IUFS Journal of Biology *Research Article*
IUFS J Biol 2014, 73(1): 31-42

Scale Surface Microstructure and Scale Size in Three Mugilid Fishes (Teleostei, Mugilidae) of Iran from Three Different Habitats

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Abstract

A substantial number of species within mugilid family has been recorded from marine, brackish and freshwater bodies of Iran. Descriptions of these species have been based mainly on morphological and anatomical data; however, the characters related to the fish scales have not up to now been intensively studied and employed for the identification of the fish species. The objective of this study is to test whether (i) scale surface morphology and microstructure, and (ii) scale size can be used to discriminate species. To achieve these objectives, scales of three species of the genus *Liza* from three different habitats, *Liza abu* (freshwater, Maharlu basin), *Liza klunzingeri* (sea, Persian Gulf) and *Liza saliens* (brackish water, Caspian Sea) have been studied using SEM images, scale measurements, and uni and multivariate statistics. It is opined that scale surface morphology and microstructure may help in distinguishing the species. In addition, scale size and J-indices, represent a valuable tool for species separation, which corroborates earlier studies for the use of these indices in fish taxonomy.

Keywords: Mugilid fish, scale ultrastructure, habitat, taxonomy
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(Received: 01.11.2013 Accepted: 18.06.2014)

Introduction

Mugilidae, commonly referred to as mullets, occurred worldwide in tropical and temperate marine and brackish water and even some in freshwater (Cardona 2006). The genus *Liza* (Jordan and Swain 1884) commonly inhabit tropical and warm-temperate estuaries where they play a crucial ecological role (Cardona 2006). These species are highly euryhaline and inhabit in a wide range of salinities and due to their euryhalinity they are often stocked in brackish coastal lagoons and are introduced into fresh water and reservios (Cardona 2006). The family comprised of 17 genera and 72 species (Nelson 2006). The family Mugilidae plays an important role in commercial fisheries and aquaculture worldwide. Consequently, a body of information exists especially on various aspects of its reproductive biology, behavioral patterns and population dynamics including those of Thomson (1957), Sarojini (1958), Anderson (1958), Abraham et al. (1966), Yashouv and Berner-Samsonov (1970), Chan and Chua (1980), Edwards et al. (1988), Hoda and Qureshi (1989), Abou-Seedo and Al-Khatib (1995), Randall (1995), Abou-Seedo et al. (2002), Golani (2002), Abou-seedo and Dadzie (2004), Dadzie et al. (2005), Bu-Olayan and Thomas (2006), Valinassab et al. (2006), Hakimelahi et al. (2010) and Bartulović et al. (2011). However, information on the ultrastructure of these fishes is scarce.

Fish scales are important structures used as versatile research material in ichthyological researches because of their unique features: They are easily available, and their preparation for image processing is very simple and their study does not require killing the specimens. However, it has been shown that, detailed structure of the fish scale can be helpful in studying the taxonomy and phylogeny of the fishes (Lippitsch 1990; Javad and Al-Jufaili 2007; Ferrito et al. 2009) age determination (Jhingran 1957; Tandon and Johal 1996; Johal and Tandon 1989; Johal and Tandon 1992; Gholami et al. 2013), 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; Esmaeili 2001), for the growth studies (Cockrell 1915; Tandon and Chaudhry 1983-84; Johal et al. 1984; Johal et al. 1996; Johal and Agarwal 1997; Chugunova 1963; Qasim 1964; Qasim and Bhatt 1966; Lippitsch 1990; Johal et al. 2001), in determining the diet of piscivorous predators, in the palaeontological analysis (Esmaeili 2001) and genetic studies pertaining to endangered fish species extraction of DNA from fish scales (Kumar et al. 2007).

Detailed structure of the fish scale can be helpful in the identification of fish up to major groups and species levels (Abraham et. al. 1966; Bartulović et al. 2011).

