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RESEARCH ARTICLE

A COMPARATIVE STUDY OF THE DISTRIBUTION AND MORPHOLOGY OF THE EXTERNAL TASTE BUDS IN THE SILUROID FISHES, MALUPTERUS ELECTRICUS AND CLARIAS LAZERA

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Abstract

The present work aimed to explore the morphology and the distribution of the different taste buds in the different regions of barbells, lips and skin of two different fishes; Malupterus electricus and Clarias lazera collected from the Nile River at Kaffr El-zayate city. Both fishes are carnivore; however they live in different habitats, the first lives among rocks or roots in turbid or black waters with low visibility and the second dwells the shallow water. On the basis of external surface morphology, four types of taste buds (TBs) are distinguished in the epithelial skin surface, lips and barbells of M. electricus. On other hand, only three types of TBs are observed in the skin surface, lips, and barbells of C. lazera.

Taste buds in the barbells, lips, and skin of M. electricus are higher in density and larger in size than in C. lazera. There are no histological differences between the TBs of both fish. It is suggested that the variable density of taste buds in the epidermis in the two studied fish may be associated with their different habitats.

Introduction

Fish living in the abyss require sophisticated means to identify, track, and locate food in the absence of solar light. Using bioluminescence at several wavelengths, lateral lines, taste buds and electrical field sensing, fishes are capable of hunting in continually dark habitats such as caves turbid river water and at night (Von der Emde et al., 2004).

It has been known that fishes in general have specialized taste organs in the epidermis of the mouth, lips, pharynx, and over the surface of the head and trunk (Iwai, 1964; Atema, 1971; Kiyohara et al., 1980; Ezeasor, 1982; Hamed et al., 1984). Moreover, in fish, the chemical senses, olfaction, and taste are highly developed and very important for a wide range of activities like feeding, orientation, and social behaviour (Hara et al., 1994; Boudriot and Rentter, 2001). Taste buds enable the animal to identify food by detecting distinct chemical substances at short distance (Kasumyan, 1997). Cyprinids and Siluroids are abundantly endowed with taste buds, not only in the oral cavity including gill regions, but also on the lips, barbells, and external skin surface (Gomahr et al., 1992). There are little attentions that have compared the location and the structure of TBs in fishes of different habitats. Nevertheless, many authors have compared the location and the structure of TBs in fishes in relation to the feeding habits (Singh and Mittal, 1990). Therefore, the present study aims to investigate the distribution and morphology of TBs with scanning electron microscopy and light microscopy in different regions of barbells, lips, and skin of two different fishes, Malupterus electricus and Clarias lazera belonging to Order Siluriformes. The two species have similar carnivorous feeding habits, but the two species living in different environments; each prefers a different bottom habitat; Malupterus electricus lives among rocks or roots in turbid or black fresh waters with low visibility where Clarias lazera dwells shallow fresh water.

Material and Methods

Eight adult specimens of Malupterus electricus and Clarias lazera, approximately 25 ± 5 cm body length, were collected from Nile River at Kaffr Elzayate city. The specimens were anesthetized, and then the bodies were washed very carefully in physiological saline to remove the mucus on the surface. Skin fragments from different regions; ventral and dorsal side of the head, trunk, tail, abdomen; upper and lower lips and different barbells; maxillary, inner mandibular, outer mandibular, and nasal barbells were fixed in 10% neutral formalin. After fixation, the specimens were dehydrated, cleared in terpineol, embedded in paraffin wax, sectioned 5 mμ thick, and stained with HE and Mallory's stains.

For scanning electron microscopy, selected specimens were cut into small pieces after prefixation with 2% gluteraldehyde in 0.2 M phosphate buffered (pH 7.3) at 4ºC for 4 hours, rinsed in 0.2 M phosphate buffered solution and post-fixed in1% osmium tetroxide in phosphate buffered (pH 7.3) at 4ºC for 4 hours. The specimens were then washed in 0.2 M phosphate buffer solution for several times before treatment with 3N hydrochloric acid at 60ºC for 30 minutes in order to remove the mucus. The samples were dehydrated in an ascending series of ethanol. After critical drying point, the specimens were mounted on stubs and coated with gold, using a Jeol vaccum unit. The coated samples were then scanned by using Jeol JSM-5300 SEM. To compare the different measures of height and width of skin, lips, and barbells of M. electricus and C. lazera, t – Students test was applied P values less than 0.05 were considered significant.

RESULTS and Discussion

Distribution of TBs along the barbells, lips, and skin:

Examination of barbells in M. electricus and C. lazera indicated that the TBs are located in the epithelium of the different types of barbells, upper and lower lips, and skin. The density of TBs varies markedly in different regions.

In M. electricus, the TBs are widely distributed in the outer surface of the barbells; the density of TBs increases gradually from the basal to the apical regions till reaches to the apical end of each barbell. They have more density in the maxillary and the inner mandibular barbells than in the outer mandibular and nasal ones.

In C. lazera the TBs are few in number, small in size, and distributed homogeneously along the entire surface of maxillary and the outer surface of mandibular barbells. Taste buds are absent in the inner mandibular and the nasal barbell.

