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Comparative Studies of the Scale Characters in four Mugilid Species (Family Mugilidae; Order Mugiliformes) from Karachi Coast, Pakistan

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ABSTRACT: The present study based on the comparative studies of some scale characters e.g., scale types, shapes and types of ctenii, arrangement of radii and position of focus on scale in order to observe their significance in determining the systematic relationship among the four selected mugilid species i.e., *Liza melinoptera, Liza macrolepis, Valamugil speigleri* and *Mugil cephalus* from Karachi coast. Both cycloid and ctenoid scales were present that shows great variations in their arrangement on the different body regions of these fishes. Two types of ctenoid scales were observed in this study such as, *Liza melinoptera, Liza macrolepis,* and *Mugil cephalus* contain basic-type of ctenoid scales, while *Valamugil speigleri* have crenate-type of ctenoid scales. Furthermore, large variations had been observed not only in the main types of scales, but also in the shapes and arrangement of ctenii and radii and position of the focus on the ctenoid and cycloid scales among these mugilid species. Hence, it had been proved now thatall thesescale characteristics could be also considered as best alternative tools for the identification, classification, phylogenetic relationships among the different genera, species or geographical variants.

Key words: Comparative studies of scale characters, scale shapes, types of ctenii, mugilid species.

INTRODUCTION

Fish scales can be define as small rigid plates that provide protections against certain diseases and predators, and also help in locomotion. The study of systematic relationships among the different mugilid species based on morphometric and meristic characters of their bodies are sometimes become confused due to the similarities in the external morphology or body shapes. Hence, it was very difficult to determine the exact taxonomic status of species. As fish scales exhibit great variations in their shapes, sizes, structures and arrangements in the different body regions of fish (Ikoma *et al*., 2003; Kardong, 2008), therefore, several workers have been used different scale characteristics for fish identification including, Agassiz (1833-34), the first scientist who classified all fishes into four main groups such as, (i) Les Placoides (ii) Les Ganoides (iii) Les Cycloides (iv) Les Ctenoides (Creaser, 1926) on the basis of their scale types. In the beginning, scale morphology had limited used in fish taxonomy. But after the application of scanning electron microscopy (SEM), the detailed studies of the scale structures by many workers like Delamater and Courtenay (1974), Johal and Dua (1994), Helfman *et al*. (1997), Esmaeili *et al*. (2007) and Jawad and Al-Jufaili (2007) have been proved that scale characters can be used as valuable tools in the identification of fish up to the genus or species level and its phylogeny. Maitland (2004) provides a key based on scale morphology of the fish families found in freshwater habitats of Britain and Ireland. Ibanez *et al*. (2009) were examined the variations in the shapes of scales in the three teleost species, two mugilid species i.e., *Mugil cephalus* and *Mugil curema* from a third phylogenetically distant species, *Dicentrarchus labrax*. The shapes of scales were also found to be varied between the different sexes belong to the same species e.g, *Upeneus vittatus* as described by Dulce-Amor *et al*. (2010).

Furthermore, besides the variation in the shape, size and arrangement of fish scales, various researchers have been frequently used some other scale structures like radii or grooves and cteniias useful tools for the identification and classification among the various genera, species and geographical variants (Kaur and Dua, 2004). Therefore, Lagler (1947) Kobayasi (1952), Roberts (1993) and Ibanez *et al*. (2011) have also been used the shapes of ctenii on ctenoid scales for the identifications, classifications and phylogenetic relationships between various genera or species. As each fish species possessed a definite scale structure and contained individual variation, hence, scale characters have been considered as some times unique for a particular species that could be helpful in its diagnosis, classification and phylogeny (Voronina, 2007). Hence, in addition to the whole scale morphology, variations have been found in the shapes of ctenii among the different fish species. In some cases, the character of ctenii was found to be species specific and could be helpful for the identification and classification of fishes.

