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Original Article

Tongue anato-histology of the oceanodromous adult *Rastrelliger brachysoma* (Bleeker, 1851) with a note on the comparison with the tongue structure of adult *R. kanagurta* (Cuvier, 1816)

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Abstract

Anatomical and histological structures of the tongue tissue of *Rastrelliger brachysoma* and *R. kanagurta* were investigated. Anatomical structure of the tongue in *R. brachysoma* was poorly developed and was of triangular shape. The histological and histochemical technique showed the tissue to be principally composed of three layers: tunica mucosa, tunica submucosa and osteocartilagionous skeleton. Stratified epithelium of its mucosal surface was interrupted with several cell types including goblet cells and taste buds. Numerous teeth with elongated shape were also found centrally on the anterior tip of the tongue. They are found among a few dermal papillae of the tongue. Tunica submucosa consisted of connective tissue, blood vessels and pigment cells. When compared anatomically and histologically with that of *R. kanagurta*, the overall morphological was quite similar. However, some histological structures including taste buds and teeth of *R. kanagurta* were rarely seen. *Rastrelliger kanagurta* also tended to have more goblet cell than *R. brachysoma*. *Rastrelliger kanagurta* tended to possess less overall histological structures of the tongue. This difference could potentially cause by different feeding adaptation between the two species. Regardless of the differences found between the two species, both are still considered as herbivores.

Keywords: *Rastrelliger brachysoma*, *R. kanagurta*, histology, Thailand, tongue tissue

1. Introduction

The teleostean digestive tract is considered as the main part of the digestive system, which is important for the metabolic and the assimilation processes of food (Genten *et al*., 2008; Groman, 1982). Previous investigations on this structure have been well documented in several fish species including *Gadus morhua* (Morrison, 1987), *Seriola dumerili* (Grau *et al*., 1992), *Gnathonemus petersii, Scyliorhinus canicula* (Genten *et al*., 2008), *Hemibagrus filamentus* (Senarat *et al*., 2013) and *Rastreliger brachysoma* (Senarat *et al*., 2015). Among the digestive regions, teleost tongue is an important tactile organ. This organ is considered as play a key role in the transportation and the swallowing of food. Also, its structure is used in the explanation of the evolution of organism life style and for the adaptation to different

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environmental conditions and feeding habitats (Iwasaki, 2002). Similarly, Yashpal *et al*. (2009) suggested that the oral modification especially the tongue structure in fishes was adapted and correlated to different environments.

Several detailed studies on teleost tongue have been carried at the light microscopic level, including *Sparus aurata* (Abbate *et al*., 2012a) and *Dicentrarchus labrax* (Abbate *et al*., 2012b). This structure consisted of several special cells particularly the taste buds, which are considered as the chemo-sensory detector cell (Ezeasor, 1982; Fishelson *et al*., 2004). Pharyngeal teeth are also seen. There has never been a report in histological study of the tongue of *Rastrelliger brachysoma* living in the Upper Gulf of Thailand. It is considered as the most economically important marine fish in Thailand. Past studies suggested that it is a plankton feeder, which covers the whole array of both phytoplankton and zooplankton (Dhebtaranon & Chotiyaputta, 1972). However, recent preliminary survey on the stomach content suggested possibility of being carnivore. The fundamental aim of this study was to understand the basic histological organization of the tongue tissue. We primarily focused on the anato-structure of tongue of *R. brachysoma* using histological and histochemical techniques and secondarily compared its structure with that of *R. kanagurta*, which is a sister species that was presumed to have niche partitioning with *R. brachysoma*.

2. Materials and Methods

2.1 Animal collection

Specimens of *Rastrelliger brachysoma* with standard length ranging from 16.5 to 20 cm were collected during the breeding seasons (December 2013 to February 2014, n = 5). They were captured by using bamboo strake trap from Samut Songkhram Province (13°16’18.4" N, 100°02’13.4" E). Specimens of *R. kanagurta* (about 16.5 cm in total length, n = 3) were purchased from gill net fisherman from Rayong Province (12°40’38.41" N, 101°42’20.55" E). Species identification of all specimens followed the identification key by FAO (2010). The *R. brachysoma* samples used in this study were the specimens from a previous study under the experimental protocol approved by the Animal Care and Use Committee of Faculty of Science in accordance with the guide for the care and use of laboratory animal prepared by Chulalongkorn University (Protocol Review No. 1423003).