Harvey and Batty (1998) in Gadus morhua, Aguirre and Lombarte (2000) in Mullus barbatus and M. surmuletus and Zhang et al. (2006) in yellow cat fish (Pelteobagrus fulvidraco) recorded that TBs are located all-over the barbells and are restricted at the middle two third regions, where there are no TBs present at the distal ends of the barbells. However, Zhang et al. (2006) recorded that the density of TBs in the basal region are low.

Similar results were reported by Boudriot and Reutter (2001) who described that the cave-dwelling Astyanax jordani, which has lost its vision, has a larger number and a more extensive distribution of external taste buds in the barbells surface than the barbells of sighted river-dwelling Astyanax mexicanus which has normal vision. Also, the taste buds of barbells are greater in demersal than pelagic Gadiformes (Harvey and Batty, 2002). Moreover, McCormick (1993), Aguirre and Lombarte (2000) recorded that the barbells of the species that inhabit water of low visibility have a higher density and a more regionalized TBs distribution than species that live in clearer water. Lombarte and Aguirre (1997) postulated that the barbells of Mullus barbatus (lives over muddy bottoms) have a greater density and number of TBs than those of M. surmuletus (lives in sandy and rocky bottoms), the greater density and number of TBs are associated with a greater sensitivity to chemical stimuli. The authors added that this is a compensatory relationship with the reduction of the vision which occurs in muddy and deeper waters.

Bailey et al. (2007) studied the roles of gustatory and tactile searching behaviour in the grenadier fish Coryphaenoides armatus. They concluded that olfactory foraging becomes ineffective at close ranges and is followed by a search phase using tactile and gustatory sensing by the barbell. The development of this sensory method probably co-evolved alongside behavioural changes in swimming mechanics to allow postural stability at low swimming speeds.

The present study indicated that the TBs in the upper lips of M. electricus are large in size and have heavy distribution density, where in the lower lips TBs are smaller in size and fewer in number. In C. lazera, the TBs in the upper lips are few in number and are absent in the lower lips. These observations are in agreement with Tripathi and Mittal (2012) who reported that the lips of Noemacheilus botia (a bottom dwelling fish) has a large number of taste buds due to the darkness and increasing of turbidity in this depth. Atema (1971) in Ictalurus natalis reported that the extreme development of taste buds all over the body, including the lips, is considered to be an adaptation owing to its weak vision. Pasha (1964) suggested that TBs on lips are absent in the Megalops cyprinoids, which is an active carnivorous fish having well developed eye. Mittal and Agarwal (1994) reported that the taste buds of the snakehead murrel, Channa striata, are poorly developed on lips; this fish is common in freshwater plains.

Viña et al. (2013) suggested that the distribution of TBs is reflection of the feeding behaviour and the habitat in which the fish live; the presence of taste buds increases the probability of detecting and locating accurately prey concealed by darkness or turbidity. Moreover, the high densities of TBs, especially on lips, gill rakers, and palatal organs, are reported by Kitoh et al. (1987) who suggested that this high density indicates the development of acute gustatory function, an adaptation to the peculiar mode of life of the minnow, Pseudorasbora parva.

In the present work, TBs in the skin of M. electricus have large size and are located with high density in the ventral head, and trunk region. However, no TBs were found on the abdomen or on the tail region. In C. lazera TBs are represented with little number in the dorsal head region, absent in the abdomen and tail regions, and are scarcely distributed on the trunk region.

Ghattas and Yanai (2010) reported that the TBs are located at the dorsal aspect of the snout of Anguilla anguilla. While, Garg et al. (2010) reported that the dorsal skin surface of Ancistrus dolichopterus, a demersal fish live near the bottom of seas or lakes, have few TBs and can be seen as an adaptive response because these algal grazers feed by night and bear long branched tentacles on the snout, which may have sufficient taste buds. Grizzle and Rogers (1976) in their study of channel catfish (inhabit muddy waters and do not depend only on visual cues) and Ohkubo et al. (2005) in their study of Zebrafish reported that the TBs are found all over the entire external surface of the epidermal skin and barbells. The last authors added that the development of TBs in the outer skin is most advantageous in muddy water where the vision is impaired; also he reported that these fish are extremely specialized for the detection of chemical stimuli in the environment.

In his studies on the cutaneous sense organs, Schemmel (1967 & 1973) described striking morphological differences in the TBs of blind cave-dwelling Anoptichthys compared with sighted river-dwelling Astyanax; he observed that in the Anoptichthys the TBs are increased in the density and propagation. Also, he recorded that TBs occur not only in the labial epithelium, as in Astyanax but also in the epithelia of the maxilla, lower jaw, and ventral head. Peters et al. (1993) and Jeffery et al. (2000) recorded that the increased number of TBs in the Anoptichthys might have evolved to compensate for the loss of vision.

Generally, TBs in the barbells, lips, and skin of M. electricus are represented with higher density and larger size than those in C. lazera. However, in both fish, the majority of TBs are more concentrated in the lips and barbells. These observations agreed with Raji and Norozi (2010) in Clarias batrachus and Serrasalmmus nattereri.

