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Distribution of Gustatory System in the lips of Spotted Snakehead, *Channa punctatus* (Bloch 1793) and Spiny Eel, *Mastacembelus pancalus* (Hamilton 1822) from India

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Abstract: The gustatory system of fish provides the final sensory evaluation in the feeding process. The primary organ of this gustatory system of fish is taste bud. Gustatory cells are comprised of group of secondary receptor cells, which are specialized epithelial cells that form synapses with gustatory nerve fibers. To determine the distribution and architecture of External Taste buds (TBs) along with others cells of the gustatory system in the lips, two specimens each of freshwater spiny eel, *Mastacembelus pancalus* and spotted snakehead, *Channa punctatus*, were studied. The external surface morphology and cellular distribution in both the lips in two fishes were explored by light microscope (LM), scanning electron microscope (SEM) and transmission electron microscope (TEM). All Microscopic analysis reveal that, mainly the Type-II and type III TBs, are prevalent in these fishes *Mastacembelus pancalus* (family - Mastacembelidae) and *Channa punctatus* (family - Channidae) while a special type of Type-III TBs mostly found in Channidae family (*Channa punctatus*). The upper and lower lips of *Channa punctatus* are associated with microridges, lacking in former one. The mucous cells, club cell, pigment cells and lymphocytes are observed together with TB in both species. The finding indicates that both fishes have different ecological station so there is differential distribution of TB and other gustatory cells in the lips of these two species. It can be postulated that, these differences in TB density and scenario among two species may be connected to differences in their foraging strategies in their microhabitats as well as environmental plasticity throughout their ontogeny in both species and family levels.

Key words: Teleost · Gustatory System · Histology · Taste Buds

INTRODUCTION

Teleost are reported to have the most taste buds (TBs) of all vertebrates [1]. Vision has prime role in the exploration of food and the preliminary evaluation of the edibility of prey items [2]. Gustatory system in the fish is a polysensory establishment and provides the final sensory evaluation in the feeding process [3]. The gustatory system in fishes is divided into two distinct subsystems, namely oral and extra oral [4]. The nonvisual organs of senses i.e. external gustatory reception thought to play a far better role in the hunting and detection of prey in bottom and near-bottom fish, as well as in fish leading a crepuscular or nocturnal mode of life or inhabiting large depths, underground water bodies, caves, etc. [5]. In fishes, irrespective of their mode of life and feeding strategy, the ultimate phase of the feeding performance is based on the function of the TBs in external gustatory system as they detect distinct chemical substances at a short distance [6]. TBs are comprised of group of 30 to 100 "secondary" receptor cells, which are specialized epithelial cells that form synapses with gustatory nerve fibers [7]. TBs are found with highest densities at the lips, the gular region, barbels and along with pectoral and pelvic fins [8]. Three types of TBs in fish are grouped on the basis of mensural variation, nature of protrusion above epithelial surface and their sensory processes [9,10]. Ultrastructural observation shows that

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the TBI is frequently situated on the dome of the epidermal papillae whereas TBII is slightly elevated and both might function as a mechanoreceptor as well as chemoreceptor. TBIII is essentially chemoreceptor and is of sunken type in nature [11].

Studies on densities and distribution pattern of cells of gustatory system especially in lips are infrequent [7, 12]. Even less abundant are comparative investigations [13, 14]. Agrawal & Mittal [15-18] and Mittal & Agrawal [19] reviewed and described the structural organization and histochemistry of epithelia of the lips and associated structures of several freshwater fishes *Catla catla, Labeo rohita, Cirrhina mrigala*, *Rita rita and Channa striata*. Pinky *et al*. [20] described the structures associated with lips of an Indian hill stream fish *Garra lamta* and later Tripathi and Mittal [21] described the keratinization in lips and associated structures of a fresh water fish, *Puntius sophore* in relation to its feeding ecology.

The aim of the present study is to compare the shape, number and distribution pattern of external TBs along with surrounding cellular structure in the lips of *Channa punctatus* and *Mastacembelus pancalus* belonging two separate families and exploiting proximate ecological niches. The hypothesis of the present study is that the TBs of the gustatory system of both the lips may show basic morphological similarities in each species but may differ in different species based on the strategy of resource utilization. The present paper describes the morphology of the gustatory system, distribution of cell types in taste buds in the lips of *Channa punctatus* (Bloch 1793) and *Mastacembelus pancalus* (Hamilton 1822) employing the resolving power of light microscope, scanning and transmission electron microscope.

MATERIALS AND METHODS

Live adult, sex-independent specimens of spotted snakehead, *Channa punctatus* (Bloch 1793) and spiny eel, *Mastacembelus pancalus* (Hamilton 1822) were collected from the ponds at Baruipur (22° 20' 58" N / 88° 26' 21" E), South-24 parganas, West Bengal, India during the premonsoon period. Fishes were identified in the laboratory by consulting taxonomic book. They were maintained in the laboratory conditions at controlled room temperature (25±2°C). Food was given *ad libitum* during their captivity on alternate days. The comparative account of two selected fishes was given in Table 1. Average length of the specimen was 7-8 cm and total weight was measured to the nearest of 2-4g using an electronic balance (Sartorius, Model No. BT 223S). After proper acclimatization skin fragments (approximately 3 x 5 mm) were cut from the lip area. Five fishes of each species were sacrificed. Both the lip tissues were fixed separately in Bouin-Hollande for light microscopy. After routine dehydration in ethanol and embedding in 58°C Paraffin, 6µm thick serial sections were prepared. The serial sections were stained in Delafield's haematoxylin and eosin. The observation of serial sections was made under Leitz and Olympus microscopes and photographs were taken in either 10 x 45 or 10 x 100 magnifications. For scanning electron microscopy tissues were dissected out and attached sediment and debris was cleaned by heparinized saline (heparin sodium salt 10000 IU mixed in 0.67% NaCl solution).Then tissues were fixed in Karonovsky's fixative (3% glutaraldehyde and 2% paraformaldehyde) for 12 hours at 25-27°C in 0.2M cacodylate buffer (pH 7.2). Samples were post fixed in 1% osmium tetraoxide for 1hour to gain better conductivity in the microscope. After the tissues were dehydrated in an ethanol-amyl acetate series the specimen were kept in 100% amyl acetate in 37°C for overnight. The tissues were dried in a critical point drier (POLARON–E–3000) using liquid CO_2 for 4 hours. Then tissues were attached to aluminum stubs and were coated with gold in a Sputter–Coater (POLARON–SC7620). Then examined under scanning electron microscope (Model: FE1–QUANTA 200) operated at 40 kV. For transmission electron microscopy, target tissues were fixed in Karnovsky's Fixative (3% glutaraldehyde and 2% Paraformaldehyde) for four hours at 4°C temperature in 0.1 M- cacodylate buffer (pH 7.2) These were post-osmicated in buffered 2% osmium tetroxide in distilled water for two hours at room temperature. After dehydration tissues were dehydrated and properly dried and finally embedded in araldite. The ultrathin sections were stained with 0.5% uranyl acetate and lead citrate and examined under transmission electron microscope (Morgagni 268D; Fei Company, The Netherlands) operated at 80 kV.

