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Scale structure of a cobitid fish, *Cobitis linea* (Heckel, 1849) using different modes of SEM

Detailed structure of the fish scale can be helpful in the identification of fishes up to major groups[1–3], species levels[4–6], phylogeny[7,8], sexual dimorphism[9], age determination[10–13] and pathology of fish scale due to water pollution of the water body[14–16] and for growth studies[17–26]. Studies on the scale structure of commercial fishes[17–21] have been undertaken and these studies have been successfully used for growth studies[22–26] and calculation of minimum harvestable size[27] so that legal fishable size can be prescribed for the determination of old age in the commercial fishes[11] and also the pollution status of the waterbody[14–16,28]. Due to some subjective reasons, in the past most of the lepidological studies are made on the commercial fishes[11,19–21,23,24,26,27].

Recently it has been reported that due to loss of fish habitat as a result of water management practices, release of effluents into the natural waterbodies and several anthropogenic activities, most of the fish species are under different types of threats as is evidenced from the squeezing of geographical limits and the reduction in the stocks of most of the fish species[29]. In some cases, the fish community structure is completely disrupted. Due to the reduction in the fish stocks, fish biologists are unable to get a large number of specimens for studies relating to fish bionomics. Under these circumstances, lepidological study is the best alternative as fish scale is considered to be the best tool in fish biology[30,31].

During a literature survey, it has been found that age and growth studies on minnows are rarely attempted and cobitids are completely ignored[11]. These two groups of fishes form one of the important links in the fish community structure in hillstreams[32–34], hence standardization of the scale structure of the fishes belonging to these two categories help in understanding their bionomics. Further, cobitids live at the bottom and are considered to be the natural scavengers. Their presence indicates that the water is regularly cleaned. Hence, their presence is encouraged in the fish aquaria for the natural cleaning of the glass walls.

An attempt has been made here to study the ultrastructure of the scale of an cobitid, *Cobitis linea* (Heckel, 1849) employing different modes of SEM collected from the streams of south-west Iran. In Iran, this group of fishes is generally known as 'sag mahi', meaning dogfish in Persian and equivalent to loach in English.

The fishes were collected by one of the authors (HE) using cast-net, scoop-net and ordinary insect net having mesh size not more than 5 mm during March 2005. The scales were gently removed with fine forceps from the left side of the body between the dorsal fin and lateral line in such a way that while removing the scales no damage is done to the scale. Immediately after their removal, the scales were cleaned mechanically using a fine brush and rinsed with triple distilled water. The cleaned scales were dehydrated in 30, 50, 70% ethanol and dried on filter paper. The scales were not put in absolute alcohol as 100% ethanol causes the scale margins to curl. To avoid curling, after 70% ethanol, the scales were kept between the microslides for 2–3 days. The cleaned and dried scales were mounted on metallic stubbs by double adhesive tape with dorsal surface upward and ventral surface sticking to the tape and coated with a 100 Å thick layer of gold in a gold coating unit. The gold coating overcomes the problem of charging and beam damage. An additional advantage of gold coating is to improve the strength of secondary electron signals from the specimen's surface. The scales were viewed under vacuum in a JEOL JSM–6100 scanning electron microscope at an accelerating voltage of 20 kV at low probe current. Various images of the scales were recorded on the photographic film. When gold-coated scales were not being viewed, the stubbs were stored in a desiccator to avoid moisture.

The different modes of SEM such as secondary electron image (SEI, low energy electrons), back scattered electron image (BEI, high energy electrons), mixed signals of both SEI and BEI and reverse polarity (negative of mixed signals) have been employed for the study of scale morphology[35] (Figure 1 *a–d*). For the details of the circuli, X and Y modulations have been use[35] (Figure 1 *e–f*).

The length and breadth of the scale of *C. linea* (Heckel, 1849) from Iranian freshwaters is almost the same having nearly circular shape (Figure 1 *a–d*). The scales maintain the same morphological proportions located on the different parts of the body. The scales present below the dorsal fin, above the lateral line are largest and those on the other parts of body are smaller in size. As the scale from this region depicts all the features, this scale has been designated as 'key scale'. The distinct focus which lies in the posterior part of the scale divides the scale into anterior (cephalic to focus), posterior (caudal to focus) and lateral fields on the lateral sides of the scale (Figure 1 *a–d*). The dorsal part is rough and the ventral part is smooth. The anterior part is embedded in the skin very firmly. The lines of growth (circuli) are distinct, overcrowded in the posterior part and widely separated in the anterior part. Each circuli is wedge-shaped, having broad base and pointed upper part (Figure 1 *e–d*). The arrangement of the circuli corresponds to the scale shape. The inter-circulus space is maximum in the anterior part, minimum in the posterior part and intermediate in the lateral part. The radii cut the circuli and annuli at right angle. The primary radii starts from the margin of the focus and reach up to the margin of the scale and are present on all fields of the scale (Figure 1 *a–d*). The secondary and tertiary radii start from the middle and near the margin of the scale respectively and reach up to the margin of the scale just like the primary radii[11]. The secondary and tertiary radii are absent in the posterior part of the scale. Some of the radii show bifurcations in the middle part of the scale, especially the primary radii which is a unique feature of the cobitid scale. The number of radii is more in the anterior part of the scale followed by lateral and posterior parts respectively. The radii formation is considered to be related to the accommodation power of the large surface area of the anterior and lateral parts of the scale in the lesser space as these two parts of the scale are overlapped by the posterior part of the preceding scale. The higher number of radii is correlated with the better nutritive conditions of the fish[11,19]. It is interesting to observe here that the scale of *C. linea* records the annuli very clearly, hence the age determination from this scale is easy and authentic. The scale in question (Figure 1 *a–f*) has recorded two clear annuli, indicating that the fish has completed two years of life and has entered the third year.

