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Comparative study of fish scale using scanning electron microscopy in two Cyprinid fishes (*Neolissochilus hexagonolepis* and *Neolissochilus hexastichus*) found in Meghalaya, North-East India.

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ABSTRACT

The scale morphology has been studied using Scanning Electron Microscope in two species of genus *Neolissochilus* viz. *N. hexastichus and N. hexagonolepis* to find out differences in scale structure and species specificity between the species. The Scanning Electron Micrograph of both the species showed the general morphological structure of a cycloid scale. Differences is more prominent in the presence of a pore in the anterior origin of the lateral line canal, the shape and distribution of tubercles, posterior filed at the base of the lateral line canal and the pattern of central regeneration pattern. Shapes of circuli, structure of lepidonts are additional distinctive tools in this comparative study. All these characteristics can be used as distinctive species specificity tool in these particular species of fishes.

Keywords: *Neolissochilus hexastichus*, *Neolissochilus hexagonolepis*, circuli, lepidonts, tubercles, chromatophores, Meghalaya.

INTRODUCTION

'Mahseer' are a group of cyprinids that belong to three genera viz. *Neolissochilus*, *Tor* and *Naziritor*. *Neolissochilus* and *Tor,* in particular, are important fish of Meghalaya and very sought after game-fishes having high angling tourism pursuit (Nguyen *et al*., 2008). The fish also act as a food fish for the local people of Meghalaya. *Neolissochilus hexagonolepis* commonly known as chocolate mahseer is considered a threatened species (McClelland, 1939; Menon, 1994) is available in almost all the rivers, streams and reservoirs of Meghalaya and a similar, superficially non-differentiable species *Neolissochilus hexastichus* commonly called brown mahseer is also found in the specific pockets of one river (Nongrum *et al*., 2015).

Dermal outgrowths found in fishes as scales are very useful in studying fish taxonomy (Kaur and Dua, 2004), Evolution, Phylogeny (Kobayashi, 1951; 1952), growth studies (Jhingran, 1957) as well as fish species identification

(Johal and Dhiman, 2007) and as pollution indicator (Johal and Dua, 1994; Johal and Sawhney, 1997). Recent studies had separated fish scales into four classes on the basis of their morphology (Vernerey and Barthelat, 2010) i.e., Placoid scales (as seen in rays and sharks), Ganoid scales (seen in sturgeon and gar fishes), Cosmoid scales(seen in fossil fishes and lungfish) and Leptoid scales (mostly the bony fishes). Different scale microstructures i.e., circuli, radii, ctenii, lateral line structures and other associated parts of a scale have been used for taxonomy (Batts, 1964; Hughes, 1981; Hollander, 1986; Dicenzo *et al*., 1998; Kaur and Dua, 2004) and the detailed structure of fish scale is helpful in fish identification up to major group and species level (Abraham *et al*., 1966; Bartulovic *et al*., 2011; Esmaeili, 2014). Variation of scale morphology had been studied by various researchers (Zahid *et al*., 2015) and precision in using SEM as a tool has help in distinguish fishes from various taxa and across species (Kaur and Dua, 2004). However, in certain fishes it is difficult to differentiate the scale structure, macroscopically, between species from the same habitat leading to the confusion in identification. Therefore, the SEM study of scale morphology can help in conclusively differentiate between fish species. The present study is conducted based on the differences in scale morphology to find out detailed variations in scale morphology of two fishes belonging to a cyprinid group under the genus *Neolissochilus* using SEM.

MATERIALS AND METHODS

In order to study the ultrastructure scales of *Neolissochilus* spp., fishes were collected from different sites using cast nets with the help of local fishermen. They were then brought to the laboratory and kept in well aerated tank. Fish scales were collected from the lateral line position of the body carefully so that no damage was done to the fish as well as the scales and the scales were washed immediately using a fine brush with double distilled water. Cleaned scales were then kept in 3% Glutaraldehyde fixative at 4˚C for 24 hours. The scales were then transferred for washing in 0.1 M Cacodylate buffer and kept at 4˚C for 2 hours. The scales samples were then again washed with 0.1M for 3 changes of 15 minutes each at 4˚C. Dehydration was carried in various strength of Acetone (30 %, 50 %, 70 %, 80 %,90 %, 95 % and 100%) and dried using TMS method (Dey *et al*., 1989), where the specimens are immersed in Tetra Methyl Silane for 5-10 minutes for two changes at 4˚C and then brought to room temperature (25-26˚C) to dry completely. The dried samples were mounted in stubs by double adhesive tape with dorsal surface upwards and posterior facing downwards and sputter coated using Gold of about 10nm thick. Scale samples were then visualised in JEOL JSM-6360 Scanning Electron Microscope under SAIF, NEHU, Shillong. This is a first attempt to study the ultrastructure of the dermal growths of the two species using SEM from North-East India. No other report was encountered by the researcher except for studying of scale morphology of *Neolissochilus hexastichus* by Ansari *et al*., 2016.

RESULTS AND DISCUSSION

The results of the present study show clear differences in the scale morphology of two species viz., *Neolissochilus hexagonolepis* and *Neolissochilus hexastichus* from Meghalaya, India. The two species look similar in their external morphology and local population treat them as one species. The taxonomy of mahseer is confusing due to the morphological variations that they exhibit (Mohindra *et al*., 2007) and an approach of this paper by studying the dermal growths hereby attempt to clarify the doubts arising based on scale morphology using SEM. After the present study of scales by SEM, it has been found that there are differences in scale morphology and they are two different species. Taxonomy is important as it plays a very important role in protecting the diversity of any organism (Nelson, 1994) and morphological characteristics is one of the taxonomical tools useful for assigning any organism to a generic or specific epithet, but classification based on morphology alone cannot clarify the differences at species level. Studies like morphometric and meristic characters, which is counting of scales is employed as the first step to clear any taxonomic ambiguities between species. Hence, lepidological study is the best alternative as one of the taxonomical tools (Tandon and Johal, 1994; Johal, 2005; Negi *et al*., 2010). In particular each of the different characteristics of a fish scale can be used for identification and this include all the important structures like Lateral line scales, Lateral line canal, Shape,size,diameter of tubercles, Circuli, Radii, Lepidonts, Ctenii and their associated arrangements. Lateral line scales have been utilized by several workers to prove their potential in fish classification and taxonomy (Delamanter, 1973).

