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Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society of Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.

The Journal publishes original research papers and reviews from any zoological discipline or from directly allied fields in ecology, behavioral biology, physiology & biochemistry. www.eajbs.eg.net



## Scale Characteristics of Three Fish Species of Genus Epinephelus From the Arabian Gulf at Kuwait

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#### **ARTICLE INFO**

Article History Received: 10/1/2017 Accepted: 1/3/2017

#### Keywords:

*Epinephelus* scale morphology circuli denticles scanning electron microscopy

## ABSTRACT

The present work has been suggested to screening and documenting the diversity of scale characteristics of three Epinephelus species, namely: E. bleekeri, E. coioides and E. latifasciatus from the Arabian Gulf at Kuwait. Scale characteristics, its detailed structure and superficial ornamentation were studied in terms of morphology and Scanning Electron Microscopic techniques. A wide spectrum of size-free interspecific variations was recorded concerning the surface morphology, shape of interradial tongues and the first circulus, the interradial circuli, most outer lateral circuli, inner lateral circuli, denticles, interradial and intercircular grooves, segmentation and granulation pattern of the caudal field and the shape of ctenii. The results of the present study has clearly indicated the potential application of scale shape variation in identification of Epinephelus species; and also, revealed that the qualitative characters of the scales of the three Epinephelus species studied exhibited species-specific valuable taxonomic characters for each species and indicated that such qualitative characters are genetically fixed and more stable.

### INTRODUCTION

*Epinephelus* is a genus belonging to subfamily Epinephelinae, family Serranidae, order Perciformes, class Actinopterygii (ray-finned fishes). The family Serranidae represents one of the valuable and economical fish families in the Arabian Gulf. From taxonomical point of view, the Arabian Gulf at Kuwait seems to be one of the least studied areas dealing with Serranidae.

Renjith *et al.* (2014) have stated that the correct identification of fish species is necessary to formulate management and conservation measures for vulnerable fish species. Scale characteristics are considered as a research material and good tools for fish identification, taxonomy and phylogeny especially with great developments of Scanning Electron Microscopy that had facilitated the application of scale microstructures to systematics (Mekkawy *et al.*, 2011 and Harabawy *et al.*, 2012). Also, the analysis of scale morphology appears to be promising as it is easy to apply, rapid, inexpensive and does not require animal kill and dissection (Renjith *et al.*, 2014).

Many authors have studied the morphological, detailed structure and some

morphometric measurements and meristic counts of fish scales of several fresh water and marine fishes; they emphasized on the validity of scale morphology and ultrastructural characteristics for fish identification, taxonomy and phylogeny especially with great developments of Scanning Electron Microscopy that had facilitated the application of scale microstructures to systematic; these studies offered valuable scale characters that can reflect a clear taxonomic status and a well-founded phylogenic tree of different groups of fishes in addition to the functional and systematic approaches. (Lippitsch, 1993; Mekkawy et al., 2003, 2011; Jawad, 2005; Mahmoud et al., 2005; Harabawy et al., 2007, 2012; Reza et al., 2009; Esmaeili and Gholami, 2011; Dapar et al., 2012; Ganzon et al., 2012; Alkaladi et al., 2013; Renjith et al., 2014; Masood et al., 2015). A wide spectrum of morphological and surface ornamentation of scales of many fish species and genera including shape of interradial tongues and the first circuli, the interradial circuli, most outer lateral circuli, inner lateral circuli, denticles and grooves among circuli, granulation pattern of caudal field, form of ctenii and lateral line canal (the shape and position) were recorded by several authors (e.g. Lippitsch, 1990, 1993; Mekkawy et al., 2003, 2011; Mahmoud et al., 2005 and Harabawy et al., 2007, 2012; Alkaladi et al., 2013; ; Renjith et al., 2014). Therefore, as the scale being a unique part of fish body, such scale characteristics can be used successfully to discriminate between species and genera and may even distinguish some taxa at the specific level.

