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Scale characteristics of *Acanthopagrus bifasciatus* (Forsskål, 1775) from the Southern Red Sea, Egypt

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Abstract
Acanthopagrus bifasciatus is a member of family Sparidae. It is a tropical coastal fish species distributed throughout the Red Sea and the western Indian Ocean. In the present study, 253 scales from ten specimens of *A. bifasciatus* (170- 350 mm Total length) were examined to elucidate their scale characteristics. A wide spectrum of intraspecific variation between different body regions of *A. bifasciatus* was recorded in terms of scale morphometric indices and primary and tertiary radii counts. The scale characters including rostral field, outer and inner lateral circuli, grooves, denticles, focus region, granulation in caudal field and lateral line canal were studied. Scale morphology and ultrastructure characteristics are important for fish identification, taxonomy and phylogeny.

Keywords: Red Sea; *Acanthopagrus bifasciatus;* scale morphometry; scanning electron microscope

1. Introduction
Family Sparidae represents one of the most important fish families in the Red Sea and *Acanthopagrus bifasciatus* is member of this family. Its members are carnivorous, marine, brackish, reef-associated and inhabit shallow coastal waters mainly aroreefs at depth ranged from 2-20 m [1, 2]. *Acanthopagrus bifasciatus* is distributed in the Western Indian Ocean from the Red Sea and Arabian Gulf to Natal in South Africa [2].
Scale characteristics have been considered relevant for fish identification, taxonomy and phylogeny especially with great developments of Scanning Electron Microscopy that had facilitated the application of scale microstructures to systematic [3, 6]. The morphological features and ultrastructure of teleost scales were studied by many authors in an attempt to discover new characteristics for scales that may be useful in fish taxonomy [7, 9, 3, 10, 12, 4, 13, 18, 5, 6]. Most of these studies aimed to provide a wide range of valuable scale characters that can reflect a clear taxonomic status.
The present study aimed to screening and documenting the diversity of scale characteristics of *Acanthopagrus bifasciatus* from the Southern Red Sea, Egypt in an attempt to determine the valid scale characