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Scanning electron microscopy of scales in two edible fishes *Notopterus kaporat* and *Etroplus suratensis* for its application in taxonomy

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Abstract

Taxonomic identification of fishes is essential in conservation study and to understand their role in an aquatic ecosystem. The study of scale morphology provides new and useful information in the field of the taxonomy of fish and paleontological analysis. The purpose of this study was to determine the scale morphology and the surface ultra-structural details like circuli, radii and focus. Other structures like tubercles, denticles, canals, chromatophores, ctenii were also noted with specifications, arrangement and numbers, on the scales. The teleost species *Notopterus kaporat* and *Etroplus suratensis* were selected in this study from the Godavari river basin in Maharashtra. Other than morphological characters using phase-contrast microscopy and SEM the microphotographs of selected characters of the scales were noted. From the result, it was concluded that fish scales have numerous hidden detail in their structures and they are a new advanced tool in fish systematics even after not getting the whole fish sample but the availability of scales only is enough.

Keywords: teleosts, scale morphology, taxonomy, Godavari river, SEM

1. Introduction

It has been estimated that the total number of all fishes is 32,500 species^[1]. Remarkably, there are more than 15,000 freshwater fish species. Comprising approximately 25% of all vertebrates, freshwater fishes are an important component of global biodiversity^[2]. Approximately 7,956 of all fish species (30%) are contained within just 6 of the 515 taxonomic families. Remarkably, 6,100 (77%) of species in these representative families live in freshwater. Much taxonomic work still remains to be done and our knowledge of fishes changes rapidly^[2]. Since 1976, an average of 305 fish species has been described as new to science per year^[3]. The study of scales on the body has one of the crucial applications in fish taxonomy besides their basic function of body protection in teleosts. In (1833-1884) scale structure was used first time for fish taxonomy^[4]. During the late 19th century and the first half of the 20th century, studies on scale morphology found increased considerably in the field of fish taxonomy^[5, 6, 7, 8, 9, 10, 11, 12, 13, 14]. The characteristics of fish body scales are very much useful in the identification as they tend to change from species to species^[15]. Circuli, radii, ctenii, and structure of lateral line canal opening on the scales, presence and absence of chromatophores and other structures associated with scales have been used authentically for fish classification^[16, 17, 18, 19, 20, 21, 22]. Scale surface microstructure, scale size can be used to discriminate species or populations to discuss the possible causes of the observed differences^[23]. Generally, elliptic, oblong, pentagonal, rectangular, square, triangular and cycloid-shaped scales are found in teleosts^[24]. A comparative study on scale morphology and squamation of *Saurida tumbil* (Bloch, 1795) was used to identify the most useful characters for future systematic studies^[25]. Extend the investigation to the microstructure of the lepidonts in ten different species of the genus *Aphanius* by using SEM to evaluate the morphological differentiation and to provide a new set of characters that could be used in the study of phylogenetic relationships and the morphology of the denticular structures (Lepidonts) that occur on the crest of the circuli seems to be a useful tool for species discrimination in the genus *Aphanius*^[26].

Fish species are differentiated based on morphometric and meristic characters of scales. Scales are a unique part of the fish body; the study showed that the morphology of scales can be used successfully to distinguish between species even at fry and fingerling stages^[27]. Therefore this research work was planned to note the morphological details of scales on selected fish species by using scanning electron microscopy (SEM), which was not been studied earlier.

