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MICROMORPHOLOGY OF THE OROPHARYNGEAL CAVITY OF THE TILAPIA FISH (*OREOCHROMIS NILOTICUS*) FROM AFIKPO RIVER IN EASTERN NIGERIA

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ABSTRACT: The micromorphology of the oropharyngeal cavity of the tilapia fish *Orechromis niloticus,* from Nigerian waters were investigated to fill the dearth of information from available literature and help understand its food prehension and pregastric apparatus biology. The lips epidermis was lined stratified squamous epithelium containing mucous cells. The lip dermal region contained dense regular collagen, melanophores and few melanocytes. Pectinate ligament were also seen in the lips. The wall of the cavity was modified into longitudinal folds lined by stratified squamous epithelium containing mucous cells. The submucosal region of the cavity wall contained loose irregular fibres, adipose tissue, blood vessels and nerve fibres. The lingual epithelium of stratified squamous cells with no taste buds makes the organ a mechanical structure involved in rolling the food back. The Pharyngeal pad was lined by stratified squamous epithelium containing mucous cells and taste buds. Pharyngeal pad teeth erupted above the epithelium. This co-localization of taste bud and teeth makes the pad the primary organ for food selection or rejection through gustation.

Keywords: taste buds, mucous cells, histology, food selection, melanophores.

INTRODUCTION:

In teleosts, the oropharyngeal cavity (OC) morphology varies greatly (Nelson, 2006; Wainwright, 2006), and is associated with suction, forward swimming and preydation. Studies have been directed on the OC of some teleost like *Catla catla* (Sinha and Chakrabarti, 1985); *Gadus morhum* (Bishop and Odense, 1966); *Orechromis alcalicus grahami* (Maina, 2000); the tongue of European sea bass (Abbate et al.,2012). In all of these and other studies, great variation has been reported and it is attributed to functional morphology and ecomorphology, mostly associated with feeding and adaptation for survival (Motta, 1988; Wainwright, 1991; Grubich, 2003).

The OC of most teleosts consist of a roof, floor and prominent teeth. The roof is made up premaxilla, maxilla, velium, palatine bones and pallate, whereas the floor comprise the dentaries, angularies, velum and tongue (El- Bakary, 2012). The rostral boundary is occupied by the upper and lower lips while the caudal extremity is demarcated by the oesophagus and septum transversum (Ikpegbu et al., 2012).

Tilapine species are widely used as an important culture species in tropical and subtropical fisheries (Caceci et al., 1997; Morrison and Wright, 1999). The fish has also been employed for vegetation control, recreational fishing and a possible source of big pancreatic islets for diabetic patients (MacKenzie, 1996; Caceci et al., 1997; Yang et al., 1997). The biology of the digestive tract of the tilapine species has attracted a lot of research interest as evidenced from available literature (Osman and Caceci, 1991; Gargiulo et al., 1996; Scocco et al., 1998; Bwanika et al., 2004; Bwanika et al., 2007). Despite the large volume of research works on the tilapia morphology, none has been described from Nigerian waters. Also there are reports from recent studies that some discrepancies exist in the earlier account of the description of the digestive tract in this species. Hence, this work is aimed at documenting our observations on the oro-pharyngeal cavity of *Orechromis niloticus* from Nigerian waters as part of our contribution to increase our body of knowledge on the morpho-adaptation to feeding in this species especially as regards to the possibility of ecomorphological differences.

MATERIALS AND METHODS:

Seven adult tilapia fish *Orechromis niloticus* sourced from Afikpo river in eastern Nigeria were used for the study. They weighed an average of 350g and measured a standard body length of 22cm. The fish were humanely immobilized by chloroform euthanasia. The oro-pharyngeal cavity was cut open through the membrane between the upper and lower jaws, and the specimens dissected out. The samples under study – lips, tongue, pharyngeal pads and cavity walls were excised, but the lips and pharyngeal pads were decalcified according to Good and Stewart 1932, before subjecting to routine histological procedure of dehydration in graded concentrations of ethanol, clearing in xylene and embedding in paraffin wax.

Sections 5µm thick were obtained with Leitz microtome model 1512. They were stained with haematoxylin and eosin for light microscopy examination (Bancroft and Stevens 1977). Photomicrographs were taken with – Motican 2001 camera (Motican UK) attached to Olympus microscope.