RESULTS

Channa punctatus

Light Microscopy

Upper Lip: In the epidermis epithelial cells are mostly polygonal in shape and at basal layer they are columnar, arranged in a single row resting on the basement membrane. Along with TBs, four types of epithelial cells (club cells, mucous cells, pigment cells and lymphocytes) are observed in the epithelium. Among them, the density distribution of mucous cells and club cells is 40-46% and 12-15% respectively. The club cells having centrally placed nucleus generally present in the outer layer and in the lower layer, lymphocytes are observed in good number enclosed within the irregular shaped lymphatic spaces (Fig. 1). The TBs are flask-shaped made of vertically elongated gustatory and supporting cells. In upper lips, Type III TBs are more frequent than TBIIs. The highest density of TBIIIs per unit area is average 52 TBs mm^{-2} but the density of the TBII is only 23 TBs mm^{-2} (Fig. 2). The average height of the TBs measured ranges from 60 to 95 µm. and the diameter at the widest part is between 25 and 65 µm. Neuromast cells are properly placed on the outward invagination of the basement membrane (Fig. 3). Type II TBs contains tall columnar sustentacular cells alternating with fusiform sensory cells and basal cells, surrounded by large mantle layer of epidermal cells. The cilium projects out through the gustatory pore to communicate with the exterior. Each gustatory cell has prominent nucleus in its enlarged end (Fig. 4).

Lower Lip: In lower lip, TBIII are present with profuse mucus cells (Fig. 5) or buried under the outer epithelial layer (Fig. 6). The density distribution of mucous cells and club cells is 20-22% and 10-12% respectively. Arrangement of other cellular composition is alike as upper lip (Fig. 7). The part of the dermis in both the lips composed of comparatively loosely arranged collagenous connective tissue fibers richly supplied with fine blood capillaries and myelinated nerves (Figs. 3 and 7). The stratified squamous epithelium with high mitotically active stratum germinativum is found to be covered by large polyhedral cells and mucus cells (Fig. 8).

Scanning Electron Microscopy

Upper Lip: The surface of the upper lip epithelium appear in folded wave like pattern with extensive network interrupted branched microridges, somewhere they lie parallel to each other (Fig. 9). Several openings of taste buds are along with globular mass of mucus signify the presence of goblet mucus cells in between the network of micro ridges (Fig.10).

Lower Lip: At low magnification, polyhedral epidermal plaques appear in regular mosaic outline and outermost ridges demarcated cell boundary (Fig.11). Each plaque had generally 4-7 prominent extensive microridges forming a whirl like pattern (Fig.12). The mucus cell apertures are present in between the whirl of the ridges of varied dimension. However, at higher magnification, microridges appear 0.8µm in height and maintain parallel arrangement in the center while more curved and branched at the periphery region. Microridges are uniform in width and equally spaced by furrows (0.2-0.4µm). Cell junctions observe in zipper like pattern separated by 0.1µm.Fine transverse connections interconnecting the adjoining microridges are called micro bridges (≅0.06µm) and the arrangement of interlocking micro bridges form an intricate maze like pattern (Fig13). A decisive majority of TBIIIs are with a so-called 'pore'(0.5µm) which is sensory zone of the TBIII is usually situated in a depression (Fig.14).

Transmission Electron Microscopy

Upper Lip: Numerous gustatory cells of atypical structure have been distinguished with electron dense cytoplasm matrix. Cytoplasm contains parallel arranged mitochondria with sparse cristae, Golgi complex and abundance of RER oriented in between the mitochondria (Fig.15). Tubular long mitochondria are extended from the basement membrane to the outer epithelium, creating a compact sheath. The width of the mitochondrial body is 0.25µm and length approximately1.5µm (Fig.16). Small microvilli are found many in number (Fig.17). Electron dense vesicles are also scatter in the sensory epithelium (Fig.18). Lower lip: The gustatory cell (may be light) cytoplasm provided with huge RER in the form of flattened vesicles or ring like structure near the apex of the cell or at the supranuclear position and rich in free ribosome. Small vesicles and fine microfilaments are present in the cytoplasm (Fig.19). Scanty of free ribosome and mitochondria occur in the perinuclear position in the cytoplasm (Fig. 20). Desmosomal connections are found in between the electron dense supporting cells (Fig. 21). The supporting cells are vertically elongated cells spanning from the base to the apex of the bud (Fig. 22). Basal cells are present in all TBs in the *Channa*, regardless of their location. The synaptic vesicles are of 40-75 nm in diameter (Fig. 23). Stacks of rER and Golgi complex are also present in this pre-synaptic cleft area (Figs. 24 and 25).

Mastacembelus pancalus

Light Microscopy

Upper Lip: The cellular architecture is closely similar with *Channa punctatus* but TBIII are present in the invagination of the basal membrane.TBIII contain parallel arrangement of gustatory cells alternating with fusiform supporting cells (Fig. 26). TBIII includes cilium, projecting out of the gustatory pore to outer epithelial layer (Fig. 27). TBII is mainly present in the dorsal aspect of the cresentic cleft in upper lip surrounded by profuse mucus cells (Fig. 28) whereas in the ventral side, large elongated club cells and goblet cells with finger like projections are found to be occupied the epithelial layer (Fig. 29).

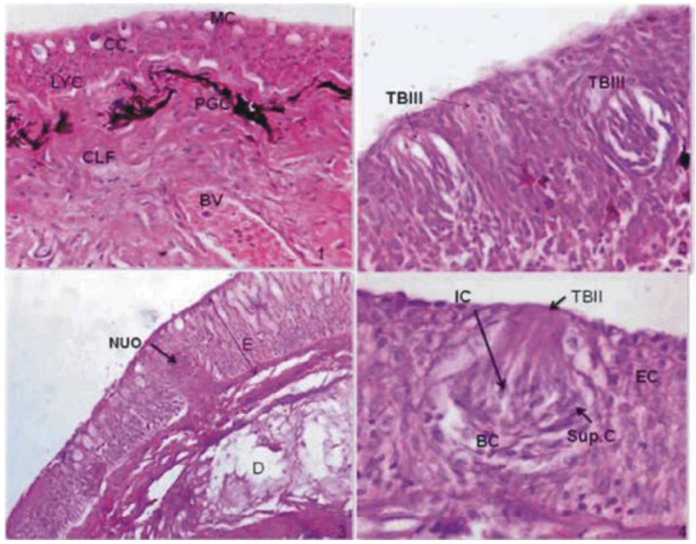

Fig. 1: A photomicrograph of a vertical section at the skin of upper lip of *Channa punctatus* showing thick epidermis of stratified squamous epithelium with club cells (CC), mucous cells (MC), aggregated pigment cells (PGC) and lymphocytes(LYC) and dermis with collagen fibres(CLF) and blood vessels (BV) (H&E,10X)

Fig. 2: A photomicrograph of a vertical section at the skin of upper lip of *Channa punctatus* showing TBIII is placed underneath of epithelial layer (H&E, 40X)