2. Materials and Methods

2.1. Area for fish sampling, Collection and Identification of fishes and processing of scales

The fish samples were collected from the four different areawise weekly markets in Nanded city, Maharashtra state, India during the year 2018-19. The source of fish to these markets is from the river Godavari flowing in the vicinity of the city and its tributaries and water bodies near Nanded in Maharashtra State. Two selected fish species were common in occurrence in all selected fish markets throughout collection periods. An international database was used as a standard and current reference <http://www.fishbase.org> to identify the fishes. Collected samples were identified as *Notopterus kapingat* (Pallas, 1769) and *Etroplus suratensis* (Bloch, 1790). For scale sampling from the fish body surface, the fish body was cleaned and washed using tap water and the scales were removed from eight different regions of the fish body to see any possible variations in the surface structure of scales. After removal and collection of scales, the samples were preserved in 4% formalin for further study. There is no requirement for more flattening of scales because these scales were small in size, delicate as well as flat hence easily mounted on the slide. 30% to 100% ethanol grades were used in ascending order for dehydration and acetocarmine stain was used for staining. To remove excess amount of stain the scale samples were slightly dipped in xylene and mounted on a slide using Canada balsam. All slides were dried in direct sunlight for one or two days then oven-dried at 40°C temperature. The dried slides were observed under the Carl Zeiss dissecting and compound binocular (primo star) microscopes and a TUCSEN eyepiece fixed camera was used for recording photo images. TUCSEN image processing software was used for scales measurements in mm (fig. 1-a and 2-a). All the scales samples and fish species have been preserved with collection reference numbers in the Department of Zoology, School of Life Sciences, S. R.T. M. University, Nanded, MS-431606, India for further reference.

2.2. Preparation of scales for SEM

For observation under the scanning electron microscopy scales were processed by dehydration process, dried and mounted on metallic stubs (aluminium blocks) using carbon double adhesive tape in the manner that the dorsal surface will remain upward and the ventral surface stick to the tape and sputtered with a layer of gold nano-particles in a gold coating unit (thickness100A, JEOL JSM-6100) then kept under vacuum using electron gun at an accelerating voltage of 20.0 kV of SEM. Then SEM images of scales were clicked at different magnifications by the digital camera of the SEM, which was connected to a monitor of a computer.

3. Results

3.1. Surface microstructures on the scales

Notopterus kapingat (Pallas, 1769) is a predatory fish and its body is laterally compressed. It is insectivorous and also feeds

on fish fry and fingerlings. *N. kapingat* is found all over India in major rivers and reservoirs. The morphological details of *N. kapingat* body scales show oval shape as the typical cycloid type of scale (Fig.1- a, and b) and (Fig. 3-a). Focus is present at the posterior end of the scale (Fig.1-b and 3-b). Besides these basic characters the radii, circuli, tubercles are also present on the scale surface. Radii are 6 to 10 in number arise from the focus and run towards the anterior margin of scale (Fig.1-b and 3-a). Each radii is intersected by many circuli. And circuli covers all the surfaces of the scale. Tubercles (Fig. 3-c) are present less in number and various tiny sizes. Other structures like chromatophores, ctenii, lepidonts are absent on the scale surface.

Etroplus suratensis (Bloch, 1790) is an exotic fish but is now established as one of the common fish in the catch from rivers and reservoirs of India. It is omnivorous fish that feed on plankton, aquatic insects, small crustaceans and young fishes. It is a food fish but also acts as a food for large predatory fishes in the aquatic food chain. The scales of this species have a rounded pentagonal shape (Fig.2 and 4-a). Circuli is closely set and many (Fig. 4-b). Focus is present at the center of the scale (Fig. 2 and 4-b). On the posterior side, tubercles have partially covered the scale (Fig. 4-b), chromatophores, Lepidonts are present. Each radii intersects the circuli which covers half of the scale but not the complete surface of scale (Fig. 2-b and 4-a). Circuli are closely set and they are many in number. The Lepidonts are small denticles or tooth-like microstructures (Fig. 4-c, d, e, f) firmly anchored to circuli of the rostral and lateral field that only become visible at high magnification of >2000 X^[23]. Lepidonts are prominent tiny, slender and sharply pointed, widely and closely spaced and firmly attached to the circuli in the deep sockets.