Scanning Electron Microscopy:

In the present investigation, the taste buds are found either singly or in groups of two or more situated in certain specialized area of the epidermis (Figs 1&2). Taste buds were recognized by their apical pores which occurred as small circumscribed clusters of villiform projections, most of which were located at the apices of elevated papillae. Four types of TBs were distinguished on the basis of external surface morphology as observed by SEM. They are distributed randomly in the epithelial surface of barbells, lips, and skin of M. electricus. These results agree with those of Fishelson et al. (2004 & 2012) who recorded four types of taste buds in the lips of Apogon angustatus, A. frenatus, and in the oropharyngeal cavity of 13 species of Starksiini.

On the other hand, only three types of TBs were observed on the barbells surface, lips, and skin of C. lazera. These observations are confirmed by Jakubowski and Whitear (1986 & 1990) in fishes, Huang et al. (1997) in Thornfish (Terapon Jarboa); they recorded that three types of taste bud have been recognized, each differing in dimensions, extent of protrusion above the epithelial surface, and the nature of the sensory processes on the cell exposed apices. Ezeasor (1982) in rainbow trout, Ohkubo et al. (2005) in Zebrafish, Zhang et al. (2006) in yellow catfish, and Elsheikh et al. (2012) in Oreochromis niloticus reported that the taste buds of fish fall into three types

based on their external surface morphology. In contrast, Tripathi and Mittal (2012) reported that in Glossogobius giuris two types of taste buds were observed in different region of lips; whereas only one type of taste buds was observed in the epithelium of the lips of Noemacheilus botia, Colisa fasciata, Puntius sophore, Cyprinus carpio var communis, and Garra lamta.

The present findings indicated that the first three types of TBs are represented in both fish. The first type of the TBs is sunken in the epithelial hillocks; it could be recognized by their apical pores and they are dispersed mainly along the epidermal surface of the barbells and trunk region of the skin of M. electricus. In C. lazera, the first type of TBs is located at the dorsal head region and at the maxillary barbell (Figs 1&2). The second type of TBs is observed as elevations project above the normal level of the neighbouring epithelial cells with sharp demarcation between the taste buds and the surrounding epidermis (Figs 3&4); the taste buds pores being flush with the acute tips of the papillae. This type are the most common and is found throughout the entire surface of the ventral head region, maxillary and the mandibular barbells, and at the upper and lower lips of M. electricus; where in C. lazera they are more restricted at the epidermal surface of the dorsal head region. Pinky et al. (2002) in the Indian hill stream fish Garra lamta recorded that this type of TBs is located on elevations of the epithelial surface, and thus are the first to come in contact with the surrounding medium, hence; the TBs may help the fish to locate food accurately and trigger a pick–up reflex. Agrawal and Mittal (1991) reported that the presence of TBs as projections at the surface on the upper lip of Catla catla correlates them with an acute gustatory sense.

In the third type, the TBs are exposed slightly above the epithelium and are surrounded by a thick mantel of epithelial cells (Figs 5&6). This description agrees with Whitear (1971), Reutter (1978), Jakubowski and Whitear (1986 & 1990), Huang et al. (1997), and Zhang et al. (2006).

In M. electricus this type of TBs are dispersed among the barbells and the upper and lower lips. In C. lazera this type is hardly to be observed and is located in the lips and trunk region.

The fourth type of TBs is dispersed solitary and not protruding above the surrounding epithelium, but is levelled with the epithelial surface and encircled by a thick ring of epithelial cells. At the summit of each taste bud, closely packed microvilli are observed. These microvilli appear to represent the taste hair originating from the sensory cells of the TBs (Fig 7). This type of TBs is restricted only in M. electricus and is observed on the lips, barbells, and the trunk region. In C. lazera this type is not detected. Fishelson et al. (2004) reported that in addition to the three types of TBs identified on various teleost fish, a fourth type of TBs is found in some cardinal fish i.e. Apogon angustatus and A. frenatus.

Table 1: The means± S.D. and ranges of height and width of taste buds of skin, lips and barbells of M. electricus and C. lazera

		N	Mean	SD	Min	Max	t (p-value)
Skin height	M. electricus	8	81.22	7.45	72.82	89.58	4.449 (0.001**)
	C. lazera	8	65.85	6.83	52.80	71.51	
Skin width	M. electricus	8	68.39	7.06	60.00	77.53	3.111 (0.008*)
	C. lazera	8	56.14	9.34	44.76	68.93	
Lips height	M. electricus	8	93.38	10.07	73.12	103.10	7.431 (0.000**)
	C. lazera	8	62.13	4.97	56.34	70.42	
Lips width	M. electricus	8	84.75	9.29	70.16	92.52	6.946 (0.000**)
	C. lazera	8	56.16	7.67	47.80	69.32	
Barbells height	M. electricus	8	57.42	3.79	50.64	61.81	3.765 (0.002*)
	C. lazera	8	51.51	2.49	49.13	55.43	
Barbells width	M. electricus	8	54.69	4.08	49.54	59.62	2.922 (0.011*)
	C. lazera	8	49.5	3.16	45.63	54.31	