Fig. 3: A photomicrograph of a vertical section at the skin of upper lip of *Channa punctatus* showing Neuromast organs (NUO) placed on a fibrous dermal fold (H&E, 40X)

Fig. 4: A photomicrograph of a vertical section at the skin of upper lip of *Channa punctatus* showing TBII is slightly elevated toward the exterior of epithelium with basal cells (BC), light cells (lC) and supportive cells (Sup.C) (H&E, 100X)

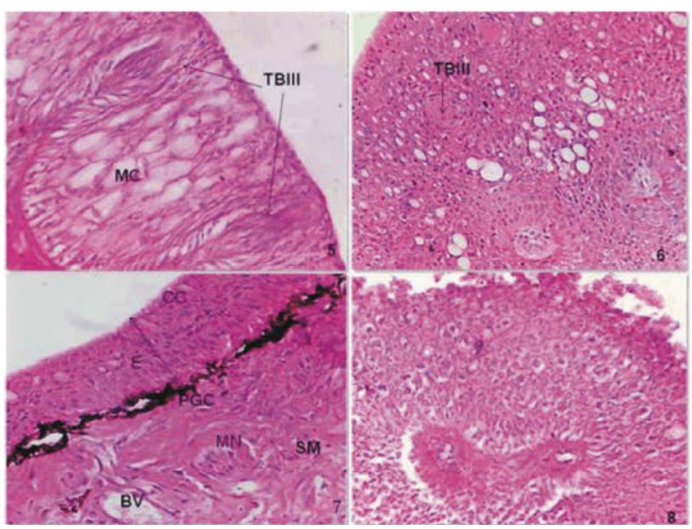

Fig. 5: A photomicrograph of a vertical section at the skin of lower lip of *Channa punctatus* showing stratified epithelium with TBIII and condensation of mucus cells(MC) through the middle and superficial cell layer (H&E, 40X)

Fig. 6: A photomicrograph of a vertical section at the skin of lower lip of *Channa punctatus* showing stratified epithelium with high frequency of mature mucus cells at the middle strata of the epidermis together with TBIII (H&E, 40X)

Fig. 7: A photomicrograph of a vertical section at the skin lower lip of *Channa punctatus* showing aggregated pigment cells (PGC) located at superficial dermal margin and dermis with smooth muscles (SM), blood vessels(BV) and myelinated nerve (MN) (H&E, 40X)

Fig. 8: A photomicrograph of a vertical section at the skin of lower lip of *Channa punctatus* showing of the stratum germinativum with epithelial cells distributed through the middle cell layer (H&E, 40X)

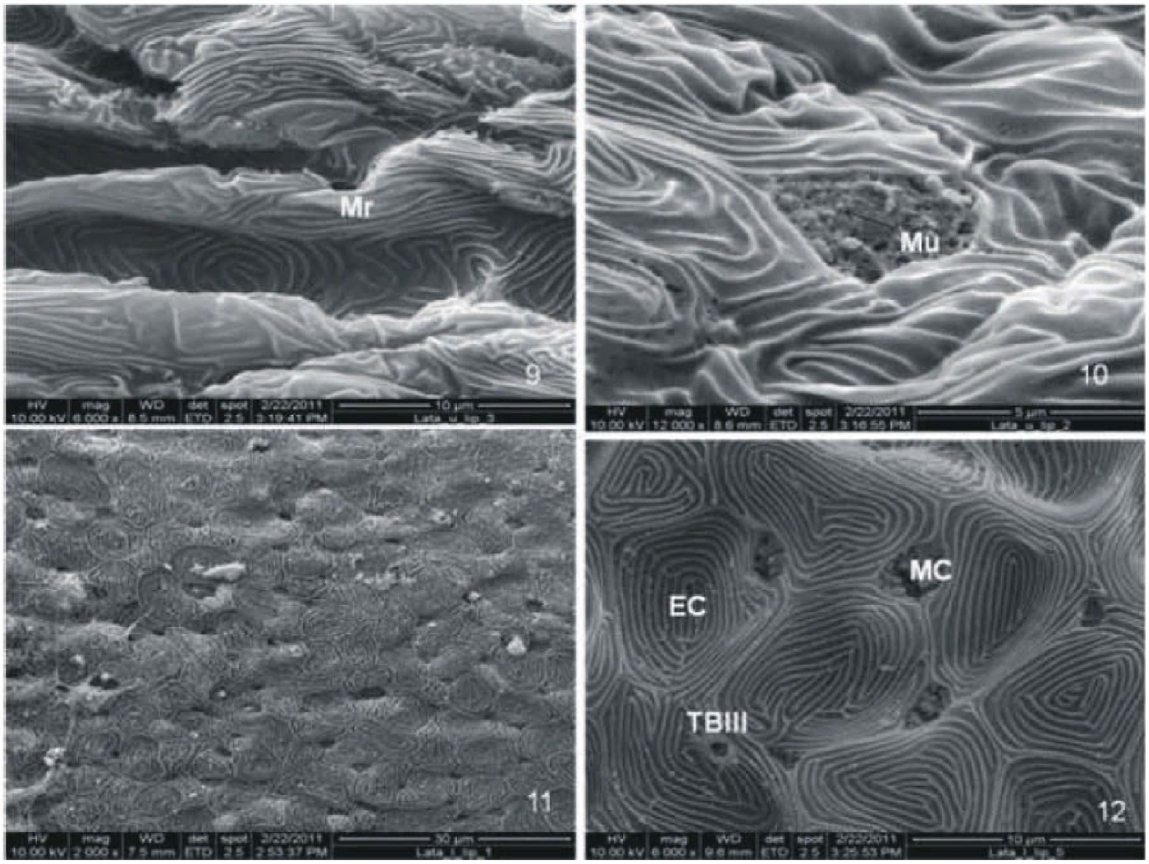

Fig. 9: Scanning electron micrograph of the upper lip of *Channa punctatus* showing wavelike pattern with extensive network of microridges and separated by distinct spaces

Fig. 10: Scanning electron micrograph of the upper lip of *Channa punctatus* showing small island of mucus cells with TBIII pore opening

Fig. 11: Scanning electron micrograph of the lower lip of *Channa punctatus* showing that adjacent epithelial cells appear as polyhedral epidermal plaques and demarcated by uniform well-defined uninterrupted double rows of microridges

Fig. 12: Scanning electron micrograph of the lower lip of *Channa punctatus* showing microridges forming a whirl like pattern and in between the whirl of the ridges varied dimension mucus cells and TBIII are seen

Lower Lip: Columnar epithelial cells are arranged in a multiple row resting on the basement membrane but in periphery mucous cells are regularly assembled in a row with density distribution of 82-87% (Fig. 30). Neuromast organ and few melanocytes happen only in the lower lip (Fig.31). Type II and III both are equally distributed in lower lip (Figs. 32 and 33) and the size of the TBs is about 50-55μmin height and about 20-25μm in width in average. TBIIIs are surrounded by mantle layer of the epidermal cells (Fig. 33).

