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Scale structure of a cyprinid fish, *Garra Rossica* (Nikol'skii, 1900) using scanning electron microscope (SEM)

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Abstract

The normal and lateral line cycloid scales of the native species *Garra rossica* (Nikol'skii, 1900), one of the most common and poorly known cyprinid fish in south-eastern Iran have been subjected to the scanning electron microscopy technique in order to study their detailed structure. Normal scale below the dorsal fin showed a clear-cut demonstration between the anterior and posterior region with the focus lying towards the anterior region that is covered by reticulate or honeycomb form structures with few mucous pores. There is a wedge shaped circuli only in the anterior and lateral fields which are partitioned by deep and narrow grooves called radii and run radially towards the focus. On the dorsal side, the anterior circuli bear numerous pointed lepidonts. In the posterior section, the scale has several rows of pigmented granules (tubercles) with different shapes from round to oval, semi-oval and even oblong structure. The lateral line scale has a canal which characteristically lies along the anterior-posterior axis, with a wider anterior opening than the posterior opening which is hidden by an eyelike extension cantilevered over it. Although the scale of *G. rossica* shows the general structure of a cycloid cyprinid scale, the shape and size of lepidonts on the circuli crest, shape and size of tubercles on the posterior region and the pattern of reticulate or honeycomb that form structures in the focus region may provide more reliable taxonomic tools. The present analysis of scale morphology in *G. rossica* may be used in combination with other morphological and molecular data in a synergic approach to the phylogenetic and systematic study of the genus.

Keywords*: Garra rossica*; scanning electron microscopy; scale; lepidont; radii; circuli

1. Introduction

General shape, size and the architectural specifications (focus, circuli, radii, tubercles, lepidonts, or scale denticles, larval marks, cteni and spines) of the fish scales using light and scanning electron microscopy have been effectively used as versatile research material in various ichthyological researches such as phylogeny, sexual dimorphism, past environment experienced by fish, discrimination between hatchery reared and wild populations, determined times of migration, periods of food scarcity, illness, the pathology of a fish scale due to water pollution, growth studies and pollution status of the water body as bio-indicator of water quality [1-17]. The scales have also been used in paleontological studies [18], to determine the diet of piscivorous predators, biomaterial design [19], as well as population genetic studies pertaining to endangered fish species (extraction of DNA from fish scales) [20].

During perusal of the literature it has been found that despite the above mentioned facts, lepidological studies on native cyprinid fishes of Iran are rarely chosen and some taxa such as the genus *Garra* are ignored.

The genus *Garra* is found throughout southwest Asia and from Africa to southeast Asia. There are about 73 species [21] and 4 are recognized from Iran: *G. persica* (Berg, l9l3), *G. rufa* (Heckel, 1843), *G. variabilis* (Heckel, 1843) and *G. rossica* (Nikol'skii, 1900) [22]. Members of the genus *Garra* from the cyprinids live at the bottom, scrape algae from rocks and are considered to be the natural scavengers [21]. This genus is characterised by a small to moderate-sized body, elongate and almost cylindrical, a rounded snout with the inferior and crescent-shaped mouth, a horny lower jaw, usually fringed upper lip continuous with the snout, and a suctorial disc with free posterior margin [21].

The present communication is a first attempt to shed light on the ultrastructural features engraved on the scales of a native cyprinid fish, *Garra rossica* (Nikolskii, 1900), collected from south-east Iran, employing scanning electron microscope. This fish is generally known as Rossbarbe and in Iran as, 'mahi-e sang lis' meaning stone licker in Persian and is widely distributed in eastern Iran [21].

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2. Material and methods

To study the ultrastructure of the scale of *Garra rossica*, the fishes were collected from the Irandegan River, Khash, Sistan and Baluchistan province in the southeast of Iran (Fig. 1) using a cast-net during the year 2009. The 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. 2) in such a way that while removing the scales no damage was being done to the scale [23]. Immediately, the scales were washed thoroughly with water by gently rubbing them between the tips of the fingers and cleared with 3% potassium hydroxide solution too. The Cleared scales were dehydrated in 30, 50, 70, and 90% ethanol for 20 minutes respectively and dried on filter paper [1]. The scales were not put in absolute alcohol as 100% ethanol caused the scale margins to curl. To avoid curling, after 90% ethanol, the scales were kept between the two microslides for 2-3 days. The cleaned and dried scales were mounted on 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 a gold coating unit (SC7640 SPUTTER COATER, Model: FISONS). The scales were then viewed under vacuum in a Leica Cambridge scanning electron microscope at an accelerating voltage of 20 kv at low probe current. Various images of the scales were photographed by a digital camera attached to the system.