* Significant at P value <0.05, ** Highly Significant at P value <0.001

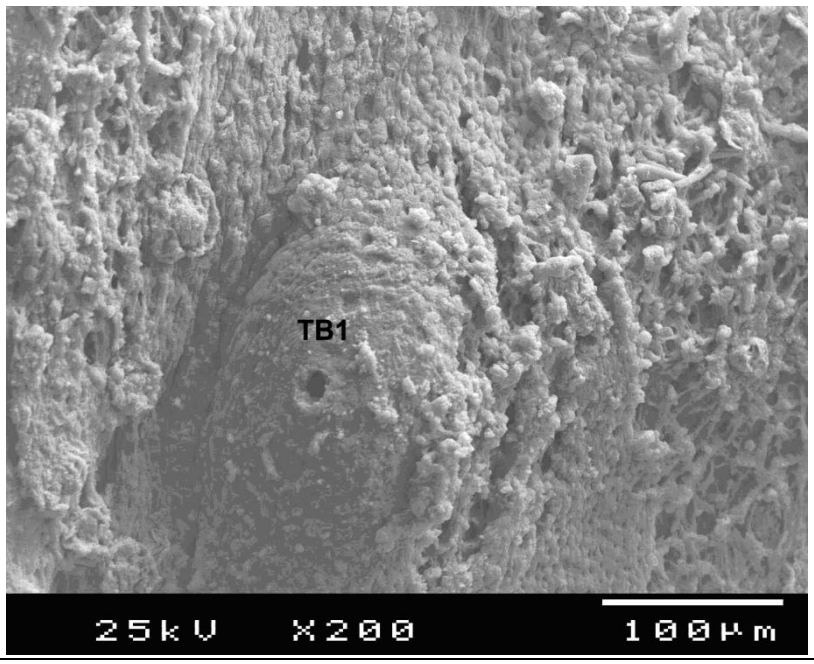

Fig 1: Type I (TB I) of taste buds in mandibular barbell of *M. electricus.*

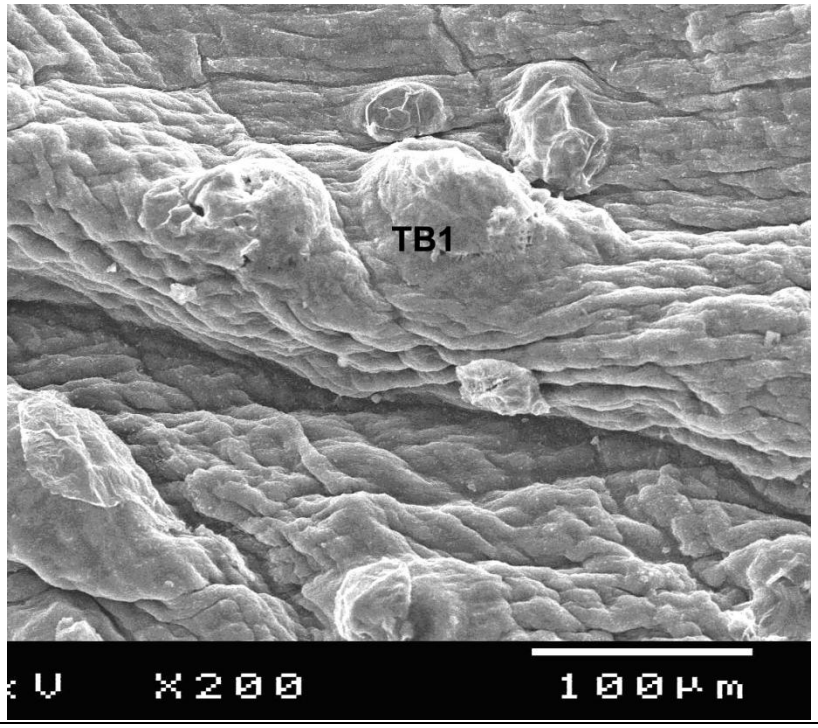

Fig 2: Type I of taste buds (TB I) in maxillary barbell of *C.* lazera

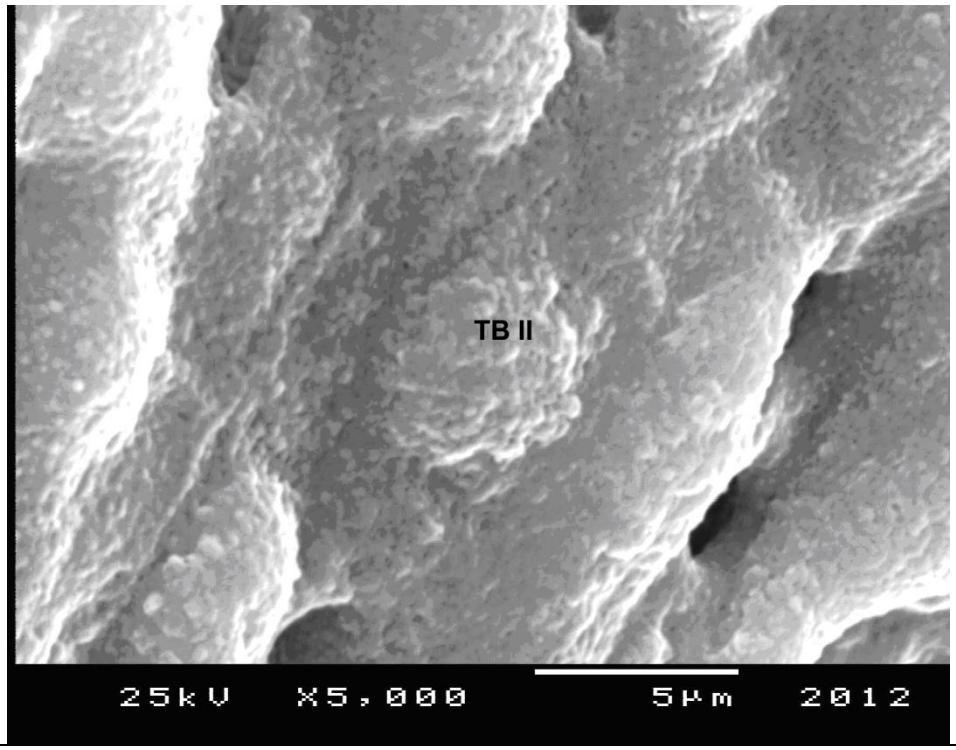

Fig 3: Type II of taste buds (TB II) in ventral head region of *M. electricus*

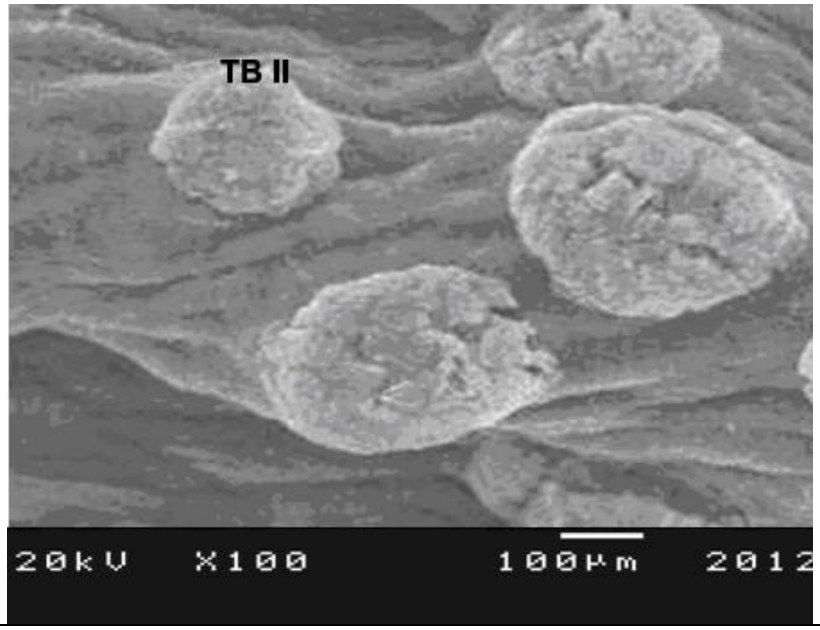

Fig 4: Type II of taste buds (TB II) in dorsal head region of *C. lazera*.

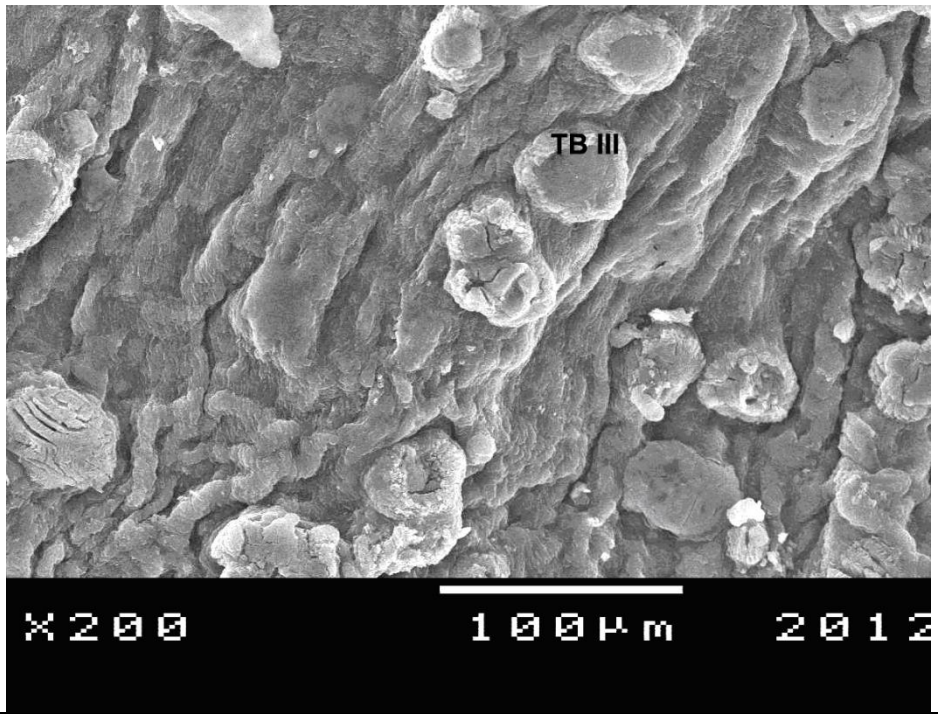

Fig 5: Type III 0f taste buds (TB III) in the upper lips of *M. electricus*

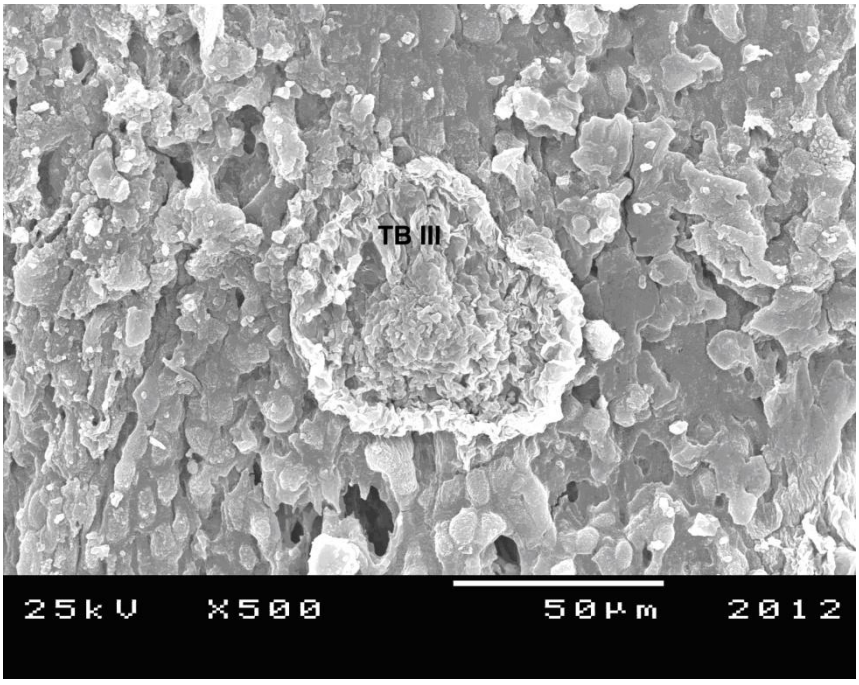

Fig 6: Type III of taste buds (TB III) in trunk region of *C. lazera*.

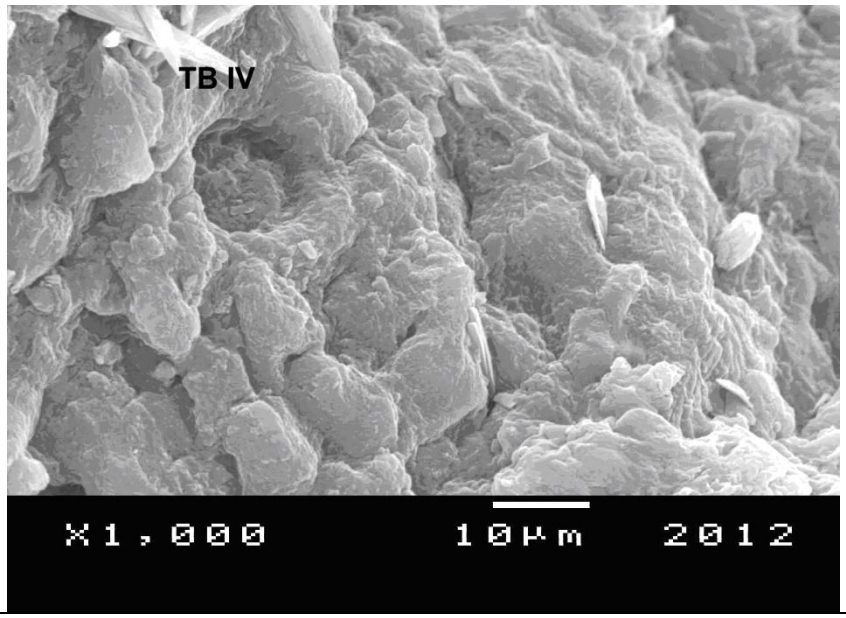

Fig 7: Type IV of taste buds (TB IV) in the trunk region of skin of *M. electricus*

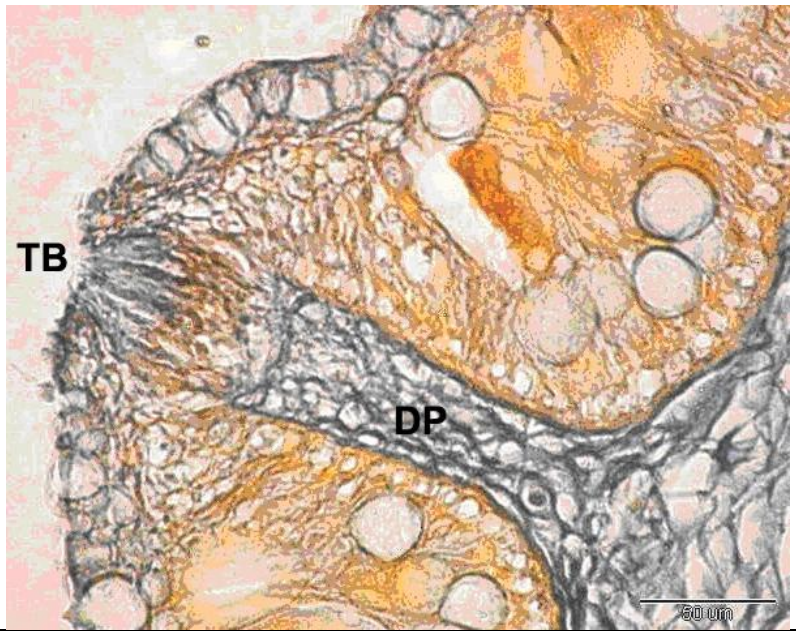

Fig 8: Photomicrograph of T.S of trunk region of the skin of *C. lazera* showing a taste bud (TB) and elevated dermal papilla (DP). Mallory triple stain.