Scanning Electron Microscopy

Upper Lip: The cresentic cleft of upper lip provided with type II Tbs, raised above the surrounding epithelium (100-200 μm) and formed dome shaped structure (Fig. 34). Papillate Tbs with taste pore are well individualized (Fig. 35). The distribution of the TBII density on the upper lip is up to 130/μm^2 (Fig. 36) and TBIII density in the ventral side of crescentric cleft is 92-98/μm^2(Fig. 37).

Lower Lip: The presence of mesh like structure with TBIII openings is prominent. The diameter of these openings is 0.3-0.6μm in average (Figs. 38, 39 and 40). At the edge of these openings, closely packed crimped edges are present. The lower lip is well revealed with numerous microvillus cells scattered in between the mucous cells. Mucus cells are present in the sensory epithelium with mucous droplets at their opening (Fig. 40).

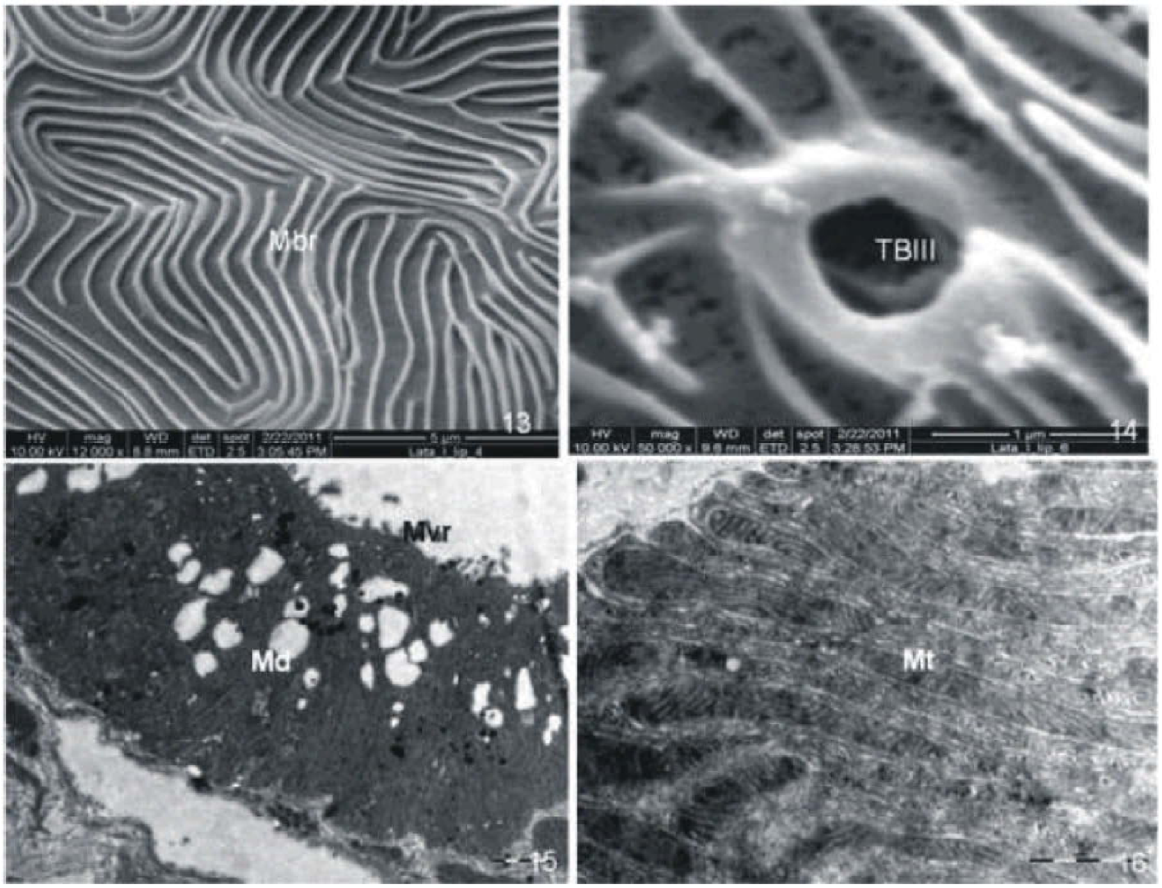

Fig. 13: Scanning electron micrograph of the lower lip of *Channa punctatus* showing fine transverse connections interconnecting microridges, micro bridges (Mbr)

Fig. 14: Scanning electron micrograph of the lower lip of *Channa punctatus* showing sensory zone of the TBIII i.e. taste pore usually situated in a depression

Fig. 15: Transmission electron micrograph of the upper lip of *Channa punctatus* showing electron dense cytoplasm matrix of gustatory cells with mitochondria, golgi complex and abundance of parallel arranged rough endoplasmic reticulum (RER), mucous droplets (Md) and small microvilli (Mvr)

Fig. 16: Transmission electron micrograph of the upper lip of *Channa punctatus* showing higher magnification of tubular mitochondria (Mt) with sparse christie.

Transmission Electron Microscopy

Upper Lip: The epithelial cells and mucus cells are arranged on a basement membrane (Fig. 41). The sensory cells have large nucleus with dense chromatin materials. Free ribosome and Golgi complex are present in cytoplasm, whereas spindle shaped mitochondria are present in perinuclear position (Figs. 42 and 43). The cell nuclei of the sustentacular cells have round oval in shape (Fig. 44). Mucus cells are characterized by abundance of rER, small secretory vesicles and Golgi complex scattered in the cytoplasm (Fig. 45). Electron dense core vesicles present in intercellular region and also in some cell cytoplasm (Fig. 46) and mitochondria with numerous cristae are also prominent (Fig. 47). Synapses between basal cells and light cells show small and irregular finger like dense projections from both side (Fig. 48). The synaptic membrane thickening is homogenous with 30–40 nm finger like projections and 20-25 nm dense-cored vesicles (Figs. 49 and 50). Membrane-bound vesicular bodies with approximately 40–50 nm in diameter locate occasionally between notches of the synaptic membranes (Fig. 51).

Lower lip has similar architecture as in upper lip. Cell types of Tbs sensory epithelium of upper and lower lip area of both the fishes are shown in the Table 2.

Table 1: Description of the comparative accounts of *Channa punctatus* and *Mastacembelus pancalus*

	Channa punctatus (Bloch 1793)	*Mastacembelus pancalus* (Hamilton 1822)
Systematic position of the species	Phylum: Chordata Class: Actinopterygii Order: Perciformes Family: Channidae	Phylum: Chordata Class: Actinopterygii Order: Perciformes Family: Mastacembelidae
Habit	Predaceous and highly carnivorous; piscivorous; feeding mainly on insects and small fishes; Air breather	Carnivorous, stenophagic, live beneath the mud; prefer submerged objects and bottom deposits; Air breather
Habitat	Freshwater; brackish; benthopelagic; potamodromous stagnant muddy swampy water	Bottom dweller-preferred muddy place; bottom feeder
Position of mouth	Mouth terminal and lower jaw longer than upper	Sub terminal or inferior in position bounded by upper and lower labial folds and surrounded by fine but firm jaws. Pointed, oblique and horizontal cresentic cleft. Snout long, tri lobed - fleshy appendage