Mullets or grey mullets contain both cycloid and ctenoid type scales (Ganguly and Mookerjee, 1947; Lagler, 1947; Roberts, 1993; Voronina, 2007). Ctenoid type of scales can be further classified into three types as recognized by Roberts (1993) in different teleost fishes, which are as follows; crenate scales, spinoid scales and basic ctenoid scales. As in different fish species, ctenii (singular ctenus) of ctenoid scale also shows some kinds of variations in their arrangements at the posterior margin or apex of scale. Therefore, according to the Roberts (1993), these ctenii can be classified into three categories, e.g., transforming ctenoid scales, peripheral ctenoid scales and a third and rare type is called whole ctenioid scale. However, Roberts (1993) reported only two types of ctenii i.e., transforming ctenii and whole ctenii on ctenoid scales of mullets. Thus, in mullets or grey mullets, variations have been observed not only in the main types of scales, but also in the shapes and arrangement of ctenii on their ctenoid scales as observed by some workers such as Jacot (1920), Kobayasi (1953), Roberts (1993), Ibanez *et al*. (2011) and Zubia *et al.* (2015). Therefore, inthe present study, instead of the shapes of scales, some other scale characteristics e.g., scale types, shape and type of ctenii, arrangement of radii and position of focus on scale were adopted in order to observe their importance in systematic classification of mullets.

MATERIALS AND METHODS

***A.** Fish samples collection*

A total of 106 specimens of the four species of family Mugilidae were collected monthly from the landings at Karachi fish harbour, during the period of April 2011 to December 2012. Each specimen was identified to species level in the field as well as in laboratory by using the FAO field guide (Bianchi, 1985; Harrison and Senou, 1999).

B. Scale samples collection

Method used for making the permanent slides of scales in order to study their complete structures follow Schneider *et al*. (2000) and Hotos (2003) with some modifications. For each specimen, scale samples were collected with the help of forceps from the following four selected body regions such as, HS = Head scales collected from the head region of the body. CS = Caudal scales collected from the caudal region. TRS = Transverse row scales collected in transverse series from the origin of dorsal fin to the origin of pelvic fin.

At least 5-6 scales were collected from each body region by using forceps. In laboratory, in order to prepared the scales for studying its complete morphology, the scales were soak in a beaker with warm water contain few drops of 10% NaOH/KOH solution for half an hour for removing all dust particles and mucous from scales. The epidermis and other tissues were also removed from the scales by rubbing fingers or soft paint brush and then these scales were dehydrated in Petri dishes contained 30, 50, 70, 90 and 100% ethyl alcohol solutions and dried on filter paper. Then these clean and transparent scales were mounted on a clean microscope slide and a drop of glycerin was kept on each scale sample, in order to protect them from drying. On each microscopic slide, at least four to five scales were mounted. Place one more microscopic slide over the first slide, press them firmly, removed the extra glycerin that comes out from these two slides after pressing, and leave them for an hour for drying. Later, a paper tape wrapped around the each end of these slides and labeled them with code number, date of collection, name and total length (TL) of species and scale type. All slides were examined under the stereomicroscope (4×10) and then measured and counts the following parameters of above mention types of scales such as, Types and shapes of ctenii arranged in horizontal row on scale, Arrangement of radii or grooves on scale and Rs = Scale radius (measured the vertical distance from the scale nucleus or focus to the outer posterior margin of the front side of the scale).

RESULT AND DISCUSSION

A. Comparative study of mullet scales

A comparative study of the different parameters of scales obtained from the four mullet species (Mugilidae) was conducted to identify the most useful characters of mugilid scales within the different body regions that can be useful for their identification and classification. Scales of the mullets possessed some characters that may not be seen or rarely seen in some other teleost fishes.

A detailed study about the scales of four selected mullet species of the present study revealed that a certain degree of variation occurs in their different scale characters i.e., scale types, position of the focus, and the shapes and types of ctenii and arrangement of radii on scale. Therefore, the main purpose of this study was to determine some useful scale characters for estimating their similarity or dissimilarity among the four mullet species and all such information's could be helpful in resolving the identification problems of these mullet species.