4. Discussions

Microstructures of scales of two teleost species studied were collected from the fish markets in Nanded city. It is located on the banks of Godavari River is a district headquarters. The fisher community from this region collects various food fishes from the river Godavari and its tributaries. The variation in scale structure of species from the family *Mugilidae* and reported that scales of the three mugilid fishes were different in the shape of focus and circuli size, shape and arrangement of lepidonts and ctenii. All these characters may be used in fish identification at the species level^[28]. In the present study, all these micro characters were studied but ctenii was absent in both species^[29]. And examined numerous hidden details of a scale in Indian goatfish *Parupeneus indicus* and concluded that the variation in the microstructures contributes effectively to the identification and classification of fishes^[29]. A similar report concluded that the detailed structures of fish scales can be helpful in the identification of fish up to the major group or species levels^[30]. In the tooth carp genus *Aphanius* (*Teleostei*, *Cyprinodontidae*) from endorheic basins in southwest Iran, they reported scale surface morphology, the microstructure of scale and scale size, shape and differences to achieve these objectives scale of three species of the genus *Aphanius* from the endorheic basin in southwest Iran have been studied using SEM images^[23]. In the present study, two species were examined in which *Etroplus suratensis* is ctenoid type and *Notopterus kapingat* is cycloid type of scales. The length and width of the scale were measured by using a microphotograph; to cover all the objectives binocular microscopy, SEM and measurement were used as recent tools of fish taxonomy. Through SEM techniques recorded that the

diversity of scale characteristics includes the form of scales and their morphometrics, radii counts, the shape of interradial tongues, circuli and their denticles or lepidonts, granulation and shape of ctenii in two species from two different genera of the family Sparidae: *Acanthopagrus bifasciatus* and *Rhabdosargus sarba* from Red Sea [31]. The present study was planned to know all characteristics and comparisons of scale ultra-structure in two species belongs to two different families (Table.1). The microstructure on the scale surface like attachment shapes and type of lepidonts on the crest of circuli in five species of the genus analyzed by scanning electron microscopy [32]. In the present study, the scale in *Etroplus suratensis* also shows lepidonts on the crest of circuli but shape and attachment structures were different. The cycloid scales of a cyprinid fish *Rutilus frisii kutum kamenskii* through SEM technique their detailed structure was reported about the focus position and rows of pigmented granular tubercles with characteristic shapes like round to oval, semi-

oval and even oblong structure [33]. In the present study *Notopterus kaporat* and *Etroplus suratensis* show tubercles in different sizes, shapes on the scale surface (Fig. 3-c and Fig. 4-b). In three *Plectorhynchus* species by SEM and recorded the shape of scales morphometrics and discrimination between the three *plectorhynchus* species for the rarity of taxonomic and biological information about three species from the Red Sea [34]. Whereas in the present study we selected two species belongs to two different families Cichlidae and Notopteridae respectively. The present study recorded a cycloid type of scale and second ctenoid type of scale and their distinct basic characteristics based on this fundamental difference in characters of scales which has taxonomical importance to identify the selected species in addition to the basic morphometrics and meristics. Even such details of body scales by SEM will also be solely useful to identify these fishes to species level without going into details of morpho-taxonomy.

Table 1: The structural details and morphological comparison of scales of two teleosts

Sr. No.	Characters	<i>Notopterus kaporat</i>	<i>Etroplus suratensis</i>
1.	Shape of the scale	Oval	Round pentagonal
2.	Focus of the scale	Present	Present
3.	Circuli	Present all over the scale	Present which covers half of the scale but not completed
4.	Tubercles	Present	Present
5.	Radii	Present 6 to 10 in number	Present 10 to 12 in number
6.	Margin of scale	Circular (cycloid type)	Wavy (ctenoid type)
7.	Chromatophores	Absent	Present
8.	Lepidonts	Absent	Present
9.	Length of scale	4.766mm	5.842mm
10.	Width of scale	2.681mm	6.632mm