RESULTS AND DISCUSSION:

Lip

The upper lip epidermis was lined by stratified squamous epithelium containing mucous cells (Fig.1). The lamina propria was composed of dense regular collagen fibres containing a single layer of melanocytes and melanophores at the immediate

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ventral region (Fig.1). The epidermal pegs interdigitated with the dermal papillae. The submucosa contained loose irregular to dense regular collagen fibres. In some areas pectinate ligament linking dermis to the subdermal connective tissue was observed and the rectangular to square shaped cavities bounded bilaterally by two pectinate ligaments bilaterally contained nerve fibres and loose connective tissue (Figs. 1 and 2). Molariform teeth were contained in the dental pad.

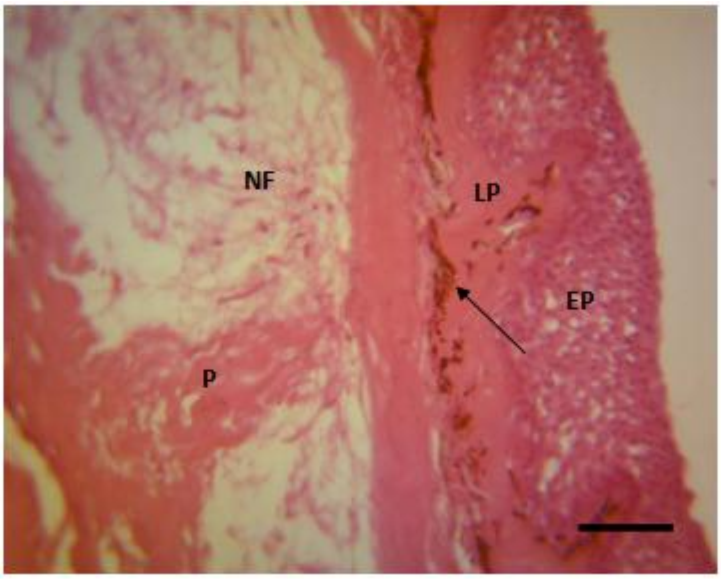

Fig.1. Section of the upper lip showing the stratified squamous epithelium E, lamina propria, melanophores (black arrow) and pectinate ligament P. Note nerve fibres NF, beside the pectinate ligament. H&E. (Scale bar = 10µm).

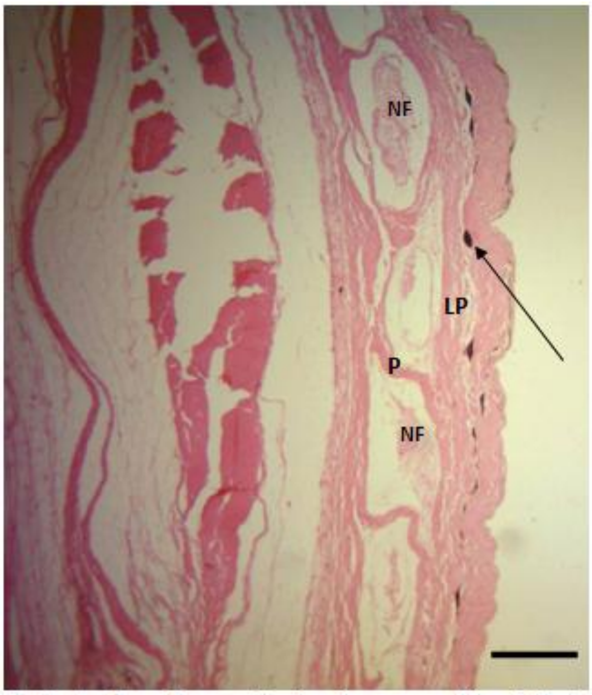

Fig.2. Section of upper lip showing nerve fibres NF, in the rectangular to square shaped cavities in-between the pectinate ligaments P. Note the melanocyte (black arrow) in the lamina propria LP. H&E. (Scale bar = 10µm).

The lower lip presented similar micro-architecture with the upper lip but the epithelium contained more layers of the stratified squamous cells with fewer PAS positive mucous cells.

Oro-pharyngeal cavity wall:

The OC wall tunica mucosa conformed into longitudinal folds lined by stratified squamous epithelium (Fig. 3) containing mucous cells. The lamina propria was composed of dense regular collagen fibres. The submucosa contained loose irregular collagen fibres, adipose tissue, blood vessels, nerve fibres and thin layer of dense regular connective tissue fibres near the tunica muscularis (Fig 4). The tunica muscularis contained skeletal muscles in circular orientation.