In the present investigation, the structure of a typical scale of these mullet species was same as described by Jacot (1920) and Pillay (1951), who used Masterman's (1913) method for the partition of mullet scale into four sections e.g., apical or posterior, basal or anterior and two lateral sections. The nucleus or focus of the scale was located near the apical portion of scale. Well developed radii were found in the basal section of scale that converge towards the nucleus in the apical portion. Ctenii were found only on the apical section of ctenoid scales. Furthermore, mullet scales obtained from the four different body regions were consist of the following structures such as focus, radii, and ctenii as observed by Pillay (1951). Thus, the scale types, the arrangement of radii, types and shapes of ctenii, and the position of focus on scales obtained from the four selected body regions of four mullet species had shown some specific variations within the different body regions (Figures b-f, g-j, k-n, o-r, s-x). Hence, all these variations can be consider as key characters for their correct identification.

(i) Types of scales in mullets: Great variations have been observed in the scale types of all four mullet species of this study. The studies of some earliest workers such as Jacot (1920), Pillay (1951), Roberts (1993), Thomson (1997) and Harrison and Senou (1999) revealed that mullets contained both cycloid and ctenoid scales.

Therefore, the result of the present study also indicates the presence of these two main types of scales in each mullet species, which was in agreements with the findings of above mention workers. However, the absence of ctenii was also observed in some scales, especially in the head region was indicating the presence of cycloid scales along with ctenoids in all four mullet species of the present study. Now it had been proved that mullets or grey mullets have two types of scales such as cycloid and ctenoid scales on their body.

In three mullet species of this study e.g., *Liza melinoptera, Liza macrolepis* and *Valamugil speigleri,* only head region contained cycloid scales along with ctenoid scales, while the rest of the selected body parts such as caudal, transverse and lateral line regions were covered with ctenoid scales only. In contrast, the entire body of *Mugil cephalus* was covered with a mixture of cycloid and ctenoid scales.

This result was in agreement with the previous study of Harrison and Senou (1999), who also observed the presence of minute cycloid scales that were superimpose on the ctenoid scales in the main body regions of *M. cephalus*. However, the formation of these two types of scales in mullet species was still not completely understood. Earliest workers like Day (1878) had been reported that habitat can influence on the nature or type of scales in fishes, therefore, mostly marine species of mullets possessed cycloid or weak ctenoid type of scales, while fresh-water species (e.g., *Mugil corsula, Mugil Hamiltonii* and *Mugil cascasia*) were found to have strong ctenoid scales. Hence, he (1878) found some kind of relationship between habitat and the type of scale formation in fish. However, the later investigation of Pillay (1951) was disagreed with Day (1878) findings. This may be because Pillay (1951) recorded the ctenoid type of scales in all those mullet species that occurred in all types of habitat i.e., fresh-water, marine water and estuaries. As the fishes with ctenoid and cycloid scales had been found in the same aquatic habitat, therefore, the exact function of these ctenii found on ctenoid scales was yet not understood (Ganguly and Mookerjee, 1947).

(ii) Types of ctenoid scales in mullets: As Roberts (1993) had further classified the ctenoid scales into three types, i.e., crenate, spinoid and basic ctenoid scales. However, in the present investigations, only two types of ctenoid scales e.g., crenate and basic ctenoid sales were reported in the four selected mullet species. This was in agreements with previous findings of Jacot (1920), Pillay (1951) and Roberts (1993) who had already been noted the occurrence of these two types of ctenoid scales in mullets (Mugilidae). The result of the present study revealed that three mullet species e.g., *Liza melinoptera*, *Liza macrolepis* and *Mugil cephalus* possessed basic ctenoid scales (Ct), while *Valamugil speigleri* contained crenate scales (Cr). The basic ctenoid scale can be easily recognized by the presence of sharp teeth-like spines or ctenii on its posterior region, while crenate scale was convered with crenae (spines) that occur in the form of simple finger-like projections and indentations at its posterior margin. Roberts (1993) had also reported the presence of these crenate scales in some other species of similar genus (*Valamugil*) e.g., *V. seheli.* Hence, the crenae found on the scales of *V. speigleri* have been used as diagnostic characters to distinguish the genus *Valamugil* from other genera of its family. In addition, both *Liza* species e.g., *L. macrolepis* and *L. melinoptera* possessed strongly ctenoid scales, while *Mugil cephalus* have weakly ctenoid scales, as shown in the Figures b-f, g-j, k-n, o-r). According to Roberts (1993), the ontogenetic studies of scale development and retention of juvenile structure in the adult scales were generally suggested that the three types of ctenoid scales i.e., crenate, spinoid and basic ctenoid scales were evolved from a generalized cycloid scale.