In this study scale ultrastructure of the three Mugilid fish from three different habitats, *Liza abu* (Heckel 1843) from a freshwater system (Maharlu basin), *Liza klunzingeri* (Day 1888) from marine environment (Persian Gulf), and *Liza saliens* (Risso 1810) from brackish water (Caspian Sea) were compared using a scanning electron microscope.

Material and methods

To study the ultrastructure of the scales of three mugilid fish species including *Liza abu* (Heckel 1843), *Liza klunzingeri* (Day 1888) and *Liza saliens* (Risso 1810), the specimens were collected using cast net and angling from the Persian Gulf, Maharlu and Caspian Sea basins of Iran respectively. The specimens were anesthetized by MS222, fixed in 10% formalin and transported to the laboratory. The scales were gently removed with fine forceps from the third row of left side of the body under first dorsal fin and also the median part of body (midline) for SEM studies (Fig. 1). To clean the scales, they were kept in 5% KOH for maximum 10 minutes. The scales were then put in 30%, 50%, 70%, and 90% alcohol (each for 20 minutes) for dehydration, and then were put on filter paper for 24 hours to be dried (Lippitsch 1990). The scales were not put in absolute alcohol as 100% ethanol caused the scale margins to curl. The cleaned and dried scales were mounted on aluminum 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 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 recorded and studied.

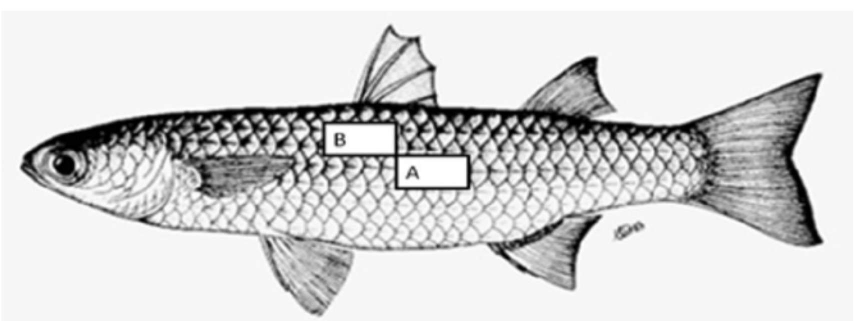

Figure 1. Schematic drawing of mugilid fish showing location of key scales used for SEM studies. A, scale from mid body; B, scale below the dorsal fin.