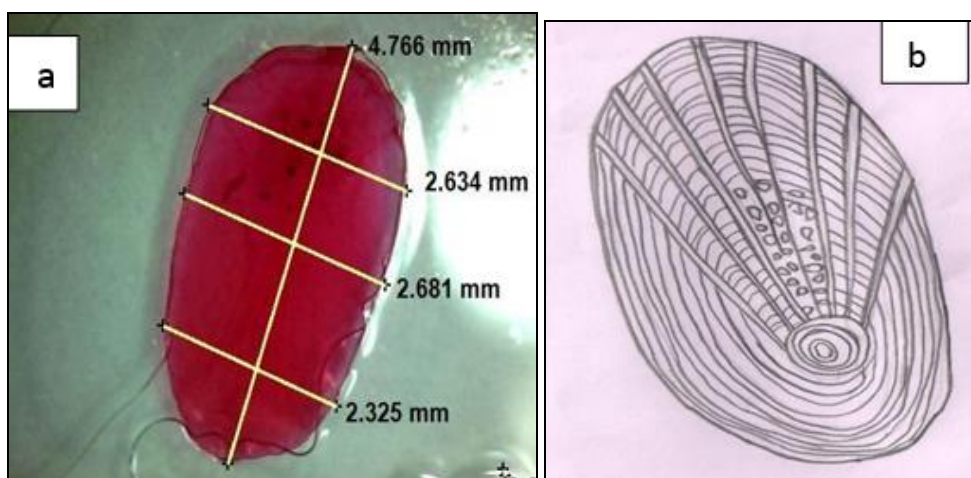


Fig 1: (a) Scale measurements and (b) diagrammatic structure of the scale of *Notopterus kaporat*

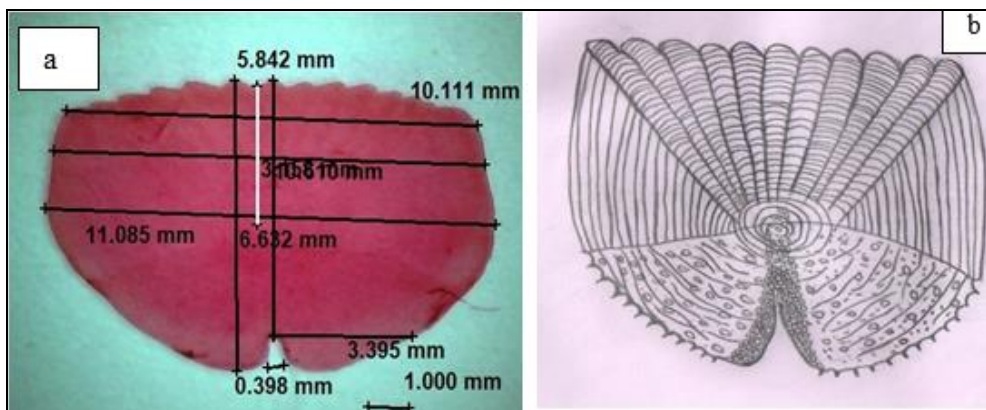


Fig 2: (a) Scale measurements and (b) diagrammatic structure of the scale of *Etroplus suratensis*

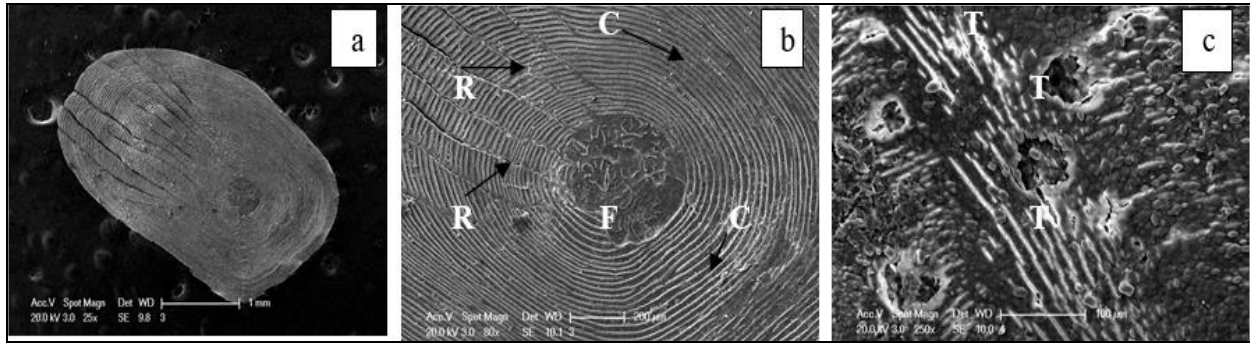


Fig 3: (a-c): (a) SEM of *Notopterus kipurat* scale, (b) Showing focus, radii and circuli, (c) Showing tubercles