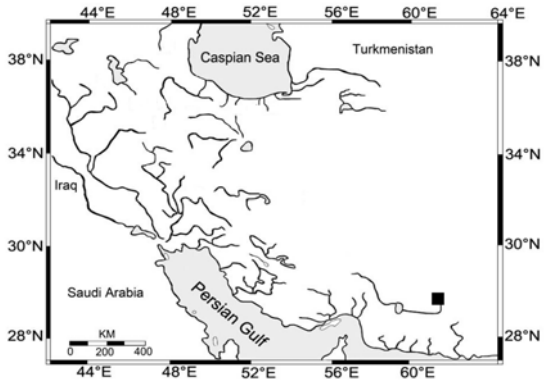

Fig. 1. Map of Iran. The black quadrangular indicate the fish collection site of *Garra rossica*

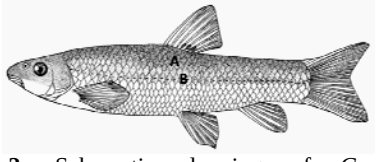

Fig. 2. Schematic drawing of *Garra rossica* (http://www.briancoad.com) showing location of key scales using for scanning electron microscopy. A) scale below the dorsal fin; B) lateral line scale

3. Results

The general structure of normal and lateral line scales of a hypothetical cyprinid fish and that of *G. rossica* from southeastern Iran is given in Fig. 3. The length of the studied scales of *G. rossica* is greater than its breadth, so it does not have a circular shape (Fig. 3).

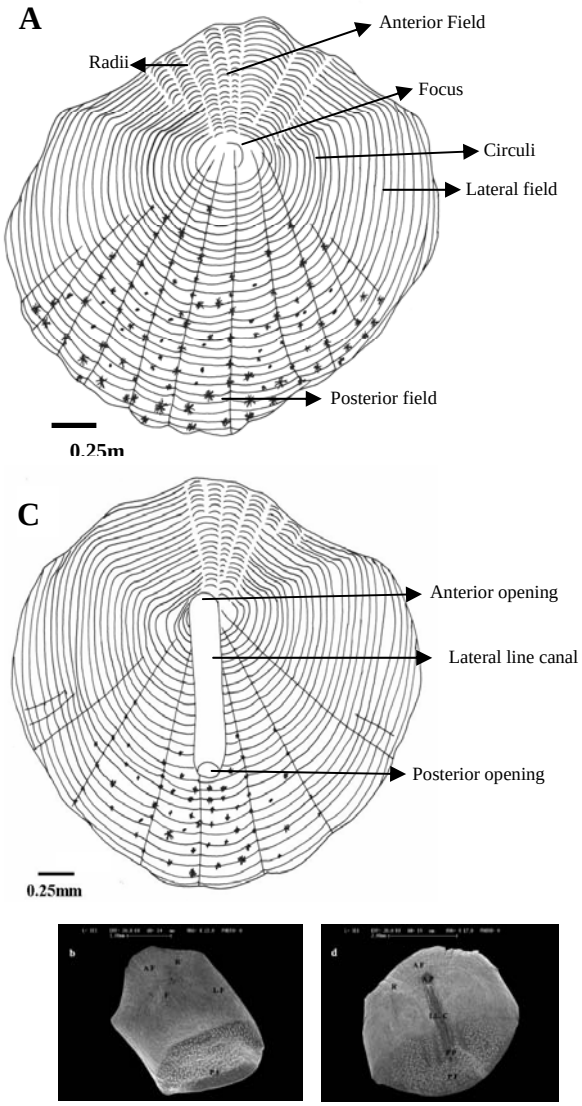

Fig. 3. (a) Schematic drawing of a sectioned cyprinid scale and (b) SEM microphotograph of a normal *G. rossica* scale and (c) schematic drawing of a sectioned cyprinid lateral line scale and (d) SEM microphotograph of *G. rossica* lateral line scale. Anterior field (AF), Anterior pore (AP), Focus (F), Lateral field (LF), Lateral line canal (LL. C), Posterior field (PF), Posterior pore (PP), Radii (R)