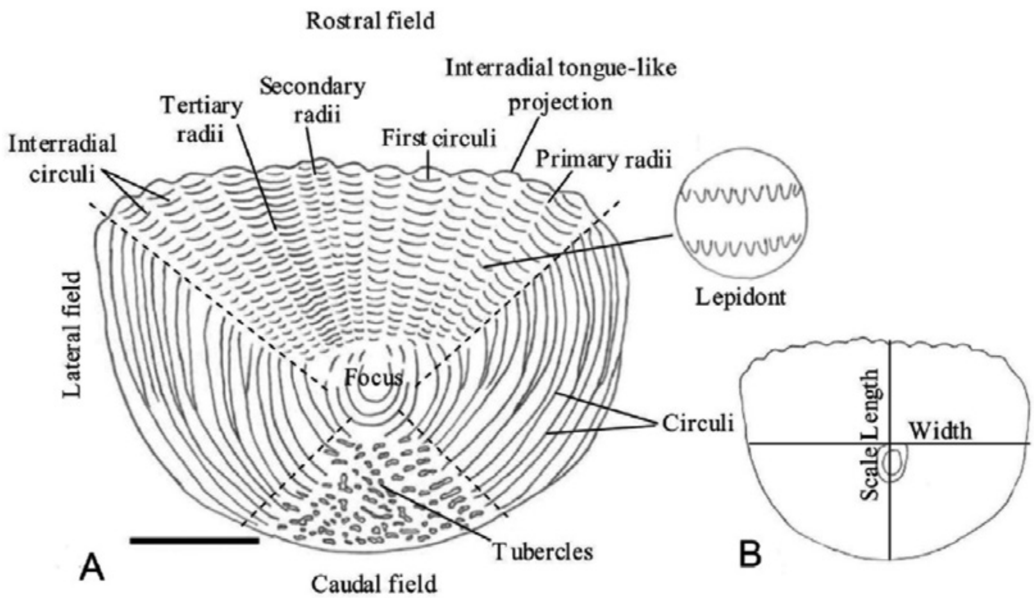

Figure 2. A) Schematic drawing of a scale showing some of the terms used in this study (after Gholami et al. 2013). B) Measurements of scale length and width (after Esmaeili 2001).

Three key scales were removed from the left side of each fish, mounted between microscope slides, and then length and width of each scale measured to the nearest 0.1 mm using a scale reader (Xerox 320). For final comparisons, scale length and width measurements were averaged to obtain single length and width values for each individual. The relative scale sizes (J-indices) for the scale length and scale width were calculated following Esmaeili (2001): J = length (width) of scale (in mm) / fish length (in mm) × 100.

Statistical analyses

The statistical analyses were carried out by using SPSS 17.0 (SPSS Inc.). Univariate analysis of variance (ANOVA, with Duncan's post hoc test, $p < 0.05$) was used to test the significance of differences in the J-indices among species. Moreover, all studied characters concerning the scale surface were coded in the form of a number (Table 3) and served as input for a dendrogram based on the Euclidean distance as a measure of dissimilarity. The between-groups-linkage method was used as the clustering algorithm to gather the phenotypic relationships (Gholami et al. 2013).

Results

In this study we compared the scale structure of three species from mugilid fish, *Liza abu, Liza klunzingeri* and *Liza saliens*. Based on the obtained results, two types of scales are present in the studied fishes including the transforming ctenoid type and cycloid scales. General terms used in this study are given in Figure 2. These scales can be divided into four fields: anterior or rostral which is frequently embedded under the preceding scale, posterior or caudal having chromatophores and lateral fields. Scales have shiny and smooth ventral surface, and the dorsal which is rough, convex and has distinct surface ornamentation, consists of granules (tubercles), grooves (radii) and ridges (circuli).

The studied scales of *L. abu* and L. *klunzingeri* are ctenoid while the scales of *L. saliens* are cycloid (Fig. 3). There is a clear distinction between these two scale types: ctenoid scales have spinous teeth called 'ctenii' on and near the caudal rim, while cycloid scales lack such teeth. In *L. klunzingeri* the ctenii show common configurations of transforming type which arise as whole spines in two or three alternating rows marginally and transform into truncated spines sub marginally. These ctenii have a conical shape with large base, average size, and is without a sharp end. In *L. abu* the posterior part of normal and lateral scales, are demarcated with the sharp conical ctenii that are in one row, and are large and very sharp at the ends. The scales of *L. saliens* have no ctenii and are cycloid type (Figs. 3 c, f). The positions of focus in all the examined species are in the posterior part of scales, but the shape of them are so different. The focus of *Liza abu* (Fig. 3 a) is wider than the other two species. In *L. klunzingeri* (Fig. 3 b), the focus in not sharp and in *L. saliens* is intermediate (Fig. 3 c). It seems that the position of the scale focus remains the same throughout life (Liu and Shen 1991; Miranda et al. 1996).