2.2 Histological procedures

All species were euthanized by rapid cooling shock (Wilson *et al.,* 2009).The tongues were kept and then fixed in Davidson’s fixative at room temperature. After routine histological technique, the sections were cut at 6-7 µm thickness and stained with Harris’s hematoxylin and eosin (H&E), Masson’s trichrome (MT), periodic acid-Schiff (PAS) and aniline blue (AB) (Bancroft & Gamble, 2002; Humason, 1979) to identify the basic structure and chemical details of two *Rastrelliger* sp. The histological structure of the tongue of these species was photographed using a Canon EOS 1100D digital camera and then they were compared using histological images.

3. Results and Discussion

3.1 Anatomy and structure in adult *Rastrelliger brachysoma* tongues

Anatomical examination of the tongue reviewed that the tongue of *R. brachysoma* was triangular in shape and poorly divided into apex, body and root (Figure 1A). All regions of the tongue under histological and histochemical techniques comprised three layers: tunica mucosa, tunica submucosa and osteocartilaginous skeleton (Figure 2A). Most of the tunica mucosa was lined with large number of a normal stratified epithelium with interrupting in several cells

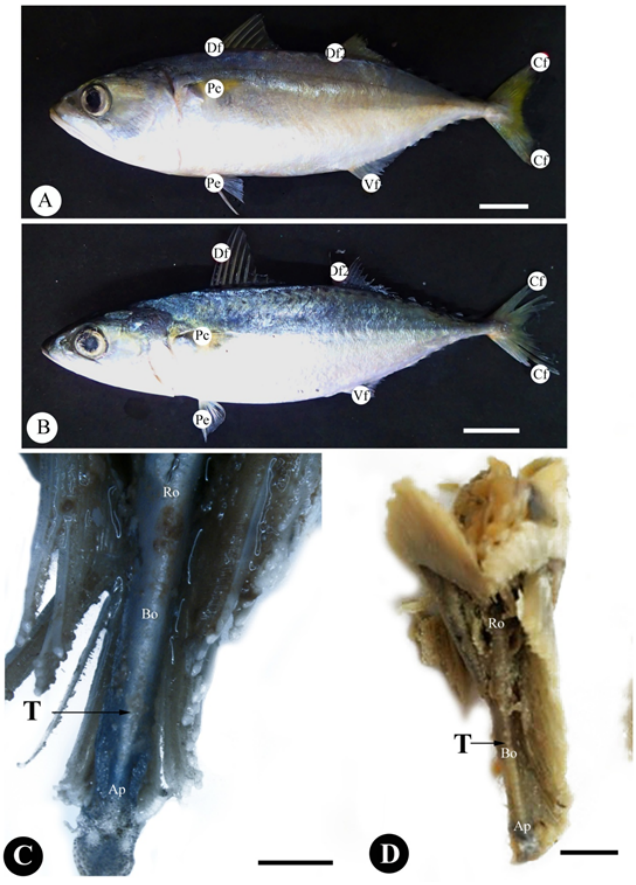

Figure 1. Anatomical structure of tongue both *Rastrelliger brachysoma* (A, C) and *R. kanagurta* (B, D). Ap = apex, Bo = body, Cf = caudal fin, Df = primary dorsal fin, Df2 = secondary dorsal fin, Pc = pectoral fin, Pe = pelvic fin, Ro = root, T = tongue, Vf = ventral fin. Note: Scale bar 0.5 cm (B); 1 cm (B).