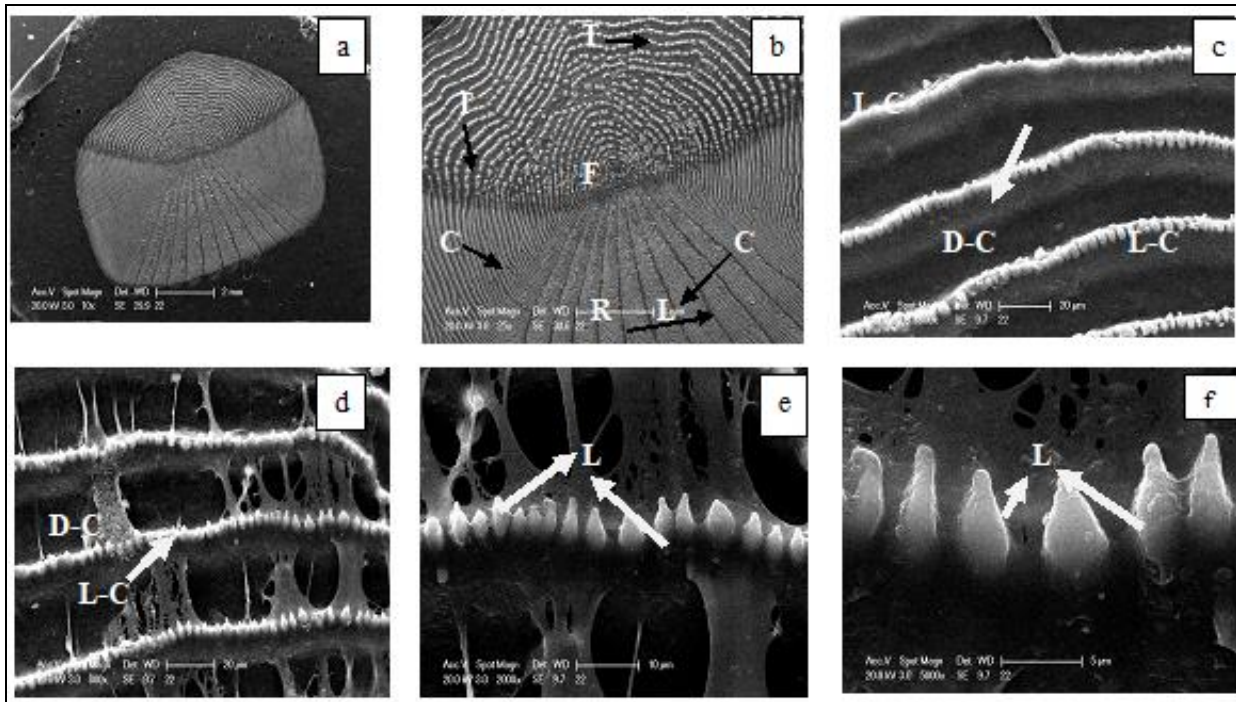


Fig 4 (a-f): (a) SEM of *Etroplus suratensis* Scale, (b) Scale showing partially covered with tubercles and partially with radii and circuli, (c) Lepidonts firmly attached on circuli, and (d, e, f) showing arrangement of Lepidonts on circuli

Abbreviations: R-radii, C-circuli, F-focus, T-tubercles, L-Lepidonts, D-C distance between two circuli, L-C Lepidonts on circuli.

5. Conclusion

In the present study, the scale structures of two teleosts *Notopterus kipurat* (Pallas, 1769) and *Etroplus suratensis* (Bloch, 1790) species were characterized. It is concluded that the different fish species were having different morphological surface structures. Due to this specific and unique impression of each fish scale that can be used in various fields like diversity and identification of fish species and as well as in food chain and conservation studies and forensics also.

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