Table 2: Cell types in the TBs sensory epithelium of upper and lower lip area in *Channa punctatus* and *Mastacembelus pancalus*

	Light Cells		Dense-core vesicle Cells		Dark Cells		Degenerating Cells		Basal Cells	
	C	M	C	M	C	M	C	M	C	M
Number per TB	21-26	20-23	0-1	1-3	30-35	25-30	0-2	0-1	0-2	0-2

(The numbers of TB cells are counted in five transversally cut TBs; Basal cells are counted in longitudinal sections of 10 TBs)

C = Channa punctatus M = Mastacembelus pancalus

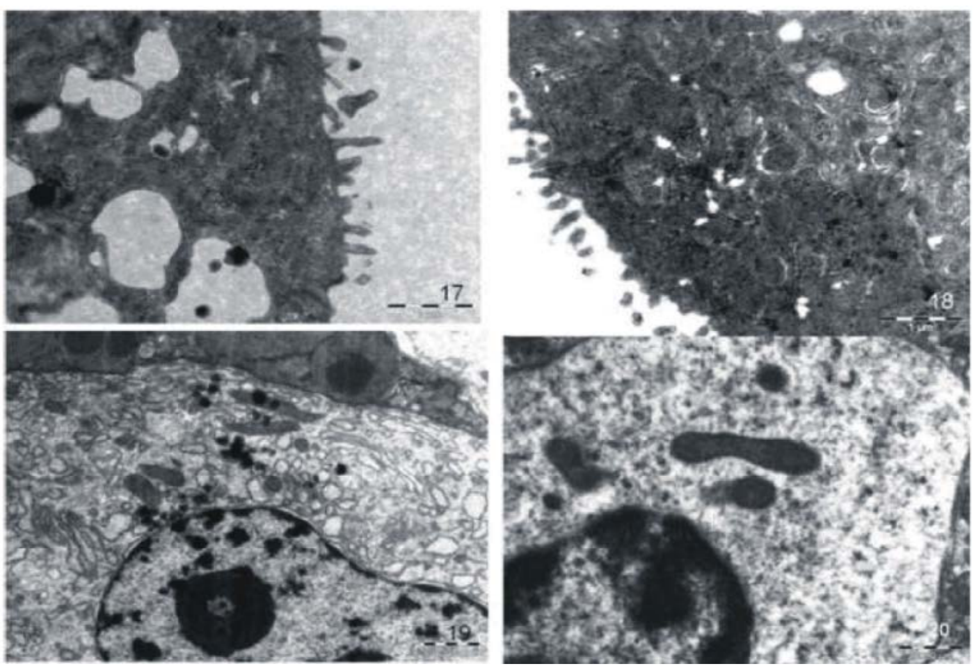

Fig. 17: Transmission electron micrograph of the upper lip of *Channa punctatus* showing outer epithelial layer occupied by mucous cells with similar degree of electron opacity

Fig. 18: Transmission electron micrograph of the upper lip of *Channa punctatus* showing electron dense vesicles scatter in the sensory epithelium

Fig. 19: Transmission electron micrograph of the lower lip of *Channa punctatus* showing cytoplasmic organelle structure and distribution of light cell

Fig. 20: Transmission electron micrograph of the lower lip of *Channa punctatus* showing free ribosome in cytoplasm and mitochondrial distribution of light cell

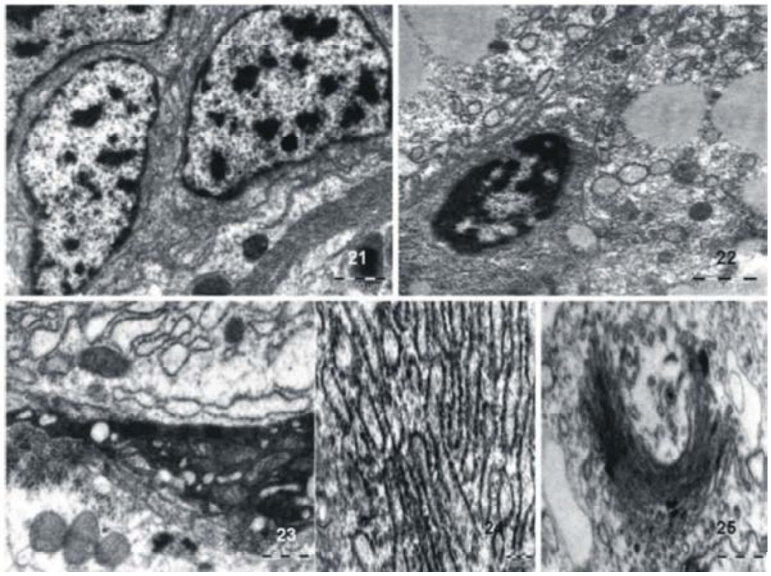

Fig. 21: Transmission electron micrograph of the lower lip of *Channa punctatus* showing desmosomal connections in between the electron dense supporting cells

Fig. 22: Transmission electron micrograph of the lower lip of *Channa punctatus* showing supporting cells with vertically elongated nucleus and other cytoplasmic organelles

Fig. 23: Transmission electron micrograph of the lower lip of *Channa punctatus* showing synaptic structure near the basal cell and nerve fibres

Fig. 24: Transmission electron micrograph of the lower lip of *Channa punctatus* showing stacks of rER in the synaptic area

Fig. 25: Transmission electron micrograph of the lower lip of *Channa punctatus* showing Golgi complex in the synaptic area

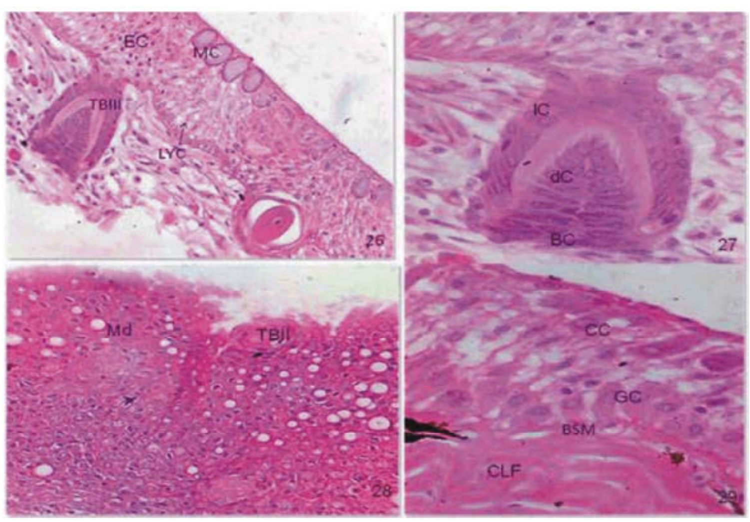

Fig. 26: A photomicrograph of a vertical section at the skin of upper lip of *Mastacembelus pancalus* showing structure of TBIII present in the invagination of the basal membrane and outer epithelium provided with mucus cells(MC) and lymphocytes(LYC) (H&E,10X).

Fig. 27: A photomicrograph of a vertical section at the skin of upper lip of *Mastacembelus pancalus* showing TBIII containing light cell (lC), dark cells (dC) and basal cells (BC) (H&E,100X).