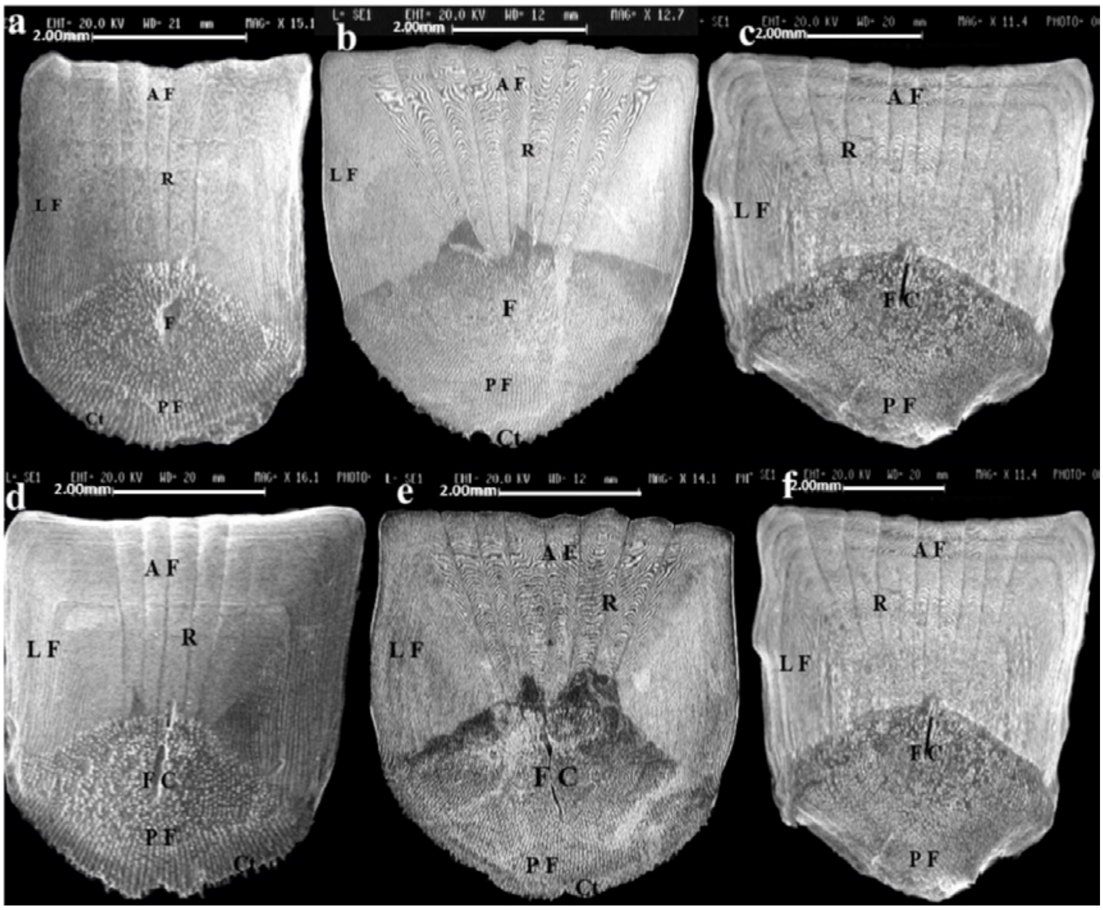

Figure 3. SEM microphotograph of normal scales in a: *L.abu*, b: *L.klunzingeri* and c: *L.saliens* SEM microphotograph of lateral pored scales in d: *L.abu*, e: *L.klunzingeri* and f: *L.saliens*. Anterior field (AF), Focus canal (F C), ctenii (Ct), focus (F), lateral field (LF), posterior field (PF), Radii (R).

The shape of the canals in the pored scales is different in three species (Figs. 3 a, b, c). In *L. abu* (Fig. 3 d) it is almost longer and wider than two other species (Figs. 3 e, f). The circuli were another studied structure in these three mugilid fishes. These are lines of growth (the ridges) that start appearing from the scale focus (Fig. 4). The space between circuli is called inter circular space. Circuli are distinct, overcrowded in the anterior part, less crowded in the lateral parts and not distinct at the posterior region due to presence of tubercles and ctenii in three studies fishes (Fig. 4). It seems that the arrangement of the circuli corresponds to the scale shape. The anterior intercirculus space is usually smaller than the lateral ones. Circuli have different shapes. In *L. abu* it is straight or slightly concave in the anterior part of scales, In *L. klunzingeri* it is almost concave and in *L. saliens* is 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 or scale-teeth. These circuli with lepidonts are found in the anterior and lateral parts of the scale. Lepidonts are almost pointed and separated and are the smallest ones in *L. abu* while they are almost blunt and less separated in *L. klunzingeri.* Lepidonts are almost bigger in *L. saliens* than the *L. abu* and *L. klunzingeri* (Figs. 4 g, h, i).