The present work aimed to screening and documenting the diversity of scale characteristics of three three *Epinephelus* species, namely: *E. bleekeri* (Vaillant, 1878), *E. coioides* (Hamilton, 1822) and *E. latifasciatus* (Temminck and Schlegel, 1842) from the Arabian Gulf at Kuwait in an attempt to determine the valid scale characters for identification of these species studied and to give an interpretation for the surface scale ornamentation in terms of functional approaches; also, to present some bases for further work on scale characteristics and their exploitation in phylogenetic investigations for Serranidae.

### MATERIALS AND METHODS

In the present work, scales from 40 specimens of three Epinephelus species, namely: E. bleekeri (Vaillant, 1878) (280 - 620 mm standard length (SL)), E. coioides (Hamilton, 1822), (260 - 600 mm SL) and E. latifasciatus (Temminck and Schlegel, 1842) (360 - 680 mm SL) were examined to elucidate their scale characteristics. These specimens were collected from the Arabian Gulf at Kuwait. The scales were gently removed with fine forceps from the left side of the body from the following positions on the body: 1)- Region A, directly below the anterior part of the dorsal fin (BDFS). 2)- Region B, post-operculum (POS). 3)- Region C, below the lateral line, between the pectoral and pelvic fins (BLLS). 4)- Region D, caudal peduncle directly above the lateral line (CPS). Scales are prepared for Scanning Electron Microscopy (SEM) examination according to the methods of Harabawy et al. (2012). Scales are cleaned by physical careful removing of the adhering tissues debris without damage in the scale surface. Then they were immersed in a solution of 10% ammonia for 24-36 hr to soften adhering tissues and to clean them. Cleaned scales were dried on a filter paper. SEM was used to study the morphology and microstructures of the scales in the most anterorostral, lateral, and caudal regions. The cleaned and dried scales were mounted for SEM examination and fixed by sticker tape on a specimen holder and coated with a 30-nm layer of gold. The electron micrographs were produced on GAOL, GSM5400LV, SEM in back scattering mode and on a Stereo Scan Cambridge Mark 2A (15 KV) in Assiut University Electron Microscope Center, Assiut, Egypt.

## RESULTS

#### Scale surface morphology:

In the present study, it was noticed that, all scales on all parts of the body of the three *Epinephelus* species studied are mainly of the ctenoid type with well-developed radii (i. e. sectioned type). Simple scales (i. e. without or with only weakly developed radii) were not recorded. The surface of scales is divided into four distinct fields, a rostral field from the focus to anterior margin, caudal field from the focus to the posterior margin and two lateral fields. The scales of *Epinephelus* species studied show a characteristic surface ornamentation which in the simplest case consists of ridges (circuli) and grooves, forming nearly circular rings around a center called focus except in the caudal region that have no circuli and alternatively, contains ctenii and granulation segments. Many regenerated scales (without any ornamentation at least at the central part of the scale) were recorded. In the anterior (rostral) part of the scale, the circuli are partitioned by deep and narrow radii (grooves that run radially between the focus and anterior rim).

#### **Rostral field:**

At the rostral rim of all scales of *Epinephelus* species studied, tongue-like projections are found in the inter-radial space (Fig. 1a-c). Such tongues and the 1st inter-radial circulus may be convex as in *E. bleekeri* (Fig. 1a) and *E. coioides* (Fig. 1b) or slightly straight as in *E. latifasciatus* (Fig. 1c). The areas among the circuli are known as intercircular spaces (grooves). Such grooves are flat and wide relative to the circulus thickness in most of scales of *Epinephelus* species studied (Fig. 2a).

## The outer circuli:

In all scales of three *Epinephelus* species studied, the most outer circuli are relatively free of denticles, the first to third circuli in anterior inter-radial circuli (Fig. 2a), and the first to second ones in the lateral margin of the scales (Fig. 2b). The outer circuli may be relatively thin as in anterior inter-radial circuli (Fig. 2a) or may be thick as in lateral field of the scales (Fig. 2b). The inner circuli bear small denticles and gradually enlarge and take their specific shape in the most inner lateral circuli towards the focus.

