Scanning Electron Microscopy of the scale morphology in Cyprinid fish, *Rutilus frisii kutum* Kamenskii, 1901 (Actinopterygii: Cyprinidae)

Esmaeili, H. R.[1]*; Gholami, Z.[2]

Abstracts

The normal and lateral line cycloid scales of a cyprinid fish *Rutilus frisii kutum* Kamenskii, 1901 have been subjected to scanning electron microscopy technique to study their detailed structure. The scales have the general morphological characteristics of the cycloid scales. In the normal scale located below the dorsal fin, the focus lies on the anterior region with few mucous pores. There is a clear-cut demonstration between the anterior and posterior region. In the posterior part, the scale has several rows of pigmented granules (tubercles) with different shapes of round to oval, semi-oval and even oblong structure. On the dorsal side, some circuli bear teeth-like structures called lepidonts can be seen which help the scale in firm attachment to the skin. The circuli may also have calcium projections. The lateral line scale has a canal which characteristically lies along the anterior-posterior axis with anterior and posterior openings. The anterior opening is wider than the posterior one and is hidden by an evelike extension cantilevered over it. Based on the obtained results it could be concluded that the shape and size of lepidonts on the circuli crest, the position, shape and size of tubercles and the presence of both anterior and posterior radii may provide reliable taxonomic tools. Changes in the circuli pattern, breakage of the circuli, loss of lepidonts and tubercles may be used to access the habitat condition of this carp especially habitat pollution.

Keywords: *Rutilus frisii kutum,* Scale structure, Lepidont, SEM, Iran

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1-Department of Biology, College of Sciences, Shiraz University, 71454, Shiraz, Iran.
2- Islamic Azad University, Babol Branch, Babol, Iran.
Correspondence Author's email: esmaeili@susc.ac.ir

Introduction

Scale morphology has proved to be a useful tool in fish classification and can be traced back to the time of Agassiz (1833–1843), who apparently was the first to use the scales for taxonomy and divided fishes into four groups accordingly: Placoidei, Ganoidei, Ctenoidei and Cycloidei. Besides its use in classification, scale morphology is an important tool in determining the diet of piscivorous predators or in the palaeontological analysis (Esmaeili, 2001). During the late 19th century and the first half of the 20th century, and with the great advancements in the field of light microscopy, the importance of scale morphology in systematics increased significantly (Cockrell, 1915; Chu, 1935; Lagler, 1947; Kobayashi, 1951, 1952). The importance of scale morphology used in classification was strengthened with the introduction and development of scanning electron microscopy (SEM) (De Lamater and Courtenay, 1974; Jawad 2005*a*, *b*; Jawad and AL-Jufaili, 2007). Detailed structure of the fish scale can also be helpful in age determination (Jhingran, 1957; Johal and Tandon, 1989a; Johal and Tandon, 1992; Tandon and Johal, 1996), past environment experienced by fish, discriminating between hatchery reared and wild populations, migration, pathology of fish scale due to water pollution of the water body (Johal and Dua, 1994; Johal and Dua, 1995; Johal and Sawhney, 1997) and for the growth studies (Cockrell, 1915; Chugunova, 1963; Qasim, 1964; Qasim and Bhatt, 1966; Tandon and Chaudhry, 1983-84; Johal et al., 1984; Lippitsch, 1990; Johal et al., 1996; Johal and Agarwal, 1997; Johal et al., 2001). In the past numerous studies on the scale structure of commercial fishes (Cockrell, 1915; Tandon and Chaudhry, 1983-84; Johal et al., 1984; Johal et al., 1996; Johal and Agarwal, 1997) have been undertaken in the world and the information available from these studies has been successfully made use for the growth studies (Chugunova, 1963; Qasim, 1964; Qasim and Bhatt, 1966; Lippitsch, 1990; Johal et al., 2001), calculation of minimum harvestable size (Johal and Tandon, 1987**)** so that legal fishable size can be prescribed, for the determination of old age in the commercial fishes (Tandon and Johal, 1996) and the pollution status of the waterbody (Johal and Dua, 1994; Johal and Dua, 1995; Johal and Sawhney, 1997; Johal and Sawhney, 1999). Due to some subjective reasons, in the past most of the lepidological studies have been on the commercial fishes (Qasim, 1964; Qasim and Bhatt, 1966; Johal et al., 1984; Johal and Tandon, 1987; Johal et al., 1996; Tandon and Johal, 1996; Johal and Agarwal, 1997; Johal et al., 2001). Recently it has been highlighted that due to loss of fish habitat as a result of water management practices, release of effluents into the natural water bodies and several anthropogenic activities, most of the fish species of developing and underdeveloped countries are under different types of threats which is evident from the squeezing of geographical limits and the reduction in the stocks of most of the fish species (Molour and Walker, 1998). In some cases the fish community structure is completely disrupted. Due to the reduction in the fish stocks, the fish biologists are unable to get large numbers of specimens

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for the studies relating to fish bionomics. Under these circumstances, lepidological study is the best alternative as fish scale is considered being the best tool in fish biology (Tandon and Johal, 1994; Johal, 2005). During the perusal of literature it has been found that there are very few published papers on scale structure of fishes in Iran (Esmaeili and Niknejad, 2007; Esmaeili et al., 2007; Esmaeili and Gholami, 2007, 2009; Esmaeili et al., 2009) and some taxa are completely ignored. Cyprinid fishes form one of the important links in the fish community structure in different water bodies hence, the standardization of the scale structure of these fishes shall help in the understanding of their bionomics. Keeping in mind the above facts, a first attempt has been made to study the scale ultrastructure of a cyprinid fish, *Rutilus frisii* employing scanning electron microscope. This fish is a benthopelagic, anadromous fish inhabit in a temperate climate. This carp is of great commercial value and is one of the most prized fish in the south Caspian Sea region. It is distributed in rivers of the northern coast of the black sea and in the Caspian Sea basins living in small schools in deep waters, but spawn in shallow water in tributaries feeding the lakes. In Iran, this fish is generally known as Sefid Mahi (Mahi Sefid) meaning white fish. Although different aspects of morphology (Abdolhay et al., 2010), biology and ecology (Coad, 2010; Yasemi and Niko, 2010) of this fish have been studied, but information on the detailed structure of its scale is rare. Hence, the purpose of this paper is to describe the ultrastructures of normal and lateral line scales of *R. frisii kutum* for better understanding of its bionomics.

Materials and methods

To study the ultrastructure of the scale of *R. frisii*, the fishes were collected from Caspian Sea basin, north of Iran (Khazar Abad, Sari) during March 2007 using cast net. More than 10 scales were gently removed with fine forceps from the left side of the body between the dorsal fin and lateral line and also from the lateral line (Fig. 1) in such a way that while removing the scales no damage was done to the scale. Immediately after their removal they were cleaned mechanically using a fine brush and rinsed with triple distilled water. Scales were then treated briefly with mild potassium hydroxide solution. Scales were studied by stereo microscopy and scanning electron microscopy. For scanning electron microscopy, cleaned scales were dehydrated in 30, 50, 70, and 90% ethanol and dried on filter paper (Lippitsch, 1990). The scales were not put in absolute alcohol as 100% ethanol caused 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 the metallic stubs by double adhesive tape with the dorsal surface upward and the ventral surface sticking to the tape and coated with a thick layer of gold in the gold coating unit (SC7640 SPUTTER COATER, Model: FISONS). 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.

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The scales were viewed under vacuum in scanning electron microscope model: Leica Cambridge 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 stubs were stored in desiccators to avoid moisture.

Results

The general structure of normal and lateral line scales *R. frisii* is given in figure 2. The scale of *R. frisii* almost has a circular shape (Fig. 2) although more other various shapes are also present. The scales present below the dorsal fin and above the lateral line are the largest, while the scales on other parts of body are smaller in size. As the scale from this region depicts all the features, hence, this scale has been designated as 'key scale'. Scale of this fish can be divided into anterior or rostral (A), posterior or caudal (P) and lateral fields (L). There is no cteni or ctenus like structures at the posterior part of the scale; hence, it is a cycloid scale. The anterior field is embedded in the skin and overlapped by the posterior side of the proceeding scale. The ventral part of the scale is shiny and smooth while the dorsal is rough, convex and has distinct structures, which consists of ridges, grooves and granules (tubercles).

Each scale has a focus, which is a part of the scale that develops first during ontogenesis (Fig. 3B). The focus which lies in the anterior part of *Rutilus frisii* scale divides the scale into anterior (cephalic to focus), posterior (caudal to focus) and lateral fields on the lateral sides of the scale (Figs. 2, 3A). The focus is sharp and clear in scales below the dorsal fin. Few mucous pores are found in the focus region (Fig. 3: B, K). From the focus lines of growth (the ridges) start appearing which are named as circuli (Fig. 3: B, F, G). The spaces between the circuli are called inter circular spaces (Fig. 3G). Circuli (the lines of growth) are distinct, overcrowded in the anterior part and almost widely separated in the lateral parts (Fig. 2: A, C). This is because of the anterior location of the focus on the scale. The posterior circuli are not very distinct due to the presence of tubercles in this region of scale. Each circuli is wedge shaped, having a broad base and a pointed upper part, although the arrangement of the circuli corresponds to the scale shape. The intercirculus space is minimum in the anterior part of the scale and maximum in the lateral parts. Bifurcation is seen in some circuli. The circuli have calcium projections (Fig. 3H). In the interradial space, some of the circuli bear small denticles or tooth-like structures that can be seen only under high magnification and are called lepidonts. They are widely-spaced blunted teeth having different size and present on the crest of circuli as seen in FigURE 3E.

In the anterior and posterior parts, the circuli are partitioned by deep and narrow grooves that run radialy towards the focus. They are called radii (Fig. 3: C, D). These scales with well developed radii are here called "sectioned".

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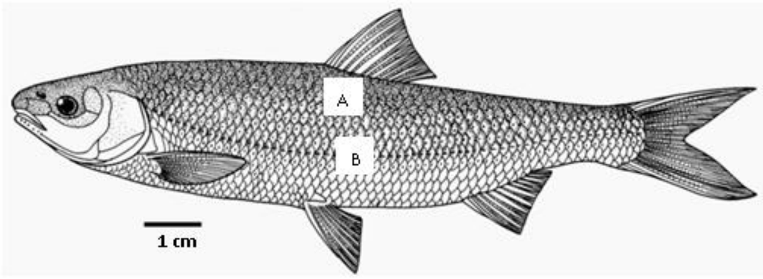


Figure 1: Schematic drawing of *R. frisii* showing location of key scales using for scanning electron microscopy. A, scale below dorsal fin; B, lateral line scale (Modified from Coad, 2010)

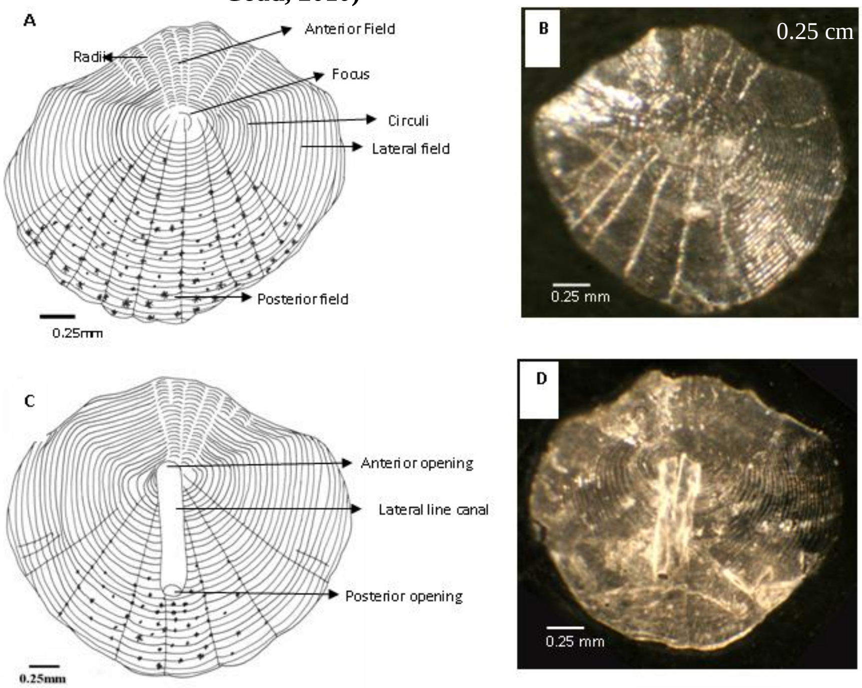
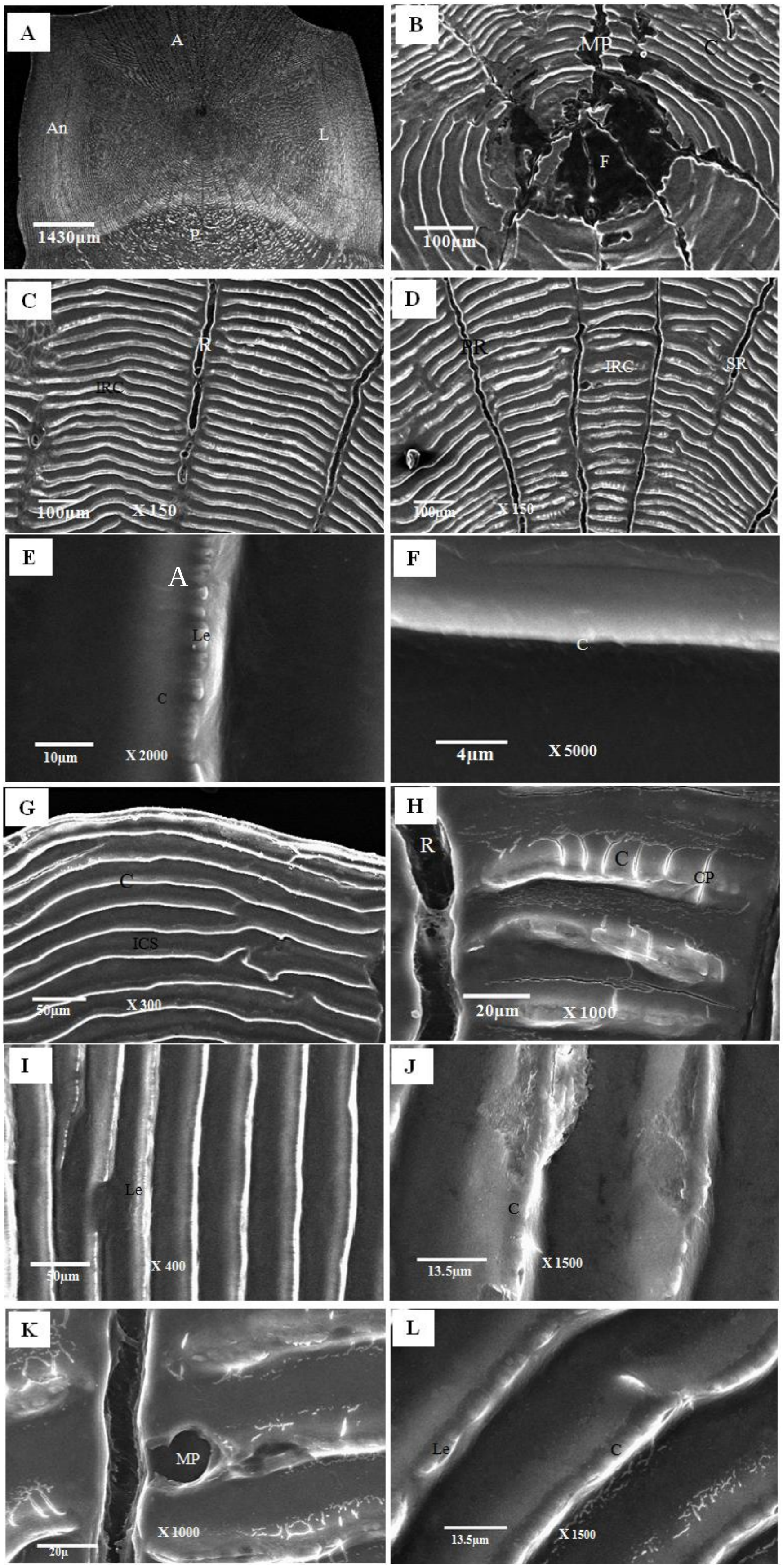