Plate 1. (a) Ctenoid scales of mullets. (Rs = vertical distance between focus and apex of scale)., (b) Head scales of *L. Melinoptera,* (c) Head scales of *L. macrolepis,* (d) Head scales of *V. speigleri,* (e) Head scales of *M. cephalus* (Cycloid), (f) Head scales of *M. cephalus* (Ctenoid), (g) Caudal scale of *L. melinoptera,* (h) Caudal scale of *L. macrolepis,* (i) Caudal scale of *V. speigleri,* (j) Caudal scale of *M. cephalus,* (k) Transverse scale of *L. melinoptera,* (l) Transverse scale of *L. macrolepis.,* (m) Transverse scale of *V. speigleri,* (n) Transverse scale of *M. cephalus,* (o) Lateral line scale of *L. melinoptera,* (p) Lateral line scale of *L. macrolepis,* (q) Lateral line scale of *V. speigleri,* (r) Lateral line scale of *M. cephalus,* (s) shows the ctenii on TRS of *L. melinoptera,* (t) shows the ctenii on TRS of *L. macrolepis,* (u) shows the ctenii on CS of *L. macrolepis,* (v) shows the ctenii on HS of *V. speigleri,* (w) shows the ctenii on CS of *M. cephalus,* (x) shows the ctenii on TRS of *M. cephalus*

On the other hand, in Percomorpha, cycloid, crenate and whole ctenoid scales of few mugilid species were seem to be derived from transforming ctenoid scales.

(iii) Types of ctenii in ctenoid scales of mullets: In this study, in addition to the whole scale morphology, variations were also recorded in the types and shapes of ctenii among the different mugilid species. Therefore, Roberts (1993) observed two types of ctenii i.e., transforming ctenii and whole ctenii on the ctenoid scales of mullets and used this character for studying their phylogeny. However, except *Valamugil speigleri*, the result of the present study revealed that the presence of only whole ctenii were observed on the scales of three mullet species i.e., *Mugil cephalus, Liza melinoptera* and *Liza macrolepis*. The presence of these whole ctenoid types of scales in *Mugil cephalus* of this study was in agreement with Roberts (1993), who also reported the presence of such type of scales only in single genus, *Mugil.* But the occurrence of these scales have also been reported in two more species of genus *Liza* in this study. Therefore, Roberts (1993) suggested that some mullet species possessed transforming ctenoid scales that represent the close resemblance with whole ctenoid scales of *Mugil cephalus*. This may be due to the reduction of their tips of ctenii sub-marginally. Such reduction or resorption of ctenial spines occurred through the osteoclast process (Sire *et al*., 1990).