# Inter-radial and inner lateral circuli, grooves and denticles in different body regions:

In region A, directly below the anterior part of the dorsal fin (BDFS), the inter-radial circuli bear different shapes of lepidonts or denticles (tooth-like structures) that can be seen only under high magnification. Two different characteristic types of denticles or lepidonts were identified on the inter-radial circuli (Fig. 3a, b). Type 1: The circuli bear denticles of variable size; the free ends of these denticles are of different shapes (rounded, pointed or flat ends) and all of them are oriented backwardly toward the focus of the scale. Such type was recorded in *E. bleekeri and E. coioides* (Fig. 3a, b). Growing denticles and bicuspid denticles are also recorded. Type 2: The circuli bear denticles with very pointed ends (claw-like ends) oriented backwardly. Such type was recorded only in *E. latifasciatus* (Fig. 3c).

In region B (Fig. 4a-c), post-operculum scales (POS), the widths of the grooves differ from species to another. The widest grooves are recorded in *E. coioides* (Fig. 4b), while the narrowest grooves are recorded in *E. latifasciatus* (Fig. 4c), but in *E. bleekeri* the grooves are in-between (Fig. 4a). The circuli bear denticles of variable sizes and the free ends of these denticles are of different shapes. The bases of

denticles are wider in *E. latifasciatus* than those of the latter two species (Fig. 4). All denticles are oriented backwardly toward the focus of the scale.

In region C (BLLS) (Fig. 5a-c), the denticles are ampula-like with pointed ends in scales of *E. bleekeri* (Fig. 5a), but the denticles which are recorded in scales of *E. coioides* and *E. latifasciatus* are heterodont in shape (Fig. 5b,c). The grooves which are recorded in *E. bleekeri* are wider than grooves of *E. coioides* and *E. latifasciatus* (Fig. 5).

In region D (CPS) (Fig. 6a-c), the denticles are conical in shape in scales of *E.bleekeri* (Fig. 6a). In *E. coioides*, the denticles are long (Fig. 6b), but in *E. latifasciatus*, he denticles are pyramidal, conical, unicuspid or bicuspid (Fig. 6c). The grooves which are recorded in *E. bleekeri* and *E. coioides* are wider than grooves of *E. latifasciatus* (Fig. 6).

#### Focus region:

The focus region of the scales of both species, *E. bleekeri* and *E. latipasciotus*, appear as elongated space surrounded by elongated ridges (Fig. 7a). But, in scales of *E. coioides*, the focus region has heart shape (Fig. 7b) and surrounded by ridges with the same shape. The focal circuli of the three *Epinephelus* species are smooth and do not bear any denticles.

### Caudal field:

#### Segmentation of the caudal field and ctenii:

Figure 8 reveals that, the posterior field of the scales of the three *Epinephelus* species studied appears as rows of segments which are arranged in oblique lines, the segments of each row occupied alternative position with the adjacent ones (as recorded in *E. bleekeri* and *E. coioides*) or in parallel lines (as in *E. latifasciatus*) and ending as long ctenii at the posterior rim of the scale. According to the pattern of segmentation, shape and arrangement of segments and shape and size of ctenii, three patterns of scales were identified in *Epinephelus* species studied. The granulation segments have pentagonal shape and extend posteriorly at the scale rim into blunt thick ctenii with weak convex end, this pattern was recorded in *E. bleekeri* (Fig. 8a). In the case of *E. coioides*, the granulation segments have unique pear-like shape and pear-like shape with transverse process; the segments of each row are adjacent to each other and the ctenii extend posteriorly at the scale rim into blunt thick ctenii with weak convex end (Fig. 8c).

#### DISCUSSION

Fish scales are bony structures that grow as fish grow in size, and producing characteristic growth circuli at the scale margin; once a circulus deposited on the scale, it remains unchanged along the entire life span of the scale (Sire and Akimenko, 2004; Harabawy *et al.* 2012).

In the present work, the scales of *Epinephelus* species studied show a characteristic surface ornamentation which in the simplest case consists of ridges (circuli) and grooves, forming nearly circular rings around the focus except in the caudal region that have no circuli and alternatively, contains ctenii and granulation segments. The morphological features and surface ornamentation characteristics of scales of fresh water and marine fishes have been studied by many authors (Lippitsch, 1993; Mekkawy, 1980; Mekkawy *et al.*, 1999, 2003; Harabawy, 2002; Ali, 2003; Basmidi, 2004; Mekkawy and Abdel-Rahman, 2005; Harabawy *et al.*, 2012; Alkaladi *et al.*, 2013; Renjith *et al.*, 2014). These studies deal with the stability of ultrastructure

and surface ornamentation of rostral and caudal field of scales. Many of these studies used these characteristics in identification of different species. Also, authors of these studies have investigated the potential of scale characteristics in fish taxonomy, and they have concluded that such characteristics are strongly determined genetically.

Ganzon *et al.* (2012) stated that the environment is considered as a powerful force in modeling the morphology of an organism during ontogeny. The epidermal cover of fishes especially those inhabiting coral reefs undergo friction forces due to water flow (Harabawy *et al.*, 2012). The circuli and its denticles of the interradial and lateral fields of the scales may play an important role to provide a resistance against these frictional forces through mechanical anchoring (Mahmoud *et al.*, 2005; Mekkawy *et al.*, 2011).

In the present work, it was noticed that, the scale characters of the three *Epinephelus* species studied were found to be size-free and the scale ornamentation were stable in shape, size and spacing of circuli and denticles; therefore, one can conclude that the size-free fixed characters are expressed through their divergent evolution (i.e. the differences among species and genera genetically controlled); and these scale features are not controlled by the environmental factors (i.e. the effects of the environmental factors were omitted). Lippitsch (1992), Mekkawy *et al.* (1999, 2003, 2006), Ali (2003), Basmidi (2004), Mahmoud, *et al.* (2005), Mekkawy and Abdel-Rahman (2005), Harabawy *et al.* (2012) and Alkaladi *et al.* (2013) have studied the stability of scale characters of several species; and they have elucidated that such stability may offer useful criteria for systematic purposes.

In the present study, the shape of the first inter-radial circulus may be convex as in *E. bleekeri* and *E. coioides* or slightly straight as in *E. latifasciatus*. These variations may be specific for different species (Lippitsch, 1993; Mahmoud *et al.*, 2005; Mekkawy *et al.*, 2011; Harabawy *et al.*, 2007, 2012; Alkaladi *et al.*, 2013); in spite of in some cases it seems to be modified by external factors (Lippitsch, 1990).

Denticles are important fine structures seen on circuli and are not homologous to breeding tubercles or contact organs (Esmaeili and Niknejad, 2007). Harabawy et al. (2012) reported that, such denticles are not considered as contact organs (to facilitate contact between males and females during reproduction) due to their microscopic size and the covered position. In the present work, the free ends of the denticles which are found on the interradial circuli of scales of Epinephelus species studied were oriented posteriorly towards the focus; also, denticles found on the inner lateral circuli are oriented at right angle on the longitudinal axis of the scale towards the scale focus. This means that the free ends of the denticles are oriented toward different directions and may be involved in the mechanical anchoring of the scale into the covering dermis acting as minute hooks to prevent movement or detachment of the scales as proposed for other fishes by many authors (Mekkawy et al., 2003, 2011; Mahmoud et al., 2005 and Harabawy et al., 2007, 2012; Alkaladi et al., 2013). Mahmoud et al. (2005) reported that, the direction of these fine hooks can explains why isolated scales always have a well-defined strip of skin adhering to it after removal. But, Zylberberg and Meunier (1981) mentioned that the scales are anchored in the surrounding tissue by the bundles of collagen fibers connecting the upper part of the scale to the overlying dermis.

In the present study, the most outer circuli are free of denticles, the first to third circuli in anterior inter-radial circuli, and the first to second ones in the lateral margin of the scales. The absence of denticles in the most outer lateral circuli may reflect that the anchoring is not important in the outer field at the newly formed circuli as proposed by Mekkawy *et al.* (2011) and Harabawy *et al.* (2012). On the other

hand, the inner denticles are oriented in various directions helping in multidirectional anchoring. These findings agree with those reported by many authors (Mekkawy *et al.*, 2003, 2011; Mahmoud *et al.*, 2005; Harabawy *et al.*, 2007, 2012; Alkaladi *et al.*, 2013).