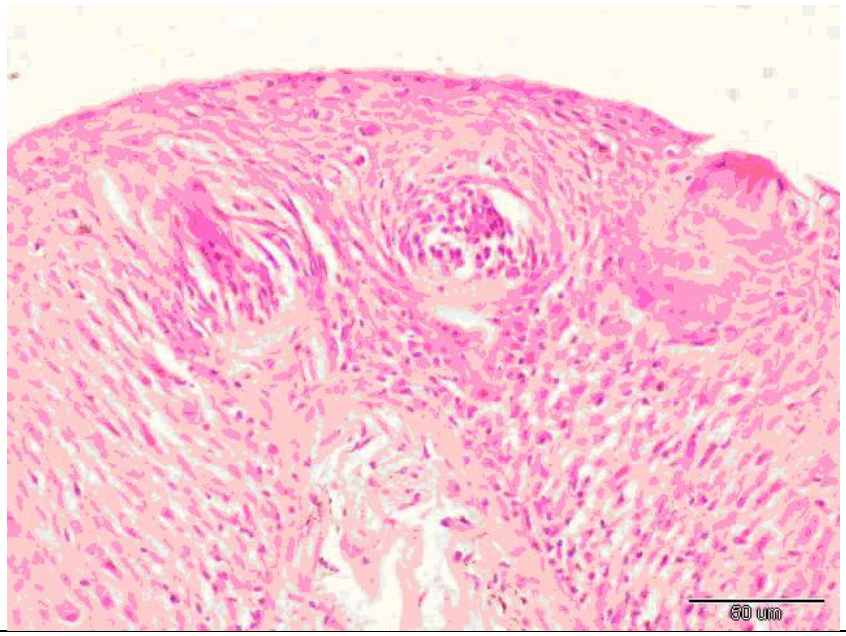

Fig 9: Photomicrograph of T.S of lower lip of *M. electricus* showing variable heights of taste buds. H&E.

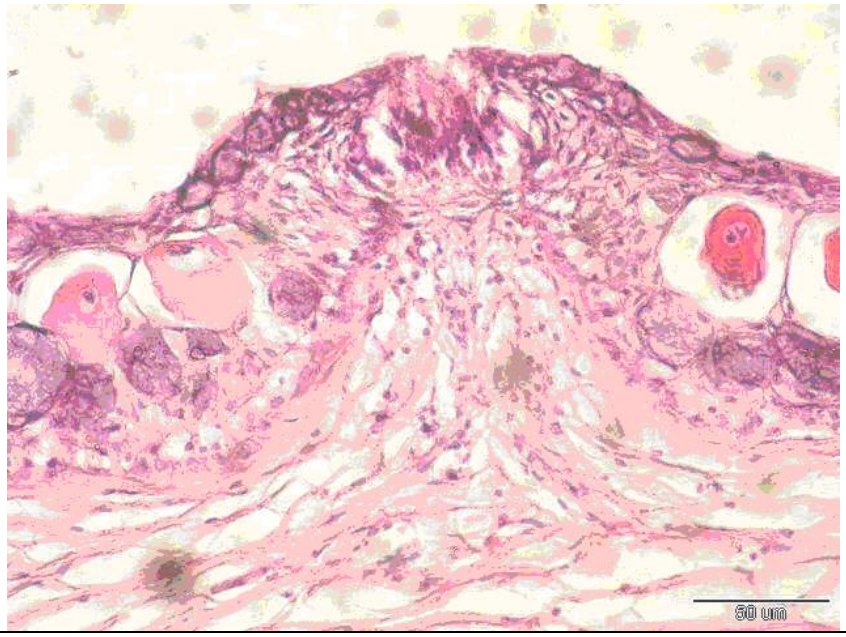

Fig 10: Photomicrograph of T.S of the dorsal head region of *C. lazera* showing the taste bud. H&E.