The scale of this fish can be divided into anterior or rostral (A), posterior or caudal (P), and lateral fields (L). There is no cteni 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 a scale is shiny and smooth while the dorsal is rough, convex and has distinct structures, consisting of ridges, grooves and granules (tubercles). Each scale has a focus, (Fig. 3) which is the first part of the scale to be formed. The focus, which lies in the anterior 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 (Figs. 3 and 4 *a*). The area enclosed by the focus is covered by reticulate or honeycomb form structures (Figs. 4 *a, b*). Few mucous pores are found in the focus region (Fig. 4 *b*). From the focus, lines of growth (the ridges) start appearing. These are named circuli (Figs. 3 and 4). The space between circuli is called inter circular space. Circuli (the lines of growth) are distinct, overcrowded in the anterior part and widely separated in the lateral parts. This is because of the anterior location of the focus on the scale. The circuli are not found on the posterior part of the scale. Interradial circuli in the anterior part of the scales are almost straight, but slightly convex or concave circuli also are observed (Figs. 4 *c-e*). 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. The interradial circuli are almost straight. In the interradial space the circuli bear small denticles or tooth-like structures that can be seen only under high magnification and are called lepidonts. These circuli are found only in the anterior part of the scale, therefore lateral interradial circuli have no lepidont. They are widely spaced, cuspidate and irregular teeth of different size on the crest of circuli as seen in (Figs. 4 *d, e*). In the anterior, posterior and lateral parts, the circuli are partitioned by deep and narrow grooves that run radially towards the focus. They are called radii. These scales with well developed radii are here called “sectioned”. On the scale of *G. rossica* the radii can be categorized into three types depending upon their point of origin on the scale including: Primary radius, extends from the focus to the margin of the scale; Secondary radius, does not extend all the way out to the margin of the scale; and tertiary radius extend between midway and margin (Figs. 4 *a, c*). Radii represent the line of scale flexibility. The scale of *G. rossica* records the annuli, hence, the age determination from this scale is easy and authentic (Fig. 4). 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 (Figs. 4 *f-h*). The shape of tubercles varies from round to oval, semi-oval and even an oblong structure. The outer surface of a tubercle is not smooth; on the contrary, it has several warts and wrinkles. They vary considerably in their size and are located in the interradial space covering a large part of the caudal field.

A schematic drawing of a lateral line scale is shown in (Fig. 3). A lateral line scale is also divided into anterior (rostral) and posterior (caudal) parts. The focus is absent in the lateral line scale. This scale has a canal which, characteristically, lies almost along the anterior-posterior axis, with two openings in the studied scales. The anterior opening of the lateral line canal is wider than the posterior opening and is hidden by an eyelike extension cantilevered over it (Figs. 5 *a, b, e, f*). The posterior part of the lateral line has several mucus pores (Fig. 5 *f*). In the anterior part, the circuli are densely spaced (Figs. 5 *a-d*). The granulation on the posterior portion is the same as normal scales located below the dorsal fin, except that the canal is extended to the granulation area.