Fig. 3. Section of OC wall showing the stratified epithelium containing mucous cells MC. note the dense regular fibres D, loose irregular fibres L and few melanocytes M in the lamina propria-submucosa. H&E. (Scale bar = 40μm).

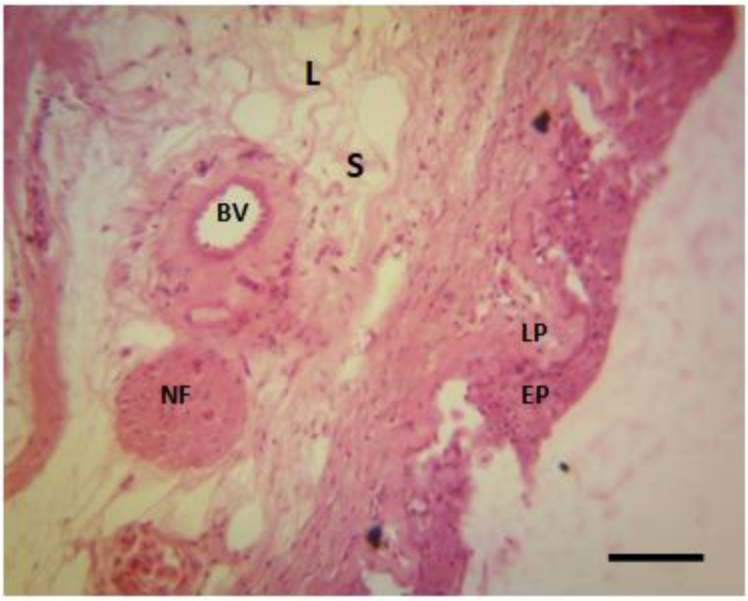

Fig.4. Section of OC wall showing the epithelium EP, lamina propria LP, submucosa S. Note the blood vessel BV, nerve fibre NF, loose connective tissue L, in the submucosa. H&E. (Scale bar = 10μm).

Tongue:

The tip of the tongue was conformed into pencil to leaf shaped longitudinal folds. These folds were lined by stratified squamous epithelium containing mucous cells (Fig 5). Some of these mucous cells contained centrally placed nuclei while other nuclei were basally to marginally located. The lamina propria of the folds contained dense regular collagen fibres. The submucosa contained collagen fibres in loose irregular to dense regular orientation. At the middle of the tongue, the epithelial mucous cells were more abundant in number and bigger in size than the mucous cells seen at the tip. The tunica muscularis in this region contained skeletal muscles and bone. The bone contained cavities and spicles. The base of tongue was similar to the middle region histologically but in addition, observed hyaline cartilage was surrounded by bone spicles.

Fig. 5. Section of the tongue tip showing the modified mucosa into longitudinal folds. Note the epithelium EP, containing mucous cells MC. Also the laminar propria collagen fibres LP. H&E. (Scale bar = 10μm).

Pharyngeal pad:

The pharyngeal pad was lined stratified squamous epithelium containing taste buds and abundant mucous cells (fig 6 and 7). The taste buds were below the epithelial surface, hence type II taste buds. The epithelium was modified into broad longitudinal fold with the each furrow between adjacent epithelia occupied by the pharyngeal tooth. The teeth were seen erupted above the epithelium and were of caniform type pointing caudally. Some teeth were seen erupting below the epithelim. At the sub-epithelial region or the core of the pad were contained bone spicles, hyaline cartilage, skeletal muscles and mucous cells. Some of these mucous cells were human foot shaped with four to five *digits.* The base of the foot (caudal or posterior end) was pointed towards the core of the pad while the digits were pointed towards the epithelial surface (Fig 8).