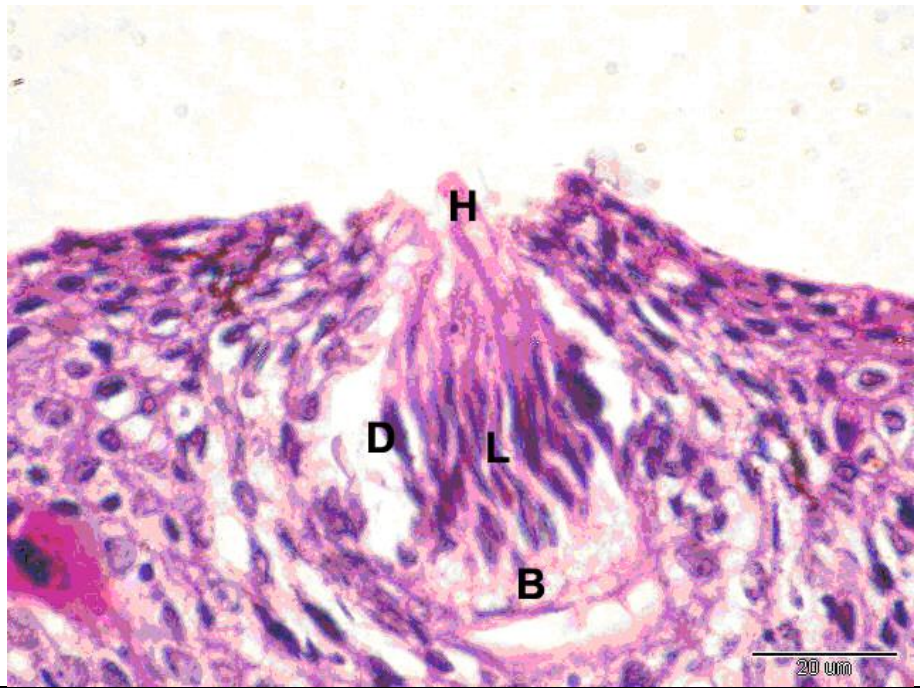

Fig 11: Photomicrograph of T.S of the barbell of *M. electricus* showing thin epidermis; dark cell (D); light cell (L);basal cell (B) delicate hair (H). H&E.

Light microscopy:

Taste buds are located in the epidermal tissue of some regions of the skin, lips, and barbells. No marked microstructure differences between the TBs of M. electricus and C. lazera is noticed. This similarity was reported by Schemmel (1967) and Boudriot and Reutter (2001) who recorded that no histological or essential ultrastructural differences between the TBs of Anoptichthys (A blind cave fish) and Astyanax (A Surface-dwelling fish) is observed.

The taste buds are small, pear-shaped structure, and are located on the apices of elevated dermal papillae; each papilla contains a single bud (Fig 8). The same histological structure was described in Catla catla (Agrawal and Mittal, 1991), Amphilius natalensis (Booth, 1996), and Pelteobagrus fulvidraco (Zhang et al., 2006).

The TBs are presented with variable heights to enable them to protrude out to a great extent from the general surface of the epithelium (Figs 9&10). These may be associated with the acute gustatory sense due to weak vision at the bottom habitat. The above mentioned description agrees with Agrawal and mittal (1991) who reported that the projected taste buds of Catla catla are the first to come in contact with surface water and enable the fish to sense the nature of food available. The elevated taste buds may have an additional mechano-receptive function as suggested by Reutter et al. (1974). Some TBs are hardly to observe, they do not project out from the general surface of the epithelium as shown in lips of M. electricus and in the dorsal region of the head of C. lazera (Fig 9). The TBs of the barbells lies on short papillae of the dermis, because the barbells epidermis is thin (Fig 11). Kiyohara et al. (1980) reported that taste buds in the minnow vary in size and shape in relation to the thickness of the epithelial layer. The TB is wide at the base and in the middle; it narrows at its upper end towards the pore, by means of which it communicates to the exterior. As shown in table 1, the TB of the skin of M. electricus measures about 81.22 ± 7.45 µm in height and about 68.4 ± 7.06 µm in width and in the lips it measures approximately 93.38 ± 10.07 µm in height and 84.75 ± 9.29 µm in width and the TBs of barbells are measuring about 57.42 ± 3.79 µm in height and 54.69 ± 4.08 µm in width. In C. lazera the TBs of the skin measure approximately 65.85 ± 6.83 um in height and 56 .14 ± 9.34 µm in width; it measures approximately 62.13 ± 4.97 µm in height and 56.04 ± 7.67 µm in width in lips. TBs of barbells measure approximately 51.51 ± 2.49 µm in height and 49.49 ± 3.16 µm in width. From table 1, it can be observed that the statistical analysis of the height and width of the taste buds of the skin, lips and barbells of

M. electricus and C. lazera showed that TBs of M. electricus increased significantly from those obtained from C. lazera. These results may be associated with their spatial distribution in the environmental habitats.

Three types of cells are distinguishable at high magnification of TBs; dark, light, and basal cells (Fig 11). The dark cells are tall columnar sustentacular cells with ovoid darkly stained nuclei. The light cells are fusiform sensory cells having lightly-stained oval nuclei and are surrounded by pale cytoplasm. The sensory cells terminate into a delicate hair. The hair projects out of the gustatory pore of barbells and lips, but it does not do so in the several region of skin. The basal cells are pyramidal in shape and are associated with the dark and light cells. The TBs are surrounded by broad peripheral cells, each with a large central nucleus. Similar structures are observed by Hansen et al. (2002) who recorded that besides the light and dark cells a third fusiform cell type of low electron-density. Whereas Raji and Norozi (2010) reported that in both the examined fish; Clarias batrachus and Serrasalmus nattereri the TBs are composed of two elongated cells, light (electron-lucent) and dark (electron-dense) cells. Ohkubo et al. (2005) blue in the Zebrafish, Danio rerio distinguished two types of the cells; dark and light cells in semithin plastic sections of taste bud stained with toluidine.

The present investigation evaluates the differences in distribution pattern, densities, and size of taste buds in the several regions of skin, lips, and barbells of the two studied fish; this may be associated with the differences in their habitats.

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