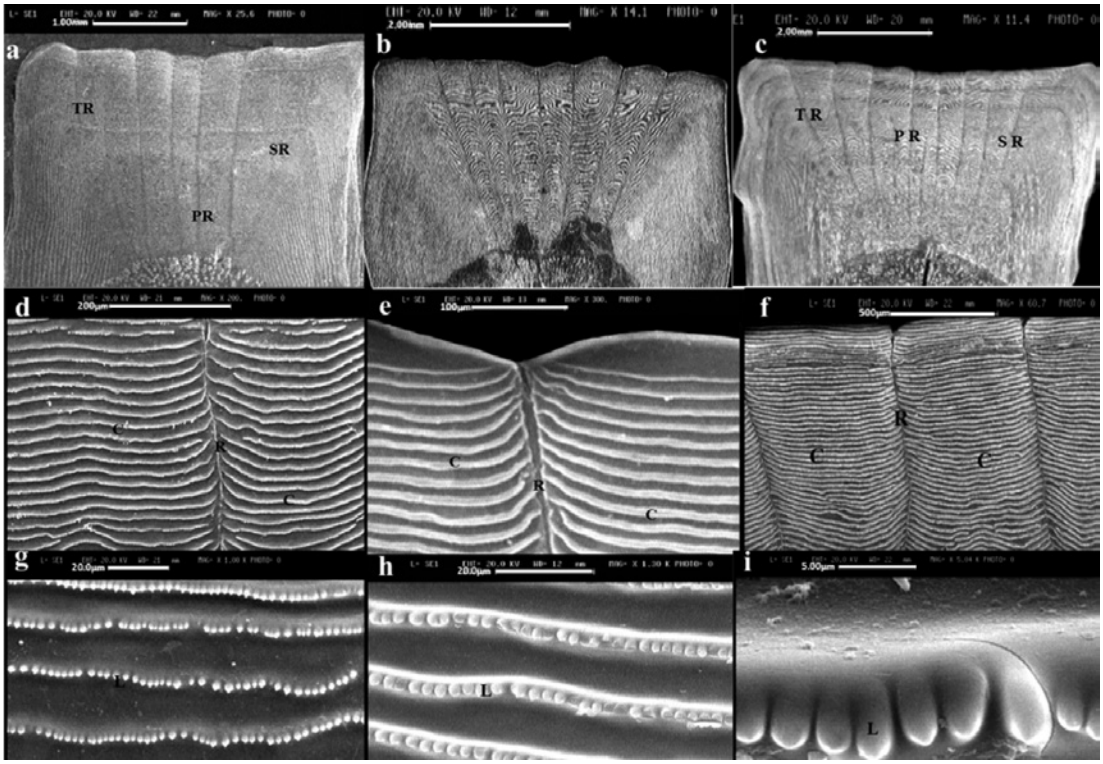

Figure 4. SEM microphotographs of Radia in normal Scales of a: *L.abu*, b: *L.klunzingeri* and c: *L.saliens*; Circuli in normal Scale of d: *L.abu*, e: *L.klunzingeri* and f: *L.saliens*; Lepidont in normal Scale of g: *L.abu*, h: *L.klunzingeri* and i: *L.saliens*. Circuli (C), lepidont (L), Radii (R), primary radii (PR), radii (R), secondary radii (SR), Tertiary radii (TR).