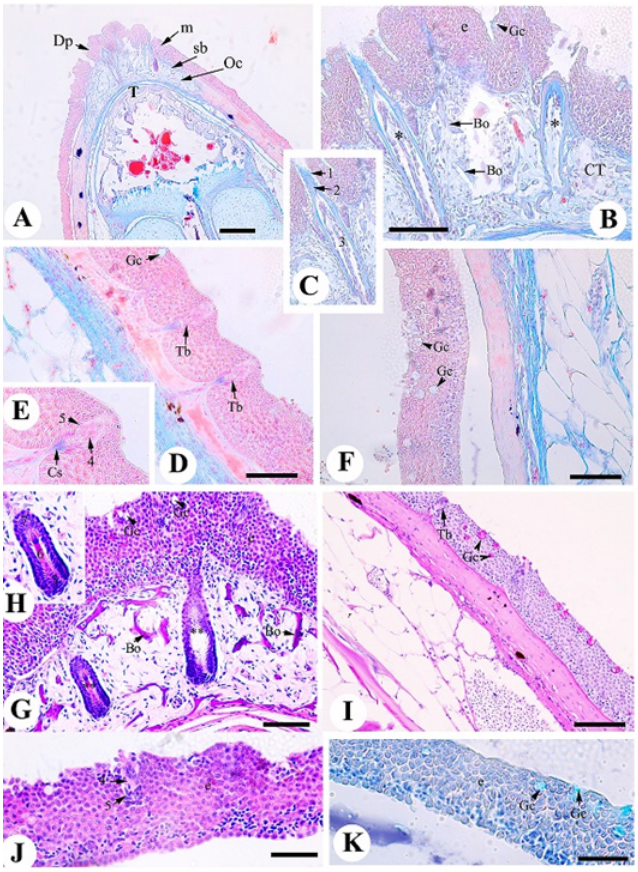

Figure 2. Photomicrograph of tongue of adult *Rastrelliger brachysoma*; Bo = bone, Cs = connective stalk, CT = connective tissue, Dp = dermal papilla, e = epithelium, Gc = goblet cell, m = tunica mucosa, Oc = osteocartilagionous skeleton, sb = tunica submucosa, T = tongue, Tb = taste bud, * = mature tooth, ** = immature tooth, 1 = enamel carp, 2 = dentine pulp, 3 = pulp core, 4 = taste cell, 5 = sustentacular cell, 6 = dentine of immature tooth. Note: Scale bar 0.5 cm (A); 1 cm (B); 100 ìm (B); 50 µm (D, F, G, H); 20 µm (J, K). A-F (MT stain); G-I (H&E); J (PAS); K (AB)

(Figure 2B). The distribution and abundance of the goblet cells were visible along the epithelium. They were negatively stained with H&E. On the other hand, they were intensively positive reacted to MT, PAS and AB techniques (Figures 2D, 2F, 2G, 2I, 2K and 3B). Scattered taste buds and mucous-secreting cells were seen as intraepithelial structures on the mucosal surface (Figure 2D, 2E and 2J). Note that they was characteristically and typically present as a pear-shaped structure, and occupied taste receptor and elongated taste cells. Sustentacular cells were also located in the periphery of the taste cells (Figure 2E). Beneath the mucosa layer was the submucosa layer. This layer was rarely contained in the loose connective tissue and it was positively bluish stained with MT. This layer was also remarkable with blood vessels and pigment cells. Additionally, several teeth in an elongated shape were found but only centrally at the anterior tip of the tongue. In addition, they were found among dermal papilla of the tongue. Like other teleosts, the teeth were similar in size and arranged in rolls, which can be replaced serially. The teeth of this species could be classified into two stages based on histological characterizations; immature and mature teeth (Figure 2B). Mature teeth were formed from dentine-like material and consisted of dentine layer, which was surrounded by (i) enamel carp (enamel coating), (ii) dentine pulp and (iii) pulp core among spongy bones. Each tooth was clearly separated by oral stratified epithelium, which contained numerous mucous cells (Figure 2C). These cells were positively stained with MT and AB reactions. Immature teeth were rarely seen among spongy bone (Figure 2G). They consisted of three parts; (i) scleroblast, which was seen in basal layer, (ii) odontoblast, which secreted to its pre-dentine, and (iii) enamel organ, which contained an outer enamel epithelium and enameloid (Figure 2G and 2H). Overall tongue structure was also supported by hyaline cartilage (MT as bluish), which was oriented from the posterior to the near the tip of the tongue.

3.2 Structure comparison of the tongue between adult *Rastrelliger brachysoma* and adult *R. kanagurta*

The tongue of *R. kanagurta* was quite similar anatomically and histologically to that of *R. brachysoma* (Figure 3D-3H). Under H&E and MT stains, when compared to *R. brachysoma*, the number of several cell types including taste buds and teeth were less. On the other hand, the goblet cells in the tongue were significantly more abundant than in *R. brachysoma*.

The tongue anatomy in both species could be poorly classified into three regions (apex, body and root). Therefore, it should be noted that it was poorly developed and closely resembled of the structure found in herbivore. In contrast, the tongue in carnivorous fish such *Esox lucius* is well developed and can be classified into three large regions including apex, body and root. (Sedeghinzad *et al*., 2014). Similarly, Abbate *et al*. (2012b) have also reported the well-structured tongue of *D. labrax* which was considered to be carnivore. The tongue of *R. brachysoma* was histologically comprised of three layers. This organization was similar to another fish (Abbate *et al*., 2012a,b). The epithelial mucosal layer of the tongue was lined by stratified epithelium, which was also found in most teleost including *D. labrax* (Abbate *et al*., 2012b) and *E. lucius* (Sadeghinezhad *et al*., 2014). Moreover, several goblet cells in the tongue of *R. brachysoma* were located among the epithelium and positively stained with PAS and AB reactions, which indicated the presence of both glycoprotein and glycolpolysaccharide. The main function of both substrates is probably concerned with protection of the tongue surface and enhanced food taste (Abbate *et al*., 2012a, b). Moreover, the taste buds were normally observed in *R. brachysoma* tongue, as similarly identified in some their fishes (Kruse & Stone, 1984; Kubitza