(iv) Shapes of ctenii on ctenoid scales of mullets: Furthermore, it was also observed that the shapes of ctenii in the ctenoid scales of *Mugil cephalus* were lanceolate type (i.e., each ctenus was narrow, slightly curved with a sharp pointed end and have expanded base) or triangular as reported by some workers such as Jacot (1920), Pillay (1951), Kobyashi (1953) and Roberts (1993) and Ibanez and Gallardo- Cabello (2005). Similar triangular shapes of ctenii have also been reported in the ctenoid scales of both *Liza* sp. (i.e., *L. melinoptera* and *L. macrolepis*) of the present study. Hence, the shapes and types of ctenii in the three mullet species i.e., *M. cephalus, L. melinoptera* and *L. macrolepis* were found identical as shown in the Fig. s-x. Therefore, such similarities in shapes and types of ctenii could be helpful in the identification of these three mullet species. As in some cases, the character of ctenii is species-specific, hence, could be helpful for the identification and classification of fishes. Therefore, various workers had used this character for their identification and classification of various fish species such as, Imamura and Amaoka (1994) identify a new Platycephalidae species, *Grammoplites knappi* on the basis of the structure of posteriorly-positioned ctenii on their lateral line pored scales. Therefore, this character also possessed a significant value in fish taxonomy (Hughes, 1981). Most mullet species (Mugilidae) have also been identified on the basis of their shapes of ctenii. For instance, Hubbs (1921) and Kobayasi (1953) observed the variations in the shapes and types of ctenii in certain mugilid species and considered them as an important taxonomic character for their identification and classification. Likewise, Ibanez and Gallardo-Cabello (2005) also identify the two closely related species of genus *Mugil* e.g., *Mugil cephalus* and *Mugil curema* due to the variations in the shapes of ctenii between these two mullet species. Recently, Ibanez *et al*. (2011) had been differentiated the two morphologically and genetically related mugilid species, *Mugil hopes* and *Mugil curema* on the basis of the shapes of ctenii on their ctenoid scales. As the character of ctenii can observe very easily on the scales of these two mugilid species, therefore, it could be helpful in identifications of these two morphologically identical species. This may be because such ctenial character had been found to be constant for all sizes and ages of fish examined as reported by Ibanez and Gallardo-Cabello (1996a & 2004).

(v) Radiion scales: In the present study, several grooves were present in the anterior part of the mullet scale, which are known as “radii”. In mullet scale, radii were found only in the anterior field, while no radii were observed in the posterior and lateral fields, which was in agreement with Pillay (1951). In the present study, in addition to the types of ctenii, large variations were observed in radii counts among the four mullet species. In general, radii were absent or less number of radii were found on head scale as compared to the scales obtained from the remaining other body regions. According to the Sudo *et al*. (2002), as the caudal fins of fish twisted backwards during swimming, therefore, the flow of water will produce numerous grooves (radii) on the surface of caudal region scales. While the head scales provide only protection to the muscles and internal organs of the fish, therefore, no ridges and grooves were observed in the scales from the head region of fish. Similar result was observed in the present study.

(vi) Vertical distance from the focus to the outer posterior margin (exposed portion) of the scale: In the present study, ‘Rs’ represent or signify the straight line distance from the focus two of the outer posterior margin (exposed portion) of the scale or it can be described as a distance measured from the focus to the apex of scale in a vertical position. Hence, the ‘Rs’ values obtained for the different mullet species in this study was in fact indicating the position of the focus on scales. In fact, each fish scale contains a focus, which may be lies in the anterior or posterior parts of scale and developed first during ontogenesis as described by Esmaeili and Gholami (2011). Thus, in the present investigation, focus was lies in the center or more towards the apical portion on scales obtained from the four selected body regions of each mullet species, which was in agreement with Pillay (1951) and Ibanez *et al*. (2007).

Furthermore, in transverse, lateral line and caudal scales of these four mullet species, focus was located at their posterior field, while the head scale mostly contains centrally placed focus. As the position of the focus also varied according to the type of scale and species, therefore, the position of focus in both cycloid and ctenoid scales of the four selected mullet species in this study was found to be different. In addition, the results of the present study revealed that in case of weakly ctenoid and cycloid type scales, focus was located more towards the anterior field or in their central portion, which ctenoid scales mostly contain focus in their posterior field or apical portion. Likewise, the position of focus have also found to be varied among the different fish species as reported by Jawad (2005) for *Lates niloticus* and *Barbus arabicus*. The posterior arrangement of focus on the scale may be due to the lateral growth of the scale rather than a mixture of anterior and posterior growths of scale as described by Roberts (1993) and Jawad (2005). While Gallardo-Cabello *et al*. (2003) also recorded an eccentric position of focus in the ctenoid scale of *Anisotremus interruptus* (Haemulidae), which may be because of the fast growth of the anterior portion than the posterior portion of scale. Position of the focus in this study was more obvious in the caudal and head scales in relation to transverse and lateral line scales, which may be due to the erosion during swimming movement. Some earliest workers such as Jawad (2005), and Liu and Shen (1991) noted that the position of the focus on the scale remain unchanged throughout the life of the individual species. Significant variation was also observed in the position of the focus lies on the scales of four mullet species in this study. Therefore, this character could be considered as a valuable taxonomic character for their correct identifications, which was in agreement with Ibanez *et al*. (2007) who also used such character for the identification of *Mugil cephalus.*