In the rostral field, the circuli are partitioned by several distinct grooves that run radially towards the focus. They are called radii. On the fish scales 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 that extends between midway and margin (Fig. 2). In all three species radii are prominent. The numbers of each kind of radii in three species are given in Table 1.

The area posterior to the focus is covered by numerous coarse granules (tubercles) with different shapes and sizes covering a large part of the caudal field. The density of tubercles in *L. klunzingeri* is higher than in the others. Tubercles in *L. klunzingeri* are almost well arranged in regular form (Figs. 3, 5). The last row of ctenii is sharper in *L. abu* than the *L. klunzingeri.* They are absent in *L. saliens* (Fig. 5).

Table 1. The average number and kinds of radii in three *Liza* species.

Species	*L. abu*	*L. klunzingeri*	*L. saliens*
Primary radii	5	6	7
Secondary radii	0	1	3
Tertiary radii	0	0	2

Table 2. The character variations in studied fishes of the genus *Liza*.

Characters	*L. abu*	*L. kluzingeri*	*L. saliens*
Focus position	posterior	posterior	Posterior
Radia	Primary 5	Primary 6	Primary 7
	-	Secondary 1	Secondary 3
	-	-	Tertiary 2
Tubercle density	Low density	High density	High density
Tubercle size	large	large	Small
Tubercle shape	No specific shape	Corn shape and regular	No specific shape
Lepidonte shape	Corn shape	Oval and regular	Wide oval
Lepidonte size	small	small	Average
Lepidonte Density	High density	Low density	High density
Ctenii	two layer and long	Multi layer and corn shape	Not existed
Focus canal	sharp	Large Cleft	
Circuli	Big or deep cleft	Straight and a little convex	cleft
Radii	Straight and a little Concave	convex	straight
		Concave	Concave

Table 3. The code for characters for dendrogram use.

Scale character	*L. abu*	*L. kluzingeri*	*L. saliens*
Focus position	1	1	1
Primary radia	5	6	7
Secondary	0	1	3
Tertiary	0	0	2
Tubercle density	1	2	1
Tubercle size	3	3	1
Tubercle shape	0	1	0
Lepidonte shape	2	1	3
Lepidonte size	1	1	2
Lepidonte density	2	1	2
Ctenii	1	2	0
Focus canal	2	3	1
Circuli	2	2	1
Radii	1	1	1

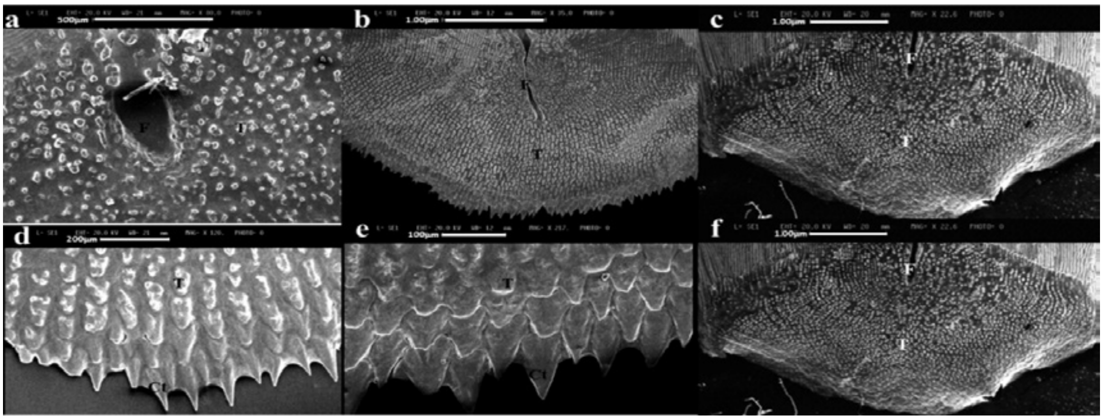

Figure 5. SEM microphotographs of Tubercle in Scales of a: *Liza abu*, b: *L. klunzingeri* and c: *L. saliens*; Ctenii in Scales of d: *L.abu*, e: *L. klunzingeri* and f: *L. saliens.* Ctenii (Ct), Tubercle (T), Focus (F).