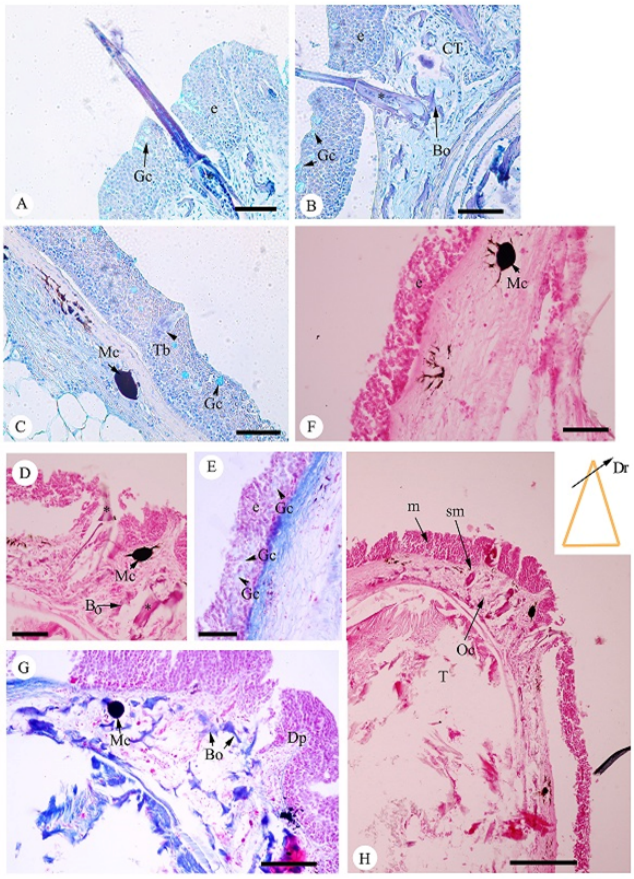

Figure 3. Photomicrograph of tongue both *Rastrelliger brachysoma* (A-C) and *R. kanagurta* (F-H) *Rastrelliger brachysoma*; Bo = bone, CT = connective tissue, Dp = dermal papilla, Dr = dorsal region e = epitheliam, Gc = goblet cell, m = tunica mucosa, Mc = melanocytes, Oc = osteocartilagionous skeleton, sm = submucosa, T = tongue, Tb = taste bud, * = mature tooth. Note: Scale bar 50 µm (A, B, C, E, F, G); 20 µm (D); 200 µm (H).

& Lovshin, 1997). To well known about function, the function of the tongue is highly conserved and plays key roles in the ultimate acceptance or rejection of potential food items (Kruse & Stone, 1984; Kubitza & Lovshin, 1997).

Furthermore, Hara (1994) described co-structural functions between teeth and cell components on the tongue surface, which interact to support food processing, tasting, lubricating the surface and ingestion as well as feeding activity. If the two parts are well developed, it is possible that the fish is a carnivores. This condition was found similarly in *D. labrax* (Abbate *et al*., 2012b). Hence, the morphological and histological characterizations suggested that *R. brachysoma* and *R. kanagurta* are herbivores. *Rastrelliger brachysoma* differed from *R. kanagurta* in the pattern of teeth and taste bud distributed along the tongue. This study is the first report for this character differences between the two species. However, the explanation for this difference is not certain. Linser *et al*. (1998) suggested the pattern and the distribution of the teeth and taste bud on the tongue of the *Micropterus salmoide*s were related to feeding for the survival of that species. It is possible that the morphology and histological differences between the two species could be a responded to differences in feeding behavior. However, knowledge of molecular and genetic variations is necessary to distinguish whether these differences are a phyletic trait or an adaptive trait.

In conclusion, the basic histological characterizations of the tongue in the two *Rastrelliger* sp. are similar to there of other fishes. However, close detailed investigation revealed *R. kanagurta* tended to have fewer taste buds and teeth when compared to *R brachysoma*. We hypothesize based on previous research that the presence/absence of cell components of the tongue of *Rastrelliger* sp. may be related to feeding as well as to behavior adaptation in the natural habitat.