The overall caudal field of the scales including granulation patterns, shape, size and arrangement of segments and ctenii of the scales of *Epinephelus* species studied were constant with fish size and exhibited interspecific variations. The great morphological variations between species and genera of the same family and a complete difference in species outside the family were evident and emphasized on the importance of the caudal field of scales as a taxonomic character not only at the level of species or genera but also at families level (Mekkawy *et al.*, 2006, 2011; Mahmoud *et al.*, 2005 and Harabawy *et al.*, 2007, 2012; Alkaladi *et al.*, 2013).

The focus area was among the important features of scales studied by SEM. The focus shows unique patterns of circuli. In the present work, the focus region of the scales of both species, *E. bleekeri* and *E. latifasciatus*, appear as elongated space, but, in scales of *E. coioides*, the focus region has heart shape. The focal circuli of the three *Epinephelus* species are smooth and do not bear any denticles. Ganzon *et al.* (2012) stated that, just the focus is formed, its position on the scale remains the same throughout the life of the individual species.

Segmentation and granulation patterns, shape and size of ctenii and overall caudal field of the scales of *Epinephelus* species studied were constant with fish size and exhibited interspecific variations. Jawad (2005) has stated that the reduction in length or resorption process of the ctenii almost stops after the tip of the spine length has been resorbed. Accordingly, such resorption process may lead to segmentation of the caudal field of the scales of *Epinephelus* species considered. Harabawy *et al.* (2012) reported that the mechanism of spine loss was interpreted as a progressive resorption rather than a sudden amputation, and, in some teleost fishes, a severe resorption leading to complete disappearance of the spine; also, the resorption of ctenial spines seems to be a characteristic of broader taxonomic groups such as genera and families rather than isolated species within these groups.

Accordingly, the results of the present work revealed that the qualitative characters of the scales of the three *Epinephelus* species studied exhibited species-specific valuable taxonomic characters for each species and indicated that such qualitative characters are genetically fixed and more stable.

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Fig. 1: Scanning electron micrographs show the inter-radial tongues (IRT), 1st Inter-Radial Circulus (1st IRC), Inter-Radial Circuli (IRC) and Radii (R) recorded in the rostral field of the scales of three *Epinephelus* species from Arabian Gulf at Kuwait: *E. bleekeri* (a); *E. coioides* (b) and *E. latifasciatus* (c).



Fig. 2: Scanning electron micrographs show the most Outer Circuli (OC) without denticles and the Inner Circuli (IC) which bear small denticles (D) in (a): anterior inter-radial tongues (IRT) and in (b): lateral region (LR) of the scales of three *Epinephelus* species from Arabian Gulf at Kuwait; G, groove between cerculi; 1st IRC, first Inter-Radial circulus.



Fig. 3: Scanning electron micrographs for scales obtained from body region A, directly below the anterior part of the dorsal fin (BDFS), of the three *Epinephelus* species considered from Arabian Gulf at Kuwait show the inter-radial circuli (C), different shapes of lepidonts or denticles (D), growing denticles (GD), grooves (G) and BD, bicuspid denticles; (a): recorded in *E. bleekeri*, (b): in *E. coioides* and (c): in *E. latifasciatus*.



Fig. 4: Scanning electron micrographs for postoberculum scales (POS), body region B, of the three *Epinephelus* species considered from Arabian Gulf at Kuwait show the inter-radial circuli (C), bicuspid denticles (BD), different shapes of denticles (D), growing denticles (GD) and different widths of grooves (G); recorded in (a) *E. bleekeri*, (b): *E. coioides* and (c): *E. latifasciatus*.