Fig. 28: A photomicrograph of a vertical section of the dorsal aspect of cresentic cleft at the upper lip of *Mastacembelus pancalus* showing with profuse mucogenic layer and TBII (H&E, 40X).

Fig. 29: A photomicrograph of a vertical section of the ventral side of cresentic cleft at the upper lip of *Mastacembelus pancalus* showing large elongated club cells and goblet cells and dermis with collagen fibres(CLF)(H&E, 40X)

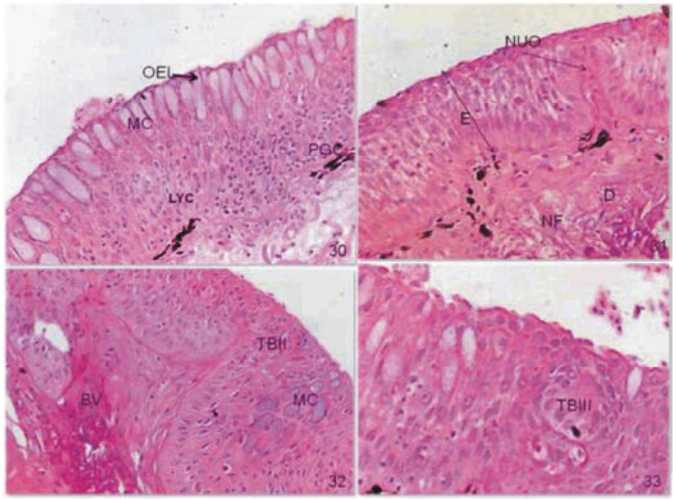

Fig. 30: A photomicrograph of a vertical section at the skin of lower lip of *Mastacembelus pancalus* showing stratified epithelium with high frequency of mucus cells arranged at the periphery (H&E, 40X)

Fig. 31: A photomicrograph of a vertical section at the skin of lower lip of *Mastacembelus pancalus* showing neuromast organs (NUO) placed on fibrous dermal fold and nerve fibres(NF) are noticed in the dermal area(H&E, 40X)

Fig. 32: A photomicrograph of a vertical section at the skin of lower lip of *Mastacembelus pancalus* showing TBII in the epithelial cell layer with the mucus cell(MC) layer(H&E, 40X)

Fig. 33: A photomicrograph of a vertical section at the skin of lower lip of *Mastacembelus pancalus* showing TBIII in the epithelial cell layer with the mucus cell(MC) layer(H&E, 40X)

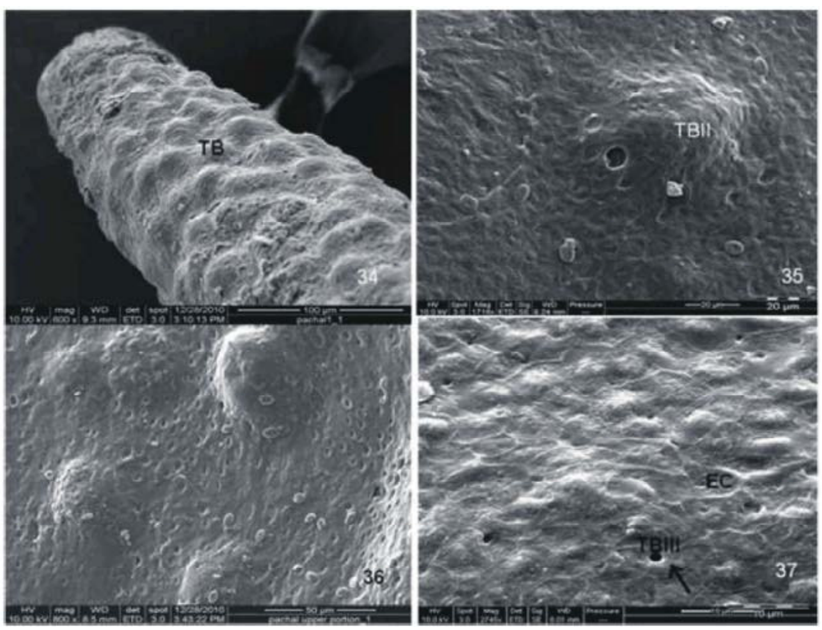

Fig. 34: Scanning electron micrograph of *Mastacembelus pancalus* showing cresentic cleft of upper lip provided with TBII, raised above the surrounding epithelium

Fig. 35: Scanning electron micrograph of the upper lip of *Mastacembelus pancalus* showing cresentic cleft of upper lip showing papillate TBIIs with prominent taste pore

Fig. 36: Scanning electron micrograph of the upper lip of *Mastacembelus pancalus* showing the distribution of the TBII density linearly arranged on the upper lip

Fig. 37: Scanning electron micrograph of the upper lip of *Mastacembelus pancalus* showing the distribution of TBIII density in the ventral side of cresentic cleft of upper lip

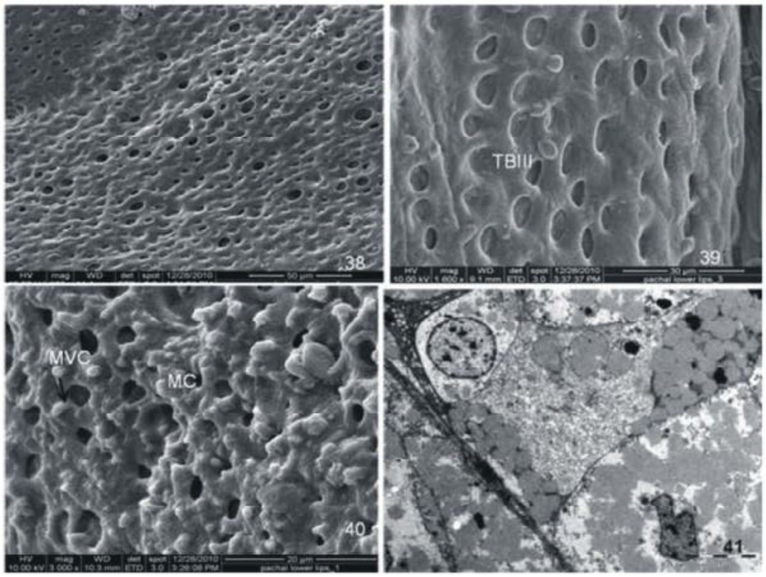

Fig. 38: Scanning electron micrograph of the lower lip of *Mastacembelus pancalus* showing presence of mesh like structure

Fig. 39: Scanning electron micrograph of the lower lip of *Mastacembelus pancalus* showing crimped edges at the boundary of TBIII openings.

Fig. 40: Scanning electron micrograph of the lower lip of *Mastacembelus pancalus* showing numerous microvillus cells (MVC) and mucus cells (MC) with TBIII in the sensory epithelium

Fig. 41: Transmission electron micrograph of upper lip of *Mastacembelus pancalus* showing epithelial cells and mucus cells are arranged on the basement membrane

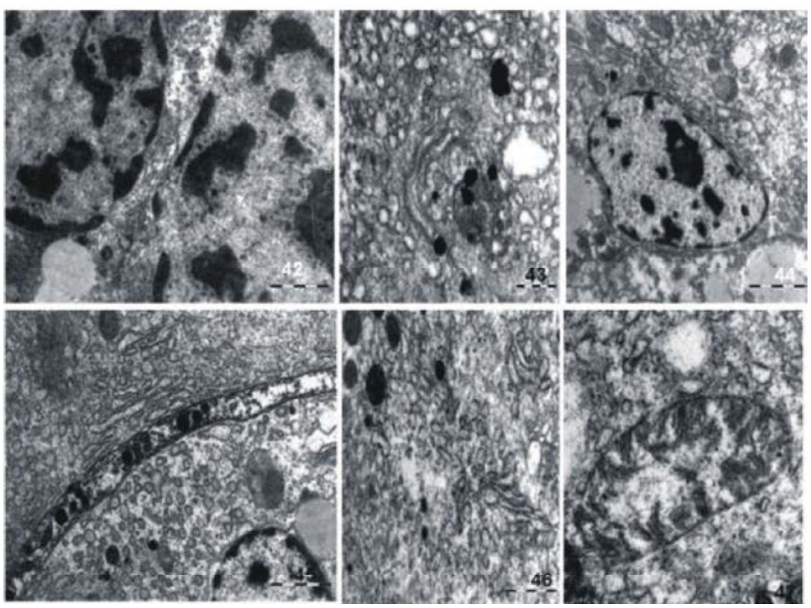

Fig. 42: Transmission electron micrograph of upper lip of *Mastacembelus pancalus* showing perinuclear zone of the sensory cells

Fig. 43: Transmission electron micrograph of upper lip of *Mastacembelus pancalus* showing concave face of golgi complex and vesicular body

Fig. 44: Transmission electron micrograph of upper lip of *Mastacembelus pancalus* showing nuclear characteristics of sustentacular cells

Fig. 45: Transmission electron micrograph of upper lip of *Mastacembelus pancalus* showing cellular pattern in mucus cell with huge RER and core vesicles

Fig. 46: Transmission electron micrograph of upper lip of *Mastacembelus pancalus* showing parallaly arranged RER and electron dense secretory vesicles

Fig. 47: Transmission electron micrograph of upper lip of *Mastacembelus pancalus* showing prominent mitochondria with cristae in the sensory cells

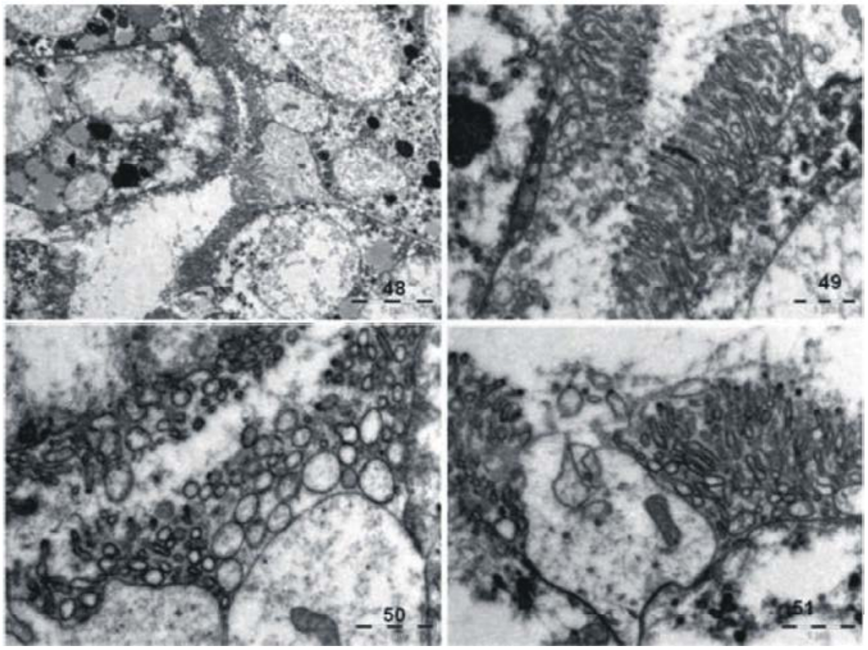

Fig. 48: Transmission electron micrograph of upper lip of *Mastacembelus pancalus* showing Synapses between basal cells and nerve fibres show small and irregularly arranged membrane-bound vesicular bodies and finger like dense projections

Fig. 49: Transmission electron micrograph of upper lip of *Mastacembelus pancalus* showing synaptic membrane thickening with 30–40 nm finger like projections

Fig. 50: Transmission electron micrograph of upper lip of *Mastacembelus pancalus* showing different shaped membrane bound vesicles in the nerve-cell junction

Fig. 51: Transmission electron micrograph of upper lip of *Mastacembelus pancalus* showing membrane-bound semi-circular bodies in the notches of the synaptic membranes

DISCUSSION

Among the teleost, the lip structure perform immense plasticity and structural adaptability for the exploitation of the diverse food items [22]. The lips of the fishes could contribute in accurate localization, capture, deglutition and predigestive preparation of food by triggering the pick-up reflex in analogy with the barbels of some fishes [23]. In terms of main structure, the pattern of TBs in the fish lips is resembled to that of other vertebrates. However they also contain some exclusive features [24]. In *Channa punctatus* and *Mastacembelus pancalus*, the TB II and TBIII are identified in the lip epithelium in high densities but the presence of TBI could not be well clarified. The development of keen gustatory function in these two fishes can be an adaptation to their specific mode of life. The presence of taste buds enhances the chance of perceiving and tracing accurate prey concealed by darkness or turbidity of habitat in which these fish live and may also permit the correct location of small food particles, which would be missed otherwise. This present findings are corroborated with the literature reported observations for Golyan fish (*Pseudorasbora parva*) [25]; *Arius felis* [26]; *Gadus morhua* [27]; *Amia calva* and *Lepisosteus oculatus*(28]*; Astyanax mexicanus, Astyanax jordani* [29]; *Danio rerio* [8], Blenniid and Gobiid fishes [10] *Pseudophoxinus antalyae* [30]; *Cyprinus carpio* [31]; *Garra rufa* [12]; in *Clarias batrachus and Serrasalmus nattereri* [7] etc. However, in *Pelteobagrus fulvidraco,* TB I, II and III were identified by Zhang *et al.* [32].

SEM studies on *Xiphophorus hellerii* [11] explained that the fish TBs could be divided into three categories based on their external surface morphology. In a comparative study of TBs in Flat fish's species (Pleuronectiformes), having diverse dietary preferences and prey activity illustrated prominent differences in the morphology of gustatory systems [33]. TBs of teleost varied in structure depending on the species examined and even on their location in the body [34]. In catfish, *Corydoras arcuatus,* only one types of TBII have been reported [35], however both the cell types, TB I and TB II was seen in another catfish species, *Ictalurus punctatus* [36]. Pinky *et al.* [20] have reported presence of only one type of TBs on the lips of *G. lamta*, where as

Fishelson *et al.* [37] found that in cardinal fish species addition to the TBIII there is another category of TB IV. However, TBIV is not properly clarified in these two selected fishes.

Normally, *Channa punctatus* and *Mastacembelus pancalus* are carnivorous, benthivory and living in turbid habitat and *Mastacembelus pancalus* specially has the habit of wriggling and burrowing in mud. Their distribution pattern of TBs can be considered to reflect their mode of gustatory feeding behavior in limited vision as well as ecological conditions as the external TBs in lips may serve for gradient search of prey, may elicit ingestion upon contact [25]. Generally, it is known that the species living in deep water and nourishing with benthic organisms have higher density of TB than those living in shallow water [14]. Fish external TBs are also crucial for orientation in the time of foraging as exhibited by Fishelson [38] on the other hand, Khanna [39] previously reported that for a predatory fish that choose its food from the mud must have the best developed gustatory faculty with numerous TBs in the lips. In both the fishes, the TBs are composed of cells having receptor characteristics, connective cells located between receptor cells; marginal cells [40, 41]. Also the cells located in TBs are called as light, dark and basal cells (42) or filamentous, tubular and basal cells (43).A third fusiform cell type with low electron density was found in the Zebra fish [30] but could not be found in present findings.

Channa punctatus is also carnivorous and predatory fish having large mouth and protruding lower jaw. In *Channa punctatus* the surface architecture is characterized by micro-ridges. The retention of secretion has been the most popular hypothesis describing micro-ridge function [44]. As the fishes are browser in habit, the microridges are may be involved in a variety of functions e.g. absorptive or secretory activities or to aid in laminar flow. Folding of micro-ridges in a wave like pattern may be resulted to increase of surface area to facilitate the channelization of mucus away from the goblet cells [45]. In the epithelia, the adjacent micro-ridges are often interconnected with each other by fine cross connections, micro-bridges. It may be possible that these structures may provide reserve surface area for stretching or distorting to enhance mechanical flexibility when scheming of ingested prey.

The peculiarity of *Mastacembelus pancalus* is pointed, oblique and horizontal cresentic cleft projects beyond over the lower lip forming an inverted ‘Y’ shaped opening. In *Mastacembelus pancalus* upper lip epithelium is exhibit papillate epidermal protrusions at irregular intervals bearing a TBII at its apex. Yashpal *et al*. [46] established generally TB I and TBII protrude above the surrounding epithelium atop of skin protrusions termed filiform papillae or earlier mentioned as ‘hillocks’ by Whitear and Moate [47]. The same may act both as chemoreceptor and mechanoreceptor. These TBs may helpful to integrate information of chemical quality with exact spatio-temporal position of prey [48]. Filter feeding mechanism in muddy habitat may be served by mesh like structure in lower lip in this fish. The sensory field of lower lip with high frequency of TBIII, may be provide guard the sensory processes of gustatory cells from excessive mechanical stimulation. At the border of the taste pore, microvillar cells with 'villi' like structure can be accountable for triggering the feeding reflex as chemo-stimulation [49]. All this architecture may be developed to compensate the indirect vision as their eyes are also minute and superior.

In the transmission electron micrograph, TBs contain a number of cells traditionally classified as “light (electron-lucent) or dark (electron-dense)” cells and basal cells but the cellular architecture and arrangement are discrete types in these two examined fishes. The longitudinal sections through the TBs show that the nuclei of dark cells lie in the half of the bud but in light cells at a deeper level. This is an observation similar to Catfish, *lctalurus punctatus* [36]. This TB cells, similar to olfactory neurons, comprise a continuously renewing population, quite unlike photoreceptors and hair cells. In these two species dense core vesicle filled cells also exist as the “dcv” cell types in sighted river fish *Astyanax mexicanus* and blind cave fish *Astyanax jordani* [29]. In these two fishes TBs basal cells generally have the same characteristics with the basal cells of other teleost [34]. The vesicle and processes of light cells towards basal cells and the close association between these two structures, suggests that a functional relationship which may support to Reutter's [50] hypothesis that the basal cell receives the taste stimulus from light cells and synaptically transmits a modified impulse to the central nervous system. [51].

Accessory cellular structure of the gustatory system other than taste buds. In both the fishes, lip epidermis is characterized by large number of unicellular secretory glands, i.e., mucous cells, uninucleate club cells, interspersed between the epithelial cells and TBs. The large numbers of mucous cells in these two fishes may be an adaptation due to their peculiar bottom-scooping habit which required amplified efficiency in keeping their lips clean; reduces surface drag. Mucus has also a remarkable power to precipitate mud held in suspension as these fish's changes its food habit with the change in seasons. Abundance of the mucous cells in the lip epidermis may also be correlated with anti-viral, bactericidal and fungicidal effects of mucus against pathogens [52]. In addition, in *Mastacembelus pancalus*, mucus plays a vital role in providing protection against abrasion and assisting them in wriggling and burrowing in mud. It appears that high density of club cells equally abundant as mucous cells may provide an effective defense mechanism. Ghattas and Yanai [53] suggested that club cells produce proteinaceous substance which initiates alarm reaction when perceived by olfactory organs of other fishes and may serve as warning of possible danger. The uninucleate club cells have been monitored in the epithelia of lips and associated structures of *Catla catla* [15] and *Cirrhinas mrigala* [18] while binucleated club cells have been noticed in the epithelium of lips and associated structures of *Rita rita* [16]. The well defined lymphatic spaces in between the cells of the stratum germinativum may be assigned to supply of nutrients for cell proliferation, can be correlated for serving a variety of activities such as immunoregulation and anti-tumoral activity. Generally the number of lymphocytes in the epidermis in tropical species is in higher than in temperate fish [54]. The pigment cells above the dermal layers may be amalgamating with the lip color with that of the substratum in the dark muddy bottom. The compactly arranged collagen fibers in the stratum compactum impart a leathery texture to lips which protect against friction in water and from scratch during nest digging [55, 56].

Scientists argue that the polymorphism and differential distribution of TBs detected in local fish are because of habitat and nourishment [26, 34]. It can be postulated that fish taste bud ultrastructure is taxon related and also might be species specific. The TBs types found in these two species have in general exhibit an orthodox plan because these two species belongs to more or less same size, age, feeding ecology and territory. However, the differences in the appearance and arrangement of TBs with other accessory cells may be due to some intriguing factors on which the variation of lip architecture is dependent. These intriguing factors may be correlated with the ontogenetic adaptability and plasticity of the gustatory system in different species enjoying proximate distinguishable micro ecological needs. The polymorphism and differential distribution of fish TBs may provide indication to the hypothesis that morphologically different taste cells and its diverse distribution pattern may be related not only to the sense of taste but to neurobiology and developmental biology as well.

CONCLUSION

The physical features of fish "lips" determine what kind of food they can eat. The cellular and morphological specialization in the lips of these two fishes may be influenced by their mode of feeding behavior and the ecological condition in which fish live. It is conceivable that such an adaptation is indispensable to carry out the feeding function in the hostile environment for survival of the individuals and species. Hence it would be hypothesized that fish taste buds do not belong to a common "fish taste bud type" as it does not exist.

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