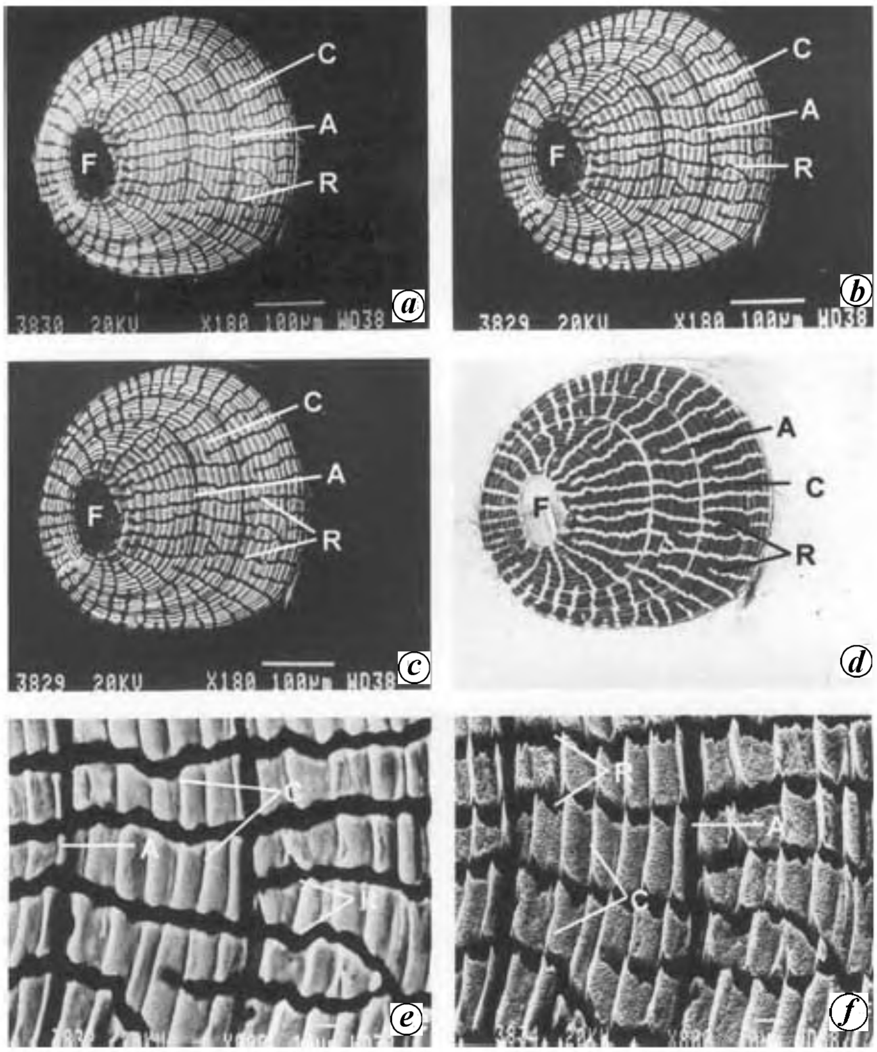

Figure 1 *a–f*. Scanning electron micrograph of the scale of *Cobitis linea* employing different modes and modulations: ***a***, Secondary electron image (SEI); ***b***, Back scattered electron image (BEI); ***c***, Mixed signals of BEI and SEI; ***d***, Reverse polarity of SEI and BEI; ***e***, Detailed structure of circuli employing X-modulation; ***f***, Detailed structure of circuli employing Y-modulation. A = annulus; C = circulus; F = focus; R = radii.

The scale of *C. linea* has been studied by employing the different modes of SEM such as SEI, BEI, mixed signals of both SEI and BEI and reverse polarity of SEI and BEI and the results are given in Figure 1 *a–d*. After the comparison of the Figure 1 *a–d*, it appears that for the study of morphology of the scale of this fish, the reverse polarity mode (Figure 1 *d*) gives better results.

For the detailed ultrastructure of the circuli and the focus of the scale of *C. linea*, Y-modulation (Figure 1 *f*) is more suitable as this modulation depicts better view of the surface layer of the circuli than X-modulation. The posterior part of the scale, which is exposed, has no granulations or chromatophores, a characteristic feature of the cycloid and ctenoid scales of carps and perches respectively[11,19–21].

The morphology and the ultrastructure of the cycloid scale of carps, both major carps and minnows, have been described by earlier workers[11,18–20,31], but the information on this aspect on the scale of cobitids is lacking. Though the scale of *C. linea* is basically similar to the cycloid scales of carps to some extent, it has its own characteristic features such as the presence of a few but very distinct circuli, prominent radii, very distinct posteriorly located focus and well-marked annuli. All these characteristics are not present in the scales of major carps and minnows[11,18–20,31]. Due to the presence of all these characteristic features on the scale of *Cobitis linea*, it is opined that this scale be designated as 'Cobitid scales'.

It is further opined that the scales of *C. linea* is most suitable for the age determination and growth studies. As morphotaxonomy of the cobitids-a group of hill stream fishes is posing problems in the identification up to species level, it may be possible to identify cobitids on the basis of scale structure. Further studies on the scale structure of other cobitids should be undertaken. Further, the scale of cobitids can be employed with great exactitude for age determination and growth studies and the age–growth data can be employed for the enhancement of cobitid populations in the hill streams.

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