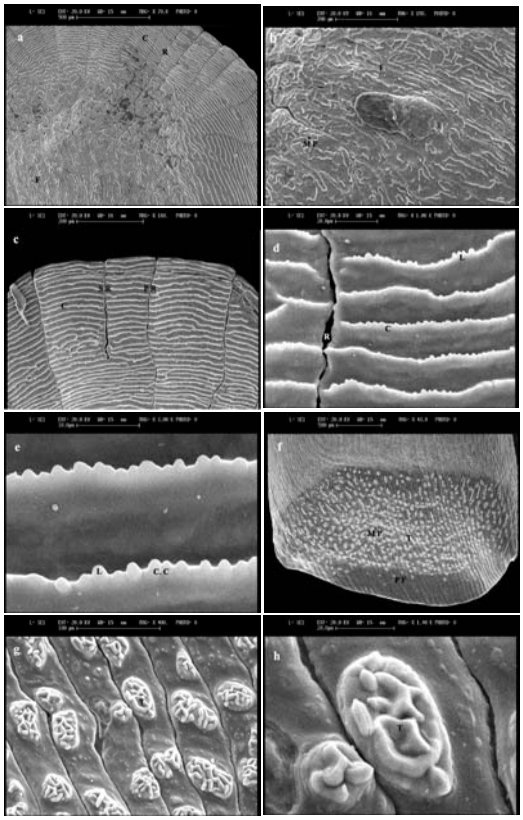

Fig. 4. SEM microphotographs of *G. rossica* scale. Circuli (C), crest of circuli (C.C), focus (F), lepidont (L), mucous pore (MP), posterior field (PF), primary radii (PR), radii (R), secondary radii (SR), and tubercle (T)

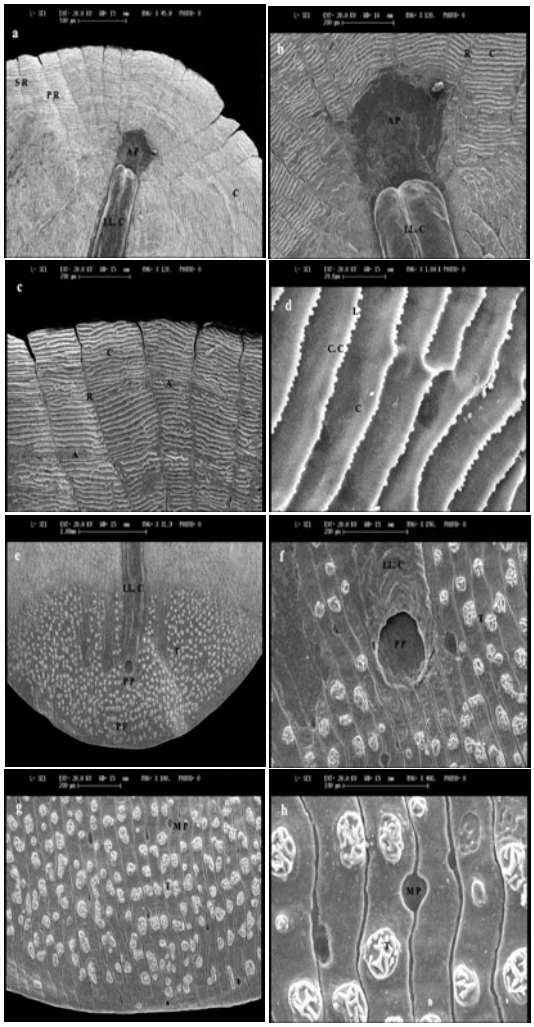

Fig. 5. SEM microphotographs of lateral line scale of *G. rossica*. Annulus (A), Anterior pore (AP), circuli (C), crest of circuli (C.C), lateral line canal (LL. C), lepidont (L), mucous pore (MP), posterior field (PF), Posterior pore (PP), Primary radii (PR), radii (R), Secondary radii (SR), and tubercle (T)

4. Discussion

The scale morphology of a cyprinid fish, *Garra rossica* (Nikol'skii, 1900) from the southeast of Iran shows the general architectural pattern of a cycloid cyprinid scale having focus, circuli and radii. The same has been reported in the scale of some other cyprinid fishes such as *Hypophthalmichthys molitrix* (Val., 1844), *Capoeta damascina* (Valenciennes in Cuvier and Valenciennes, 1842), *Catla catla* (Hamilton-Buchanan, 1822) and *Tor putitora* (Hamilton–Buchanan, 1822) [10, 23-24]. The focus of the scale is distinct and located in the anterior field and is the first part of the scale to be formed during ontogenesis. Its position and shape in different species may vary, being oval, circular, rectangular, or triangular [17].