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References

Abbate, F., Guerrera, M. C., Montalbano, G., Ciriaco, E., & Germaná, A. (2012a). Morphology of the tongue dorsal surface of gilthead seabream (*Sparus aurata*). *Microscopy Research and Technique, 75*, 1666–1671

Abbate, F., Guerrera, M. C., Montalbano, G., Carlos, F. D., Suárez, A. A., Ciriaco, E., & Germaná, A. (2012b). Morphology of the European bass (*Dicentrarchus labrax*) tongue. *Microscopy Research and Technique, 75*, 643-649.

Bancroft, J. D., & Gamble, M. (2002). *Theory and practice of histological techniques*. London, England: Churchill Livingstone.

Dhebtaranon Y., & Chotiyaputta K. (1972). Review of the mackerel fishery (*Rastrelliger* spp.) in the Gulf of Thailand. *Indo-Pacific Fish Council Proceeding, 15(3)*, 265-286.

Ezeasor, D. N. (1982). Distribution and ultrastructure of taste buds in the oropharyngeal cavity of the rainbow trout *Salmo gairdneri* Richardson. *Journal of Fish Biology, 20*, 53-68.

Food and Agricultural Organization. (2010). *Report: First workshop on the assessment of fishery stock status in South and Southeast Asia*. Rome, Italy: Author.

Fishelson, L., & Delarea, Y. (2004). Taste buds on the lips and mouth of some blenniid and gobiid fishes: Comparative distribution and morphology. *Journal of Fish Biology, 65*, 651-665.

Genten, F., Terwinghe, E., & Danguy, A. (2008). *Atlas of fish histology*. Enfield, NH: Science.

Groman, D. B. (1982). *Histology of the striped bass*. Bethesda, MD: American Fisheries Society.

Grau, A., Crespo, S., Sarasquete, M. C., & Gonzalez de Canales, M. L. (1992). The digestive tract of the amberjack *Seriola dumerili*, Risso: a light and scanning electron microscope study. *Journal of Fish Biology, 41*, 287-303.

Hara, T. J. (1994). Olfaction and taste in fish: An overview. *Acta Physiolgica, 152*, 207-217

Humason, G. L. (1979). *Animal tissue techniques (4th ed.)*. San Francisco, CA: W. H. Freeman & Company.

Kruse, K. C., & Stone, B. M. (1984). Largemouth bass (*Micropterus salmoides*) learn to avoid feeding on toad (Bufo) tadpoles. *Animal Behavior, 32,* 1035 - 1039.

Kubitza, F., & Lovshin, L. L. (1997). Salmoides feeds and training strategies. *Aquaculture, 148*, 299-312.

Morrison, C. M. (1987). *Histology of the Atlantic Cod, Gadus morhua. Na Atlas*. *Part one: Digestive tract and associated organs. Canadian special publications in fisheries and aquatic science*. Ottawa, Canada: Canadian Government Publishing Centre.

Iwasaki, S. (2002). Evolution of the structure and function of the vertebrate tongue. *Journal of Anatomy, 201*, 1-13

Linser, P. J., Carr, W. E., Cate, H. S., Derby, C. D., & Netherton, J. C. (1998). Functional significance of the co-localization of taste buds and teeth in the pharyngeal jaws of the largemouth bass, *Micropterus salmoides*. *The Biological Bulletin, 195*, 273-281.

Sedeghinzad, J., Rahmati-holasoo, H., Fayyza, S., & Zargar, A. (2014). Morphological study of the northern pike *Esox lucius* tongue. *Anatomical Science International.* doi: 10.1007/s12565-014-0254

Senarat, S., Kettratad, J., Poolprasert, P., & Yenchum, W. (2013). Histological structure of the esophagus and stomach in yellow Mystus, *Hemibagrus filamentus* (Fang & Chaux, 1949). *Thammasat Journal of Science and Technology, 22*, 46-54.

Senarat, S., Kettratad, J., Jiraungoorskuland, W., & Kangwanrangsan, N. (2015). Structural classifications in the digestive tract of short mackerel, *Rastrelliger brachysoma* (Bleeker, 1865) from Upper Gulf of Thailand. *Songklanakarin Journal of Science and Technology, 37*(5), 561–567.

Wilson, J. M., Bunte, R. M., & Carty, A. J. (2009). Evaluation of rapid cooling and tricaine methanesulfonate (MS 222) as methods of euthanasia in zebrafish (*Danio rerio*). *Journal of the American Association for Laboratory Animal Science. 48*, 785-789.

Yashpal, M., Kumari, U., Mittal, S., & Mittal, A. K. (2009). Morphological specializations of the oral cavity in relation to the food and feeding habit of a carp *Cirrhinus mrigala*: A scanning electron microscopic investigation. *Journal of Morphology, 270*, 714-728.