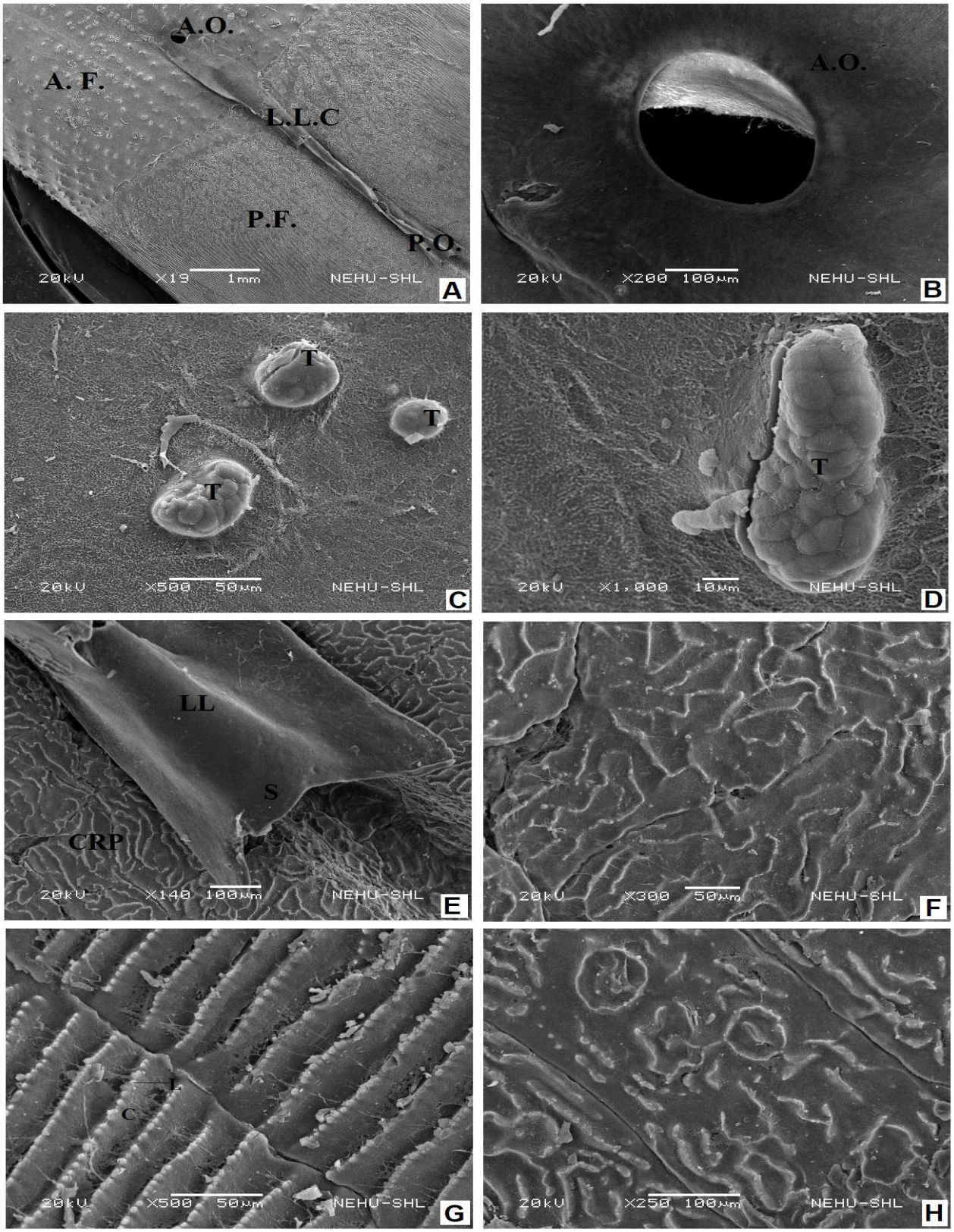

Fig. 1- Scanning Electron Micrographs of Scale morphology of lateral line scale of *Neolissochilus hexastichus.*

A. Posterior opening (P.O), Anterior Field (A. F.), Posterior Filed (P. F.), Lateral Line Canal (L.L.C.) **B.** Enlarged view of Anterior opening , **C.** Tubercles (T) bearing chromatophores **D.** Enlarged view of tubercle (T) **E.** End of covering sheath (S), Lateral line (LL), Central regeneration pattern (CRP), **F.** Enlarged view of Central Regeneration Pattern (CRP) **G.** Lepidonts (L), Circuli (C) **H.** Region below Focus