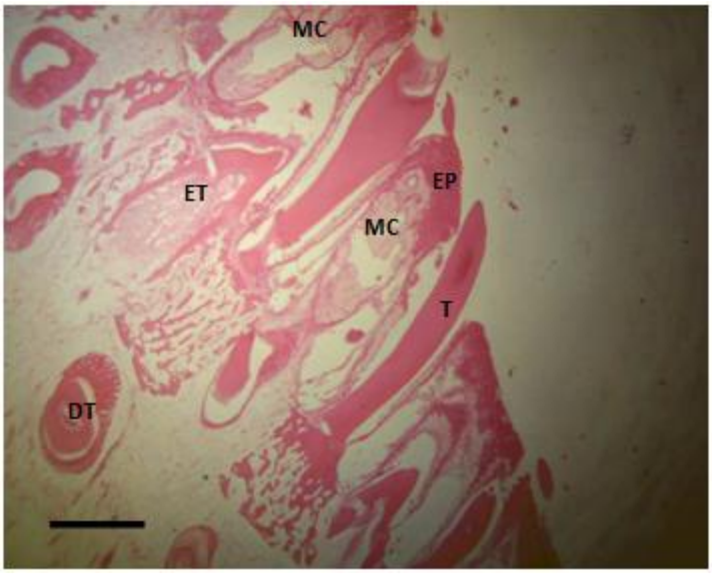

Fig.6. Section of oropharyngeal pad showing the epithelium EP, erupted tooth T, mucous cells MC, erupting tooth ET, and developing tooth DT. H&E. (Scale bar = 4μm).

Fig.7 . Section of oropharyngeal pad showing the stratified squamous epithelium EP, taste bud TB, taste bud nerve bundle NF, and mucous cells MC. H&E. (Scale bar = 40µm).

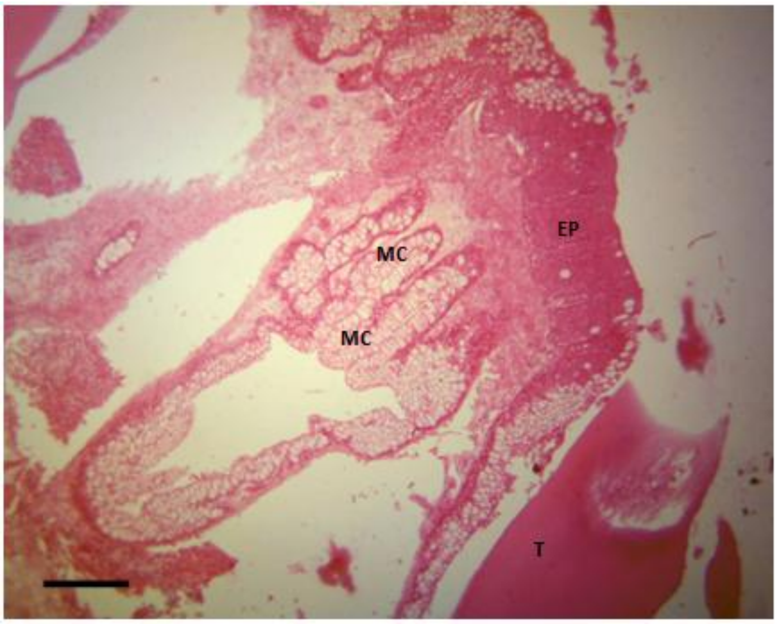

Fig. 8. Section of oropharyngeal pad showing the stratified squamous epithelium EP, tooth T, and *human foot-shaped* mucous gland MC. H&E.(Scale bar = 40µm).

This paper for the first time in available literature presents the microanatomy of the tilapia OC from Nigerian waters. The lip serves as the primary organ of prehension, hence the protective epithelium of stratified squamous cells (Agrawal and Mittal, 1991). The mucous cells will produce mucin on the surface to help protect the lip from abrasion or pathogenic agents like bacteria (Neuhaus, 2007; Elbal and Agulleiro,1986). The melanocytes produce the melanin stored in melanophores (Hussain et al., 2009). The melanin is responsible for the dark colouration of the lip skin and protects it from ultraviolet ray effects. The presence of melanin has been reported in other fish and several reports have suggested that colorful signals including dark coloration of melanin can become modified to serve both as a warning to predators and as a display to attract mates or deter competitors, because conspicuousness can be important in all of these contexts (Eagle and Jones, 2004; Price et al., 2006). Also there reports that melanic individuals are more aggressive than non-melanic forms as exemplified in the mosquito fish where melanic males are more aggressive that the silver breeds, even to potential mates (Ducrest, 2008). This melanin in fish is regulated by the melanin concentrating hormone, MCH (Kawauchi, 2006). This MCH is now been associated in mammals with the regulation of dynamic physiological functions from food intake and energy expenditure to behavior and emotion (Shi, 2004).