As mullets or grey mullets are mostly morphologically identical fishes, therefore, there were great difficulties to identify them on the basis their external morphological characters. Traditionally, most researchers such as Katselis *et al*. (2006), Begum *et al*. (2008), Manimegalai *et al*. (2010) and Akombo *et al*. (2011) had been utilized various morphometric and meristic characters for the identifications of fishes belongs to same or different species. However, it should be rather difficult to use great number of these characters for observing the clear discrimination among the species or local populations in the field study. On the basis of skeletal features and mitochondrial DNA sequences, earliest workers such as De Silva (1984), Patterson (1993), Stiassny (1993), Crosetti *et al*. (1994), Corti and Crosetti (1996) suggested that mugilids were either placed with Perciformes or Atherinomorpha. Roberts (1993) classified the Atherinomorpha as a sister group of Percomorphs, which together form an order Acanthopterygii. However, Nelson (1994) had placed them in a separate order Mugiliformes. But the systematic and phylogenetic positions of mugilids based on all these characters was greatly confusing the earliest researchers, so various workers have been used scale characters e.g., shapes of scales and ctenii, number and arrangement of radii and ctenii, and the position of focus in fish taxonomy. Hence, fish scales were considered as quick and cheap materials that can be easily obtained from fish without giving any damage. Therefore, even rare or endangered species can discriminate because of their scale characteristics (Ibanez *et al*., 2007). Thus, scale characters were considered as useful taxonomic characters that could be used in correct identification and classification of any particular fish species because they reduced the misclassification rates of fish species (Hiilivirta *et al*., 1998). Though earliest workers such as Jawad (2005), Ibanez *et al*. (2009), Dulce-Armor *et al*. (2010), Garduno-Paz *et al*. (2010) and Ibanez and Paul-O'Higgins (2011) have proved that shapes of scales are species specific, therefore this characters could be used as useful tool in fish taxonomy. However, not only the scale shapes, earliest workers also observed the great variation in various types of scale structures i.e., position of focus, shape of ctenii, number and arrangement of radii, and used them for correct identification, classification, phylogenetic relationships and various other purpose (Lagler, 1947; Esmaeili *et al*., 2007). Therefore, all these scale characters had also considered as best alternative tools for the correct identification and classification of certain species. Hence, in the present study, a comparative study of mullet scales obtained from the four different mullet species was conducted for providing rich information about their scale structures and their great utility in systematic research.

CONCLUSIONS

The identification of any fish species or population is quite necessary for the fisheries conservation and management. Therefore, it is necessary to identify any specimen during the investigations of various biological traits like growth, mortality, fecundity, tropic relationship, parasitic relationship and paleontological events. Traditionally, identification of any fish specimen is usually based on morphometric and meristic characteristics (Katselis *et al*., 2006). However, all these methods are sometimes unreliable. Therefore, several workers have been used various scale characters in the systematic classification of fishes. As fish scales were commonly used by several workers for the examination of ages and growth in fish (Campana, 2001, Hotos, 2003), but after the analysis of different scale characteristics by several workers proved that fish scales can also be considered as useful tools for systematic classifications of the different fish species.

Hence, in the present study, it had been proved that these unique scale characters of a particular species may consider as significant taxonomic characters that would be useful in its identification and classification, and might be helpful for providing valuable information about its phylogeny. Furthermore, all such variations in scale characters might be related to the differences in their life history, habitat use and ecology (Garduno-Paz *et al*., 2010).

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