Table 4. Descriptive analysis of J-indices based on fish total length (TL), standard length (SL) and fork length (FL) for three mugilid species.

Species	J index	N	Min	Max	Mean	SD.
L. abu	JL.TL	16	.64	.84	.7281	.06091
	JL.SL	16	.76	.99	.8768	.07356
	JL.FL	16	.66	.85	.7541	.06074
	Jw.TL	16	.64	.84	.7281	.06091
	Jw.SL	16	.76	.99	.8768	.07356
	Jw.FL	16	.66	.85	.7541	.06074
L. kluzingeri	JL.TL	8	.22	.36	.3181	.04334
	JL.SL	8	.29	.46	.3993	.04996
	JL.FL	8	.25	.40	.3533	.04480
	Jw.TL	8	.22	.36	.3181	.04334
	Jw.SL	8	.29	.46	.3993	.04996
	Jw.FL	8	.25	.40	.3533	.04480
L. saliens	JL.TL	2	.24	.27	.2541	.02230
	JL.SL	2	.29	.32	.3092	.02007
	JL.FL	2	.26	.29	.2711	.02169
	Jw.TL	2	.24	.27	.2541	.02230
	Jw.SL	2	.29	.32	.3092	.02007
	Jw.FL	2	.26	.29	.2711	.02169

JL, index of scale length; JW, index of scale width; SD., standard deviation.

J-indices have been employed for the study of scale sizes, i.e. relative scale length (Jsl.sl) and relative scale width (Jsw.sl). The J-indices analyses for three species have shown that the average J-indices length and width in *L. abu* are significantly larger (ANOVA, $p<0.05$) than the others and in *L. saliens* J-indices are the smallest (Table 4).

Codes of scale surface structures (Tables 2,3) served as input for the analysis of between-groups-linkage method based on the Euclidean distance. The dendrogram of the between-groups-linkage method sorts the *Liza* species into two groups (Fig. 6). *Liza saliens* from Caspian Sea basin represent a separate cluster and two other species are closely related.

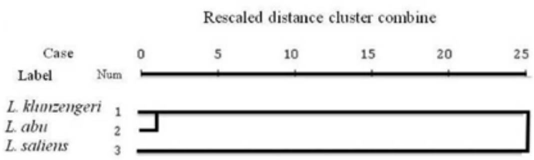

Figure 6. Dendrogram of phenotypic relations among three studied mugilid fish of Iran based on scale characters given in Tables 2,3.

Discussion

Study of mugilid fish scale morphology in fish classification was recognized almost 175 years ago by Louis Agassiz who classified the scale of mugilid fishes as Les Ctenoides scale. He classified fishes on the basis of four scale types:"Les Placoides" (e.g., "Pastenagues, Raies, Squales") with spine-like denticles of enamel and dentine, "Les Ganoides" (e.g., "Esturgeons, Polypteres, Lepisostes, Goniodontes, Silures, Scleroderms, Lophobranches") with thick plates of ganoine and bone, "Les Ctenoides" (e.g., "Mugiloides, Gobiodes, Cottoides, Scienoides, Sparoides, Scorpenoides, Percoides, Pleuronectides, Chaetodontes, Polyacanthes, Aulostomes") having thin plates with comb-like posterior borders, and "Les Cycloides" (e.g., "Cyprinoides, Clupes, Salmones, Esocides, Gadoides, Anguilliformes, Blennoids, Atherines, Scomberoides, Labroides") having thin plates with smooth borders (Agassiz 1834; Patterson 1977). Although this classification was short-lived and unnatural, the nomenclature introduced by Agassiz has been fully incorporated into ichthyology.