Fig. 5: Scanning electron micrographs for scales obtained from body region C, below the lateral line between the pectoral and pelvic fins (BLLS), of the three *Epinephelus* species considered from Arabian Gulf at Kuwait show inter-radial circuli (C) bearing different shapes of denticles and different widths of grooves (G); recorded in (a): *E. bleekeri*, (b): *E. coioides* and (c): *E. latifasciatus*.



Fig. 6: Scanning electron micrographs for scales obtained from body region D, caudal peduncle directly above the lateral line (CPS), of the three *Epinephelus* species from Arabian Gulf at Kuwait and show inter-radial circuli bearing different shapes of denticles, and different widths of grooves (G); recorded in (a) *E. bleekeri*, (b): *E. coioides* and (c): *E. latifasciatus*.



Fig.7: Focus region of the scales below the anterior part of the dorsal fin (BDFS) of three *Epinephelus* species from Arabian Gulf at Kuwait; (a): focus region appear as elongated space surrounded by elongated ridges, recorded in *E. bleekeri* and *E. latifasciatus* (b): heart shape focus surrounded by ridges with the same shape recorded in *E. coioides*. RF: rostral field, CF: caudal field, F: focus, FC: focal circuli.



Fig. 8: Different forms of ctenii and granulation segments of scales below the anterior part of the dorsal fin (BDFS) of the three *Epinephelus* species from Arabian Gulf at Kuwait; (a): The granulation segments have pentagonal shape and extend posteriorly at the scale rim into blunt thick ctenii with weak convex end, recorded in *E. bleekeri*. (b): The granulation segments have unique pear-like shape and pear-like ctenii at the rim of scales, recorded in *E. coioides*. (c): The granulation segments have pear-like shape with transverse process, ctenii extend posteriorly at the scale rim into blunt thick ctenii with weak convex end, recorded in *E. latifasciatus*.

# ARABIC SUMMERY خصائص القشور لثلاثة أنواع من جنس إبينيفيلس من الخليج العربي – الكويت

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تم اقتراح هذه الدراسة لفحص وتوثيق تنوع خصائص القشور لثلاثة أنواع من جنس إبينيفيلس هى إبينيفيلس بليكيرى، إبينيفيلس كويويدس و إبينيفيلس لاتيفاسياتوس من الخليج العربى – الكويت. وقد درست خصائص القشور وتفاصيل تركيبها و الزخارف السطحية للقشور بدلالة الصفات المورفولوجية وتقنية الميكروسكوب الإلكتروني الماسح. ولقد سجل ووثق طيف واسع من التباينات المورفولوجية البين نوعية من خلال مورفولوجية السطح، شكل اللسان الموجود بين الشعاعيات، أول حلقة، الحلقات البين شعاعية، الحلقات الجانبية الخارجية ، الحلقات الجانبية الداخلية، الأسنان الدقيقة على الحلقات، الميازيب التي بين الحلقات والميازيب التي بين الشعاعيات، وكذلك الزخارف الموجودة في الجزء الخلفي من القشرة من حيث التجزءات وأشكال الحبيبات الموجودة وكذلك شكل الأسنان الموجودة على حافة القشرة الخلفية. وقد أشارت نتائج هذه وأشكال الحبيبات الموجودة وكذلك شكل الأسنان الموجودة على حافة القشرة الخلفية، وكثفت أيضا أن وأشكال الحبيبات الموجودة وكذلك شكل الأسنان الموجودة على حافة القشرة من حيث التجزءات الدراسة بوضوح إمكانية تطبيق تباين شكل القشور في تعريف أنواع جنس إبينيفيلس، وكشفت أيضا أن وأشكال الحبيبات الموجودة وكذلك شكل الأسنان الموجودة على حافة القشرة الخلفية. وكثلات أيضا أن وأشكال الحبيات الموجودة وكذلك شكل القشور في تعريف أنواع جنس إبينيفيلس، وكشفت أيضا أن وأشكال الحبيات الموجودة وكذلك شكل القشور في تعريف أنواع جنس إبينيفيلس، وكشفت أيضا أن وأوضحت أن مثل هذه الصفات النوعية ثابتة وراثيا وأكثر إستقرارا.