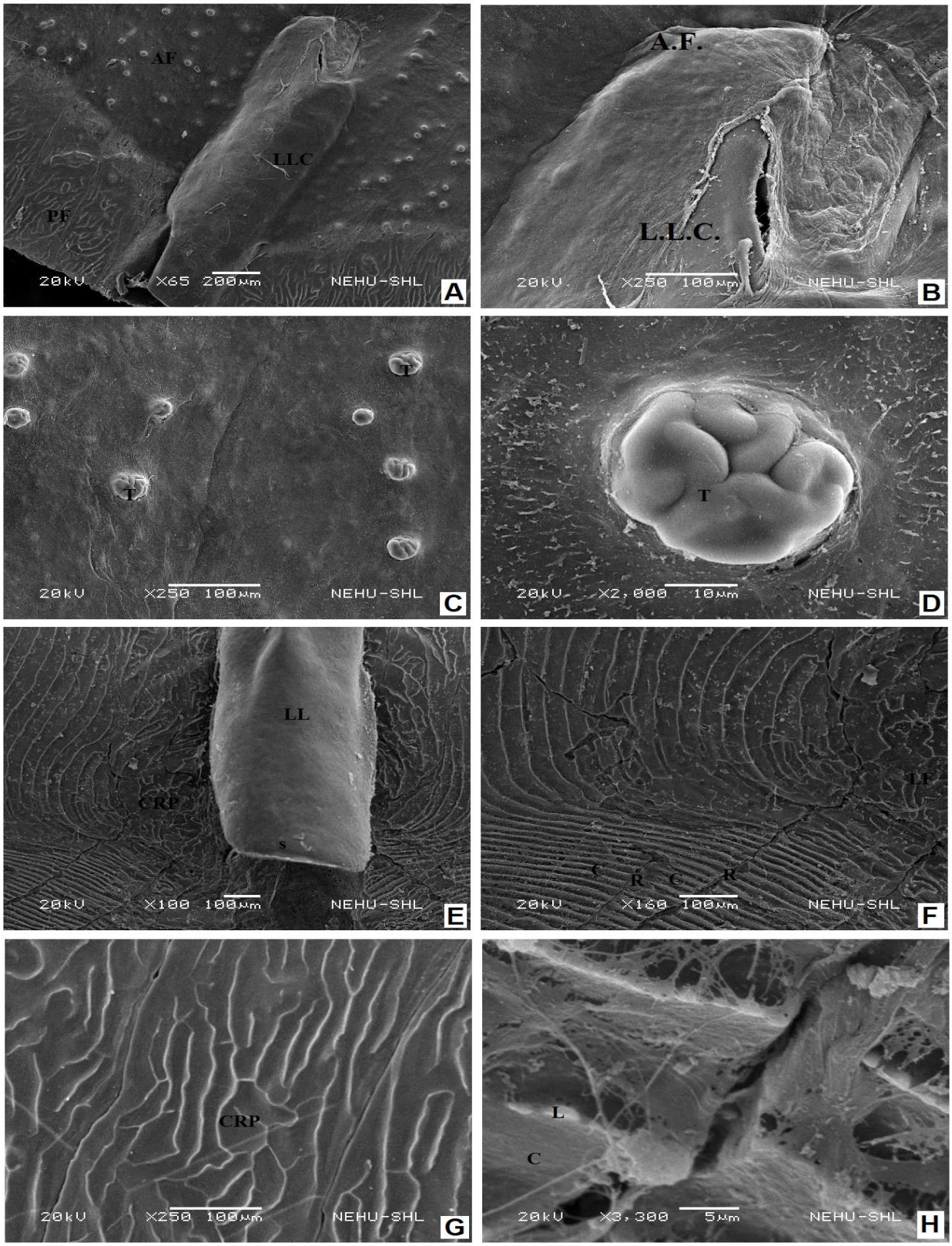

Fig. 2- Scanning Electron Micrographs of Scale morphology of lateral line scale of *Neolissochilus hexagonolepis*.

A. SEM of hexagonolepis showing absence P.O. **B.** Scale showing Posterior Field **C.** Scale of *hexagonolepis* showing tubercles (T) **D.** Enlarged view of Tubercle (T) **E.** Lateral Line (L.L.), Central Regeneration Pattern (CRP) **F.** Micrographs showing circuli and radii **G.** Enlarged View of Central Regeneration Pattern (CRP) **H.** Scales showing Lepidonts (L) and Circuli (C)

Circuli indicates the deposition of calcium salt in the scale and the distances of circuli indicate the fast and slow growth period and lepidonts are teeth-like structures found on circuli (Esmaeili and Gholami, 2011). Primary, secondary, and tertiary radii is considered as a growth phenomenon (Alkaladi *et al*., 2013) and less influenced by genetic characteristics of a fish (Lippitsch, 1990). Studies by various researchers (Esameili *et al*., 2009; Kaur and Dua, 2004; Jawad and Al-Jufail, 2007) shows that lepidonts can be an important tool in species distinctiveness and characterize genera and may even distinguish taxa at a specific level (Delmater and Courtenay, 1973).

The unique differences found, during the present study, is the presence of a round pore on the anterior field of scale of *Neolissochilus hexastichus* (fig. 1A) which is not found in the scale of *Neolissochilus hexagonolepis* (fig. 2A). However, though the shape of the origin of the lateral line canal is similar in both the species but the presence of the pore in the origin of the scale of *Neolissochilus hexastichus* mark a big difference in the scale morphology in these species. Secondly, the tubercles were found to be more in number and clustered in the anterior field of *Neolissochilus hexastichus* (fig. 1C, 1D), their shape ranging from round, oval and oblong. The shape of tubercles in the fish species varies in these two species and they impart specific colour to the fish species as they contain chromatophores in the outer surface (Esmaeili, *et al.,* 2012). Whereas in *Neolissochilus hexagonolepis* (fig. 2C, 2D), the tubercles were less in number and most of them are round in shape. The posterior opening in *Neolissochilus hexastichus* (fig. 1E) is flat and the Central regeneration pattern (CRP) is well separated, obstructed and not well-defined whereas in *Neolissochilus hexagonolepis* (fig. 2G) CRP is joined, continuous and well-defined. There is a difference in the position and appearance of the lepidonts in the circuli of the two species. The spacing between the circuli is different in two species (fig. 1G and fig. 2H) and the differences is also seen in the region below the focus (Johal *et al*., 2014) regarding size, thickness and arrangement of bony ridges.

In conclusion, scales of the two species of *Neolissochilus* viz., *N. hexastichus and N. hexagonolepis* are different in terms of the shape of their scales, presence of circuli, arrangement patterns of lepidonts in the body. Tubercles impart color to the fish scales as they contain chromatophores in the posterior part (Esmaeili, *et al.,* 2012) and hence this shows the difference in color in the two species of mahseer.

Conflict of Interest

The author declares that there is no conflict of interest.

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