Today five basic types of scales (placoid, cosmoid, ganoid, cycloid and ctenoid) are recognized. However, including all scales with spines on their posterior margins under the term ctenoid is an over simplification of the situation (Johnson 1984; Roberts 1993). Today, all the spined scales have been categorized into three different, general types: (i) crenate, with simple marginal indentations and projections; (ii) spinoid, with spines continuous with the main body of the scales; and (iii) ctenoid, with ctenii formed as separate ossifications distinct from the main body of the scale (Roberts 1993). Crenate scales occur widely in the Elopomorpha and Clupeomorpha; spinoid scales occur widely in the Euteleostei; peripheral ctenoid scales (whole ctenii in one row) occur, probably independently, in the Ostariophysi, Paracanthopterygii, and Percomorpha; and transforming ctenoid scales (ctenii arising in two or three rows and transforming into truncated spines) are a synapomorphy of the Percomorpha. The scale morphology of *L. klunzingeri* from the south of Iran shows the general architectural pattern of a transforming ctenoid scales in most parts of the body (except the head region which is cycloid) having focus, circuli, radii and ctenii. The presence of both ctenoid and cycloid scales has been reported in other fishes (e.g. *Iranocichla hormuzensis,* Esmaeili et al. 2001). They have the advantage of being imbricate, overlapping like shingles on a roof, which gives great flexibility compared with cosmoid and ganoid scales. Our results show that the studied scales of these species are transformed ctenoid and cycloid scales, which are characterized by a discrete center of ossification at the posterior margin of the scale and grows into a separate fully formed ctenus comprising distal spine and proximal lobate base for ctenoid scales. It has been reported in other fish species (Roberts 1993). The cycloid scales are without ctenii (as seen in *L. saliens*). The ctenii in *L. abu* and *L. klunzingeri* may improve the swimming dynamics and so, reduce the water resistance.

In fishes with ctenoid scales the focus of the studied scales is distinct and located in the posterior field and is the first part of the scale to be formed during ontogenesis. Transforming ctenial ontogeny has already been studied by Roberts (1993). The spinous part of the ctenus then degenerates into a truncated spine and base separate from its neighbors. Finally, the spinal remnant and associated base become fused with adjacent spinal remnants and bases to form a solid calcified plate. A similar development is shown by subctenii, except that subctenii are usually asymmetrical due to their lateral position, and they initially arise out of one or more circuli before becoming truly separate elements.

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. 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 which may be false annuli. 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.

The presence of lepidonts on the crest of circuli is another characteristic of this scale which is different in three studied species in size and shape. It is in agreement with other studies (Kaur and Dua 2004; Esmaeili et al. 2007; Javad and Al-Jufaili 2007). According to them the taxa usually differ with regard to shape, texture, attachment and orientation of lepidonts on the crest of circuli

Lepidonts help the scale to anchor on the fish body.

Since *L. saliens* has cycloid scales it suggests that the scale of *L. saliens* is anintermediate form in the evolution of typical ctenoid mugil scales or can be due to reversal characteristics during its phylogeny.

In contrast to the scale shape, surface morphology and microstructure, we found that relative scale size (J-indices), which has received little attention to date in the field of fish taxonomy, is a reliable character with which it is possible to distinguish the three studied species. This is partially in agreement with the observations of Esmaeili et al. (2012) and Gholami et al. (2013), who opined that J-indices are suitable for the separation of *Aphanius* (Cyprinodontidae) species.

The J-indices in the studied species have revealed that the scales of *L.abu* are significantly bigger than the others and could be used in fish identification. However for better justification, it is suggested more specimens be included in the analysis.

In conclusion, scales of the three mugilid fishes are different in terms of scale type, focus shape, circuli shape, size, shape and arrangement of lepidonts and also ctenii shape and size which may be attributed to the their habitats and these characteristics may be used in fish identification. Moreover, J-indices were found to be a reliable tool for species discrimination, even in the case of closely related species.

Acknowledgments

The authors are thankful to Engineering College of Shiraz University for providing SEM facilities.

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