The dermal dense regular collagen lends support to the epidermis while the pectinate ligament is to help in reinforcing the rigidity and attachment of the epidermis to the dermis and subdermal region. The presence of pectinate ligament has been reported in the lips of Africa catfish- *Clarias gariepinus* (Ikpegbu et al., 2004). The nerve fibres contained in the cavities formed by pectinate ligaments will make the lips very sensitive to touch. The position of these nerve fibres may also be an adaptation to help protect the fish from hard objects or evading predators' as the lip will quickly through nervous stimulation and relay inform the fish to move away as no eosinophilic club cell was observed from this study. This feature will compliment lateral line system. Eosinophilic club cells when present secret alarm substances that helps the fish in fight or flight when danger appears in the environment (Diaz et al., 2006; Cao et al., 2009). The molariform teeth is used for grinding the mostly plant based feed. The presence of molariform teeth has also been reported in *Crenicichla taikyra* and *Cichlasoma minckleyi* (Trapani, 2004; Casciotta et al., 2013).

The conformation of OC wall into longitudinal folds lined by stratified squamous epithelium containing mucous cells will increase the quantity of mucin produced and protect the underlying structures. This tunica mucosa conformation was not reported in the OC of African catfish- *Clarias gariepinus* but was seen in the oesophagus (Ikpegbu et al., 2012). The dense regular collagen may be serving as modified stratum compactum, thus supporting the epidermis. The presence of stratum compactum has been reported in other teleosts where it is associated with a protective, supporting and strengthening layer, which keeps the distension of OC wall within bounds (Ezeasor, 1986; Khojasteh et al., 2009). The adipose tissue in the OC wall is for metabolite storage. This adipose layer has been described in the African catfish skin where it is referred to as stratum adiposum (Guerra et al., 2006). The tunica muscularis of skeletal muscles in circular orientation will help in voluntary contraction during grinding of food by the molariform teeth in the dental pad.

The tongue tip mucosa that was modified into longitudinal fold will help reduce the rate of epithelial sloughing off by the rough plant diet. This epithelial structural modification will be aided by the mucin from mucous cells to protect the tongue. The mucus cells number and size that increased caudally on the tongue towards the base may reflect the need to protect the tongue from increasing concentration of abrasive materials in feed. The absence of taste buds in this species makes it a mechanical organ that may be involved in rolling food caudally towards the pharyngeal pad. Taste buds have been observed on the tongue of *Salmo garidneri* (Ezeasor, 1982). This difference may be due to species variation. The skeletal muscles function in voluntary movement of the tongue in the desired direction. The bone and hyaline cartilage will provide support and provide rigidity to the tongue but the bone in addition serves as origin and insertion of the skeletal muscles (Lehner et al 1989).

The co-localization of taste buds and teeth on the epithelial surface makes the pharyngeal pad organ the site of food selection or rejection by gustation. This has been reported in literature (Hobbler and Merchant, 1983; Northcott and Beveridge, 1988; Linser et al., 1998; Ikpegbu et al., 2012b). OC teeth have been also associated with shredding, grinding of food, and rupturing cells of the tissues to release their contents, thus helping in making food nutrients biologically available for the fish (Linser et al., 1998; Tibbetts and Carseldine, 2003; Kumari et al 2005; Kumari et al., 2009; Ikpegbu et al., 2012b). The presence of heavy mucification of the OC represents need for lubrication of occlusal tooth surfaces in the pharyngeal pad, pregastric digestion, help in extraction of nutrients from plant material digested by fish, reduction of adhesion to thereby ensuring their continued effectiveness, protection and addition of carbohydrate moieties to food since the teleosts have been documented to lack salivary glands (Tibbetts, 1992; Murray, 1994; Tibbetts, 1997; Scocco et al., 1998; Tibbetts and Carseldine, 2003). The caniform teeth will help in tearing and sharing of food suggesting that in addition to plant materials, the Tilapia fish under study maybe involved in consumption of animal flesh. The oro-pharyngeal pads seen in this study has been reported in the *Mugil tade* (Pillay, 1953); *Mugil cephalus* (Kawamoto and Higashi, 1965); *Microptenes salmoides* (Linser et al., 1998). In the *Aphanius persicus*, a teeth-bearing pharyngeal bone at roof of the oral cavity has been reported (Monsefi, 2010), but no oro-pharyngeal pad was reported in the *Odonthesthes bonariesis* (Diaz et al., 2006), and *Pelteobagrus fulvidraco* (Cao and Wang, 2009).

CONCLUSION:

From this study, the Tilapia from Nigerian waters under study is similar to others from previous reports in other locations, but the presence of nerve fibres and pectinate ligaments in the lips has not been reported in other tilapian fish. The tongue is just a mechanical organ with no gustatory ability. The presence of stratum compactum is also very significant for support to the epithelial in food mastication. This study fills the knowledge gap and will serve as baseline in further investigative studies.

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