Figure 2: A, a schematic drawing of a sectioned cyprinid scale. B, a microscopic photograph of a normal *R. frisii* scale. C, a schematic drawing of a sectioned cyprinid lateral line scale. D, a microscopic photograph of *R. frisii* lateral line scale

The relative number of posterior radii is more than the anterior one. On the scale of *R. frisii* the radii can be categorized into three types depending on their point of origin on the scale including: Primary radii, originating from the focus, reaching the margin of the scale (Fig. 3D); Secondary radii, originating midway between the focus and the margin (Fig. 3D) and tertiary radii originating between midway and the margin (Fig. 4B). When compared to the tertiary radii, the relative number of primary and secondary radii more. There is no significant relationship between the number of radii and the scale size, as the numbers of radii depend on the location of the scale on the fish body. Like many other cyprinid fishes, the scale of R. *frisii* records the annuli, hence, the age determination from this scale is easy and authentic (Fig. 3A). In the posterior part of the scale, which is exposed, the circuli lose their characteristic features. In this part, the scale is covered with epidermis and has several rows of pigmented granules (tubercles) whose concentration depends on the location of the scale on the fish body (Fig. 3M, N). The shape of tubercles varies from round to oval, semi-oval and even oblong structure.



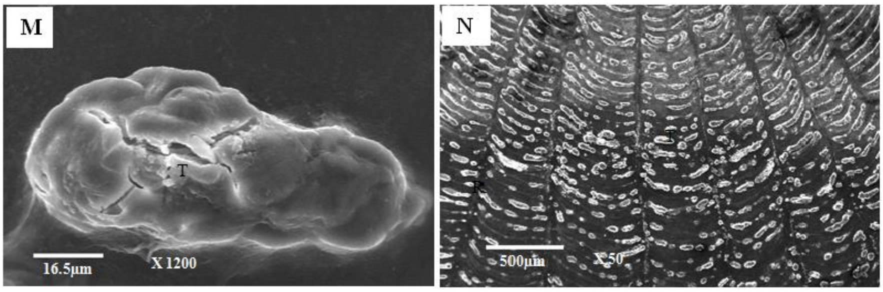


Figure 3, A-N: The scale structure of *R. frisii*. Anterior (A), Posterior (P), Lateral (L), radii (R), circuli (C), lepidont (Le), mucous pore (M.P), focus (F), Primary radii (P.R), secondary radii (S.R) and tertiary radii (T. R), Interradial circuli (IRC), Inter circular space (ICS), Calcium projection (CP), Lateral line canal (LLC), Tubercle (T), Annuli (An).