The circuli formation is due to the excess calcium salts secreted by the skin and their subsequent deposition on the scale. So, the distance between circuli indicates a fast or slow growth period. This is especially useful in temperate waters where pronounced retardation of growth of body and scales occurs in fall and winter, causing the spacing between the circuli to decrease and thus leaving a band on the scales called an annulus or periodic zone [10]. Bhatia [25] stated that production of the periodic zones in *Salmo irideus*, a synonym of *Oncorhynchus mykiss* (Walbaum, 1792) is entirely or primarily dependent on the abundant or deficient food supplies of the fish. Fisher and Pearcy [26] indicate that seasonal change in the spacing of scale circuli is a useful indicator of seasonal change in the growth rate of Coho salmon, *Oncorhynchus kisutch* (Walbaum, 1792) in the ocean. However, interpreting such marks as annuli requires caution since any retardation in growth may leave a mark. The stress of spawning, movement from fresh to salt water, parasitism, injury, pollution, and sharp and prolonged change in temperature may all leave marks on the scales similar to annuli [17]. Scales grow in a direct relationship with body growth, making it possible to measure the distance between annuli and back calculate the age at different body sizes [17]. The arrangement of the circuli corresponds to the scale shape and its arrangement around the focus has already been given importance for its role in species specificity [5]. According to Lippistsch [1], the shape of the first interradial circuli (convex, straight, or concave) is characteristic within species of cichlid fishes, but in some cases seems to be modified by external factors.

Lepidnots of different size and shape have been reported in many fish species [1, 5, 10, 13, 27]. These denticles are also important structures known to support species distinctness. The taxa usually differ with regard to shape, texture, attachment and orientation of lepidonts on the crest of circuli [5, 28]. Lepidonts are not homologous to breeding tubercles and contact organs. 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 [27]. Jawad and Al-Jufaili [13] stated that there is no variation in the shape and size of the scale denticles (lepidonts) from different regions of the body of greater lizardfish.

Radii may be present in different fields: only anterior, as in pickerels (*Esox*); only posterior, as in shiners (*Notropis*); anterior and posterior, as in suckers (Catostomidae); or even in all four fields, as in barbs (*Barbus*) [17]. The relative number of

primary and secondary radii is more as compared to the tertiary radii. There is no significant relationship between the number of radii and scale size, as the numbers of radii depend on the location of the scale on the fish body. However, according to Jawad and Al-Jufaili [13], in some other teleostes such as *Mullus surmuletus* L., 1758 and *M. barbatus* L., 1758, the number of radii is correlated to fish size. It was shown that in greater lizard fish, the number of radii is three and remains constant on a given scale during ontogeny. The consistency in the radii number makes it a good taxonomic criterion to identify this fish in a further comparative study. The presence of primary and secondary radii is a growth phenomenon and obviously only weakly influenced by genetic factors [1]. 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 correlate to the better nutritive conditions of the fish [10,29-30].

Tubercles are formed by the aggregation of the 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 the presence of chromatophores on the posterior part of a scale is a characteristic feature of the cycloid and ctenoid scales of carp and perch respectively [29-32]. Kaur and Dua [5] have made an attempt to study the importance of these tubercles in species specificity of the four species of *Labeo*. They claim differences were with regard to their relative abundance and distribution in the posterior region, reflecting a general difference in the colour of fish species. Distinctness was, however, more profound for their size and shape.

Lateral line scales have been utilized by several workers to prove their potential in fish classification and taxonomy [5, 28]. The number, position of canal, its alignment, viz. straight or oblique, perforations, i.e. anterior, posterior or lateral, are important features that have been given substantial relevance for fish classification [5]. According to DeLamater and Courtenay [28] scanning electron microscopy of lateral line scales of teleostean fish demonstrates a wide range of structural variation of the 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 for the anterior opening.

The results presented in this lepidological study reveal that although the scale of *G. rossica* shows the general structure of a cycloid cyprinid scale, the shape and size of lepidonts on the circuli crest, shape and size of tubercles on the posterior region and the pattern of reticulate or honeycomb that form structures in the focus region may provide more reliable taxonomic tools.

Focus shape and position, circuli, chromatophores, lepidonts, position of radii, regenerated region and lateral line canal are features present on the fish body scales that constitute important criteria for separating the taxonomic groups [5, 9, 10, 14-15], therefore, a general agreement can be concluded for accrediting these structural specifications towards species characterization and discrimination.

The present analysis of scale morphology in *G. rossica* may be used in combination with other morphological and molecular data in a synergic approach to the phylogenetic and systematic study of the genus.

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