rita rita*, mucous cells show significant differences in their shape as well as in their histochemical reactions at different locations. They are of two types—elongate and goblet-shaped on the lips, and goblet-shaped only on the rostral cap and skin fold. Furthermore, although most mucous cells secrete mixed acid and neutral mucopolysaccharides, a few elongate mucous cells secrete only neutral mucopolysaccharides. In *Cirrhina mrigala* too, though

most mucous cells appear goblet-shaped and secrete mixed acid and neutral mucopolysaccharides at different locations on the lips and associated structures, those cells on the dorsal side of the upper lip secrete only acid mucopolysaccharides (Agrawal and Mittal, in press). No firm conclusions can be drawn, at this stage, with respect to the significance (if any) of the differences in shape and histochemical nature of the mucous cells. Studies on the epidermis of fishes have also shown great variations in the shape as well as in the histochemical nature of the secretions of mucous cells, suggesting that taxonomy is no guide to the type of mucus secreted. Such secretions may change at different life history stages and also in response to changing environmental conditions (Whitear, 1986).

Although most elongate mucous cells in the epithelia of the lip of *Rita rita* are often indistinguishable from adjacent epithelial cells using histological techniques alone e.g. HE, PS and VHE, they are invariably visualized with histochemical techniques e.g. PAS and AB/PAS. The use of histochemical techniques may thus be emphasised as an aid for location of mucous cells. This becomes significant as previous workers (Girgis, 1952; Pasha, 1964; Kapoor et al., 1975; Sinha and Moitra, 1978; Agrawal and Mittal, 1991) described the distribution of mucous cells without using histochemical techniques.

The club cells, as in fish epidermis (Mittal, 1968; Singh and Mittal, 1990), appear binucleated in the epithelia of the lips and associated structures of *Rita rita*, unlike those in the carps, *Catla catla* and *Cirrhina mrigala*, in which they are uninucleated (Agrawal and Mittal, 1991, in press). The contents of these cells in *Rita rita* show protein reactions, which may be due to helical filaments reported electron microscopically from the epidermis of several fishes (Whitear and Mittal, 1983). Functionally, they are mainly protective and have been associated with the secretion of pheromones (Pfeiffer, 1974, 1982) and hemagglutinin (Suzuki, 1985; Suzuki and Kaneko, 1986).

The taste buds, primarily gustatory in function (Hara, 1971), on the lips and rostral cap of *Rita rita* may serve to enable accurate localization of prey and the trigger of a pick-up reflex. This may be considered as an adaptation to the sluggish, feeding habit of the fish, compensating for its reduced eyes as well as the restricted visibility in the foul, turbid water which it inhabits. In the elopid *Megalops cyprinoides*, an active carnivorous fish having well developed

eyes, taste buds on lips are absent (Pasha, 1964). In Ictalurus natalis, extreme development of taste buds all over the body, including the lips, is considered to be an adaptation owing to its reduced vision (Herrick, 1904; Atema, 1971). Schemmel (1967) has shown that in the cave-dwelling Mexican characin. Astyanax mexicanus, which has lost its vision, the distribution of external taste buds is more extensive than in the river-dwelling Astyanax mexicanus, which has normal vision. In the cyprinid Pseudorasbora parva, which has the habit to swimming along the bottom of shallow waters, grazing or picking up small organisms such as worms, the lips are equipped with a high density of taste buds (Kiyohara et al., 1980). Presence of taste buds in the epithelia of the rostral cap and often in groups of 3 or 4 at the apical region of the upper and lower lips in the cyprinid Cirrhina mrigala has been associated with the acute sense of taste necessary for this fish which has the habit of scooping pond mud in its search for food (Agrawal and Mittal, in press).

The ampullary organs are considered to be electro-receptors, primarily to detect signals of external origin (Bennett, 1971; Bullock, 1974; Szabo, 1974; Szabo and Fassard, 1974; Srivastava and Seal, 1981). The presence of these structures on the lips and rostral cap in *Rita rita* suggests their playing a significant role in complementing the taste buds in the location of food. Previous workers have not reported the presence of ampullary organs on the lips or associated structures of fishes.

The folds of skin between the lateral regions of the lower lip and the ventral head skin in *Rita rita*, which has non-protrusible jaws, may act to facilitate an increase in the gape when feeding. Folds of skin have also been reported between the upper lip and rostral cap as well as between the lower lip and ventral head skin in *Catla catla* and *Cirrhina mrigala*, and facilitate protrusion of the jaws during feeding (Agrawal and Mittal, 1991, in press). Taste buds and ampullary organs are not located on the skin fold, as they have little advantage in this region, which often remains concealed. In *Catla catla* and *Cirrhina mrigala* (Agrawal and Mittal, 1991, in press) also, such structures are not reported on the skin folds.

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インド産ナマズの1種 Rita rita の唇上皮とその付随構造の形態ならびに組織化学

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ナマズの1種 Rita rita の上唇は、吻冠と皮膚褶を備えた下唇 と連結している。 吻冠および下唇の上皮は、本種の特異な摂食行 動との関わりで変化しており、粘液原性である。粘液細胞のほか に表層上皮細胞も活発な粘液多糖類の分泌を行う. 粘液は, 魚類 表皮における多様な機能とは別に,底性砕片中から餌を探査捕 獲する際に受ける機械的傷害から上皮を守るために、上皮をな めらかにする. 上皮は、その粘液産生性や防御性を備えるため に,適応して厚くなっている. 棍棒細胞の内容物は,他魚類の表 皮で報告されているように、らせん状細糸と関連してたん白性 である. 唇表面にある特徴的な隆起は、食物摂取動作を助けるた めに適応したものである. 隆起間の溝へ分泌される粘液は, 円滑 な運動をするために、摩擦を減少させ、異物の沈着を防ぐ、皮膚 褶は,口を大きく開くことに役立つ.両唇と触冠にある味蕾およ び瓶器は、餌の位置を定め、摂取反射を引き起こすことに関与す るが、下唇と腹側頭皮との間にかくれている皮膚褶には存在し ない. 組織学的研究補強のための組織化学的分析の重要性が強 調された.