The outer surface of tubercle is not smooth; on the contrary, it has several warts and wrinkles. They vary considerably in their length and are located in the interradial space covering a large part of the caudal field. The scale tongue is very clear at most posterior parts of the scale of this carp (Fig. 4C). Schematic drawing of the lateral line scale is showed in (Fig. 2C). The lateral line scale is also divided into anterior (rostral) and posterior (caudal) parts. Focus is absent in the lateral line scale due to the position of the lateral line canal. This scale has a canal which characteristically lies along the anterior-posterior axis (Fig. 4A), slightly towards the posterior part with two openings (Fig. 2C). The anterior part of the lateral line has several mucus pores. In the anterior part, the circuli are densely spaced. The granulation on the posterior portion is the same as the normal scales located below the dorsal fin, except that the canal is extended to the granulation area.

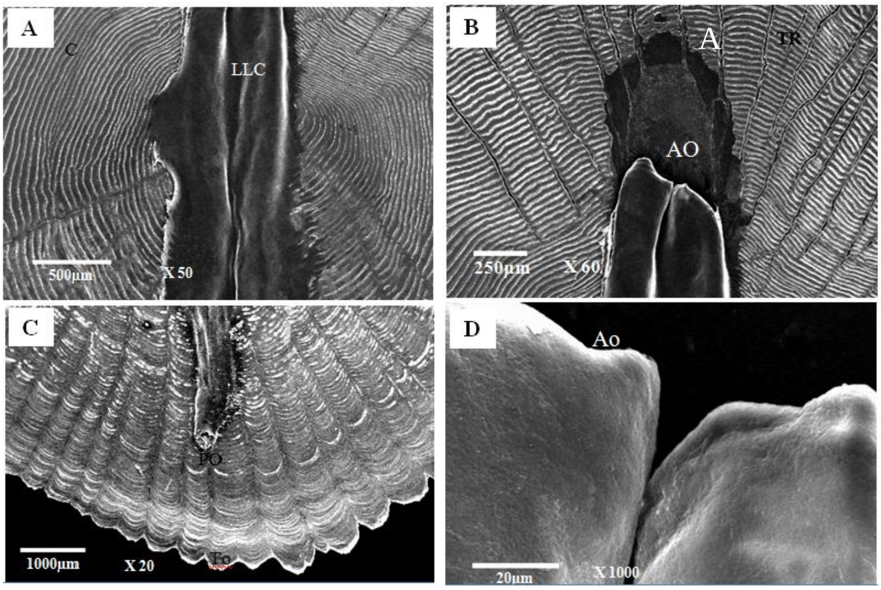


Figure 4: The SEM structure of the lateral line scale of *R. frisii*. Circuli (C), Tertiary radii (T. R), Lateral line canal (LLC), Tubercle (T), Tongue (To), Annuli (An), Anterior Opening (AO), Posterior Opening (PO)

Discussion

This study of scale morphology in *R. frisii kutum* illustrates the general characters present in other cyprinid species including focus, radii, circuli, intercirlcular space and tubercles. Each circuli is wedge shaped, having a broad base and a pointed upper part although the arrangement of the circuli corresponds to the scale shape. The intercirculus space is minimum in the anterior part of scale and maximum in the lateral parts. The circuli formation is due to the excess calcium salts secreted by the skin and their subsequent deposition on the scale. Therefore, the distance between the circuli indicates fast and slow growth period. The interradial circuli are almost straight. According to Lippitsch (1990) the shape of the first interradial circuli (convex, straight, or concave) is a characteristic within species of cichlid fishes, but in some cases seems to be modified by external factors. Some circuli bear lepidonts which are widely-spaced blunted teeth with different sizes. Lepidonts of different size and shape have been reported in many fish species (Delmater and Courtenay, 1974; Lippitsch, 1990; Javad and AL-Jufail, 2007). Delmater and Courtenay (1974) illustrated these structures for several fish genera and suggested that they might characterize genera and may even distinguish some taxa at the specific level. Lepidonts are not homologous to breeding tubercles and contact organs (Delmater and Courtenay, 1974). The microscopic size and the covered position of lepidonts make the function as contact organs (to facilitate contact between males and females during reproduction highly improbable (Delmater and Courtenay, 1974). Javad and Al-Jufaili (2007) stated that there is no variation in the shape and size of the scale denticles (lepidons) from different regions of the body of greater lizardfish (Javad and Al-Jufail, 2007).The surface structures of the overlapped region of the scales of *R. frisii* do not change after they are formed. This stability suggests that the shape, size and spacing of the denticles may provide useful criteria for systematics, as already proposed in other teleosts (Delamater and Courtenay, 1974). This hypothesis requires test and refinement by comparisons of these denticles with those in other cichlid species. In contrast, the tubercles and ridges of the free region change in shape, and increase in surface and thickness, during ontogeny. Consequently, the ornamentation patterns are specific to different ontogenetic stages, and this should be taken into account in systematic studies. Growth phenomena are evident in *R. frisii* scales by the presence of primary and secondary radii (Lippitsch, 1992; Kuusipalo, 2000). There is no significant relationship between number of radii and scale size, as the numbers of radii depend on location of the scale on the fish body. However, in some other teleosts such as *Mullus surmuletus* L., 1758 and *M. barbatus* L., 1758, the number of radii is correlated to fish size (Javad and Al-Jufail, 2007). Javad and Al-Jufaili (2007) showed that in greater lizard fish, the number of radii is three and remains constant on a given scale during ontogeny. The consistency in radii number makes it a good taxonomic criterion to identify this fish in a further comparative study (Javad and AL-Jufail, 2007). The presence of primary and secondary radii is a growth phenomenon and obviously only weakly influenced by genetic factors (Lippitsch, 1990). 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 (Tandon and Johal, 1996; Johal et al., 1984). Radii represent the line of scale flexibility. The focus in this cyprinid fish is sharp and clear in scales below the dorsal fin. Few mucous pores are found in the focus region. The same has been reported in the scale of some other cyprinid fishes such as *Catla catla* (Hamilton–Buchanan, 1822) and *Tor putitora* (Hamilton–Buchanan, 1822; Johal et al., 1999). Tubercles are formed by aggregation of epithelial layer of the skin which covers the posterior part of the scale. They impart specific color to fish as they contain chromatophores in the outer surface. Granulations or presence of chromatophores on the posterior part of scale is a characteristic feature of the cycloid and ctenoid scales of carps and perches respectively (Johal et al., 1984; Johal et al., 1996; Tandon and Johal, 1996; Johal and Agarwal, 1997). The scale of *R. frisii* shows signs of resorption (Fig. 3B). Resorption of teleost scales occurs under various physiological and experimental conditions and has been suggested to be initiated during periods of increased calcium demand such as during sexual maturation when oestradiol – 17B (E_2) induce vitellogenin synthesis and binding of calcium ions to vitellogenin (Persson et al., 1999). According to Persson et al. (1995), E_2–induced calcium mobilization from scales in rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) is known to result from an increased osteoclast activity.

The anterior opening of the lateral line canal is wider than the posterior opening and is hidden by an evelike extension cantilevered over it (Fig. 4: B, C, D). The same structure has been reported in some other fishes (Delmater and Courtenay, 1973). According to Delmater and Courtenay (1973) scanning electron microscopy of lateral line scales of teleostean fishes demonstrates a wide range of structural variation of lateral line canal from a simple direct or slightly oblique perforation to an extended canal with or without simple to highly complex cantilevered extensions acting as covers anterior opening.

The results presented in this lepidological study reveal that although the scale of *R. frisii* shows the general structure of a cycloid cyprinid scale but the shape and size of lepidonts on the circuli crest, presence of both anterior and posterior radii may provide reliable taxonomic tools. Changes in the circuli pattern, breakage of the circuli, loss of lepidonts and tubercles may be used to access the habitat condition of this carp such as temperature, food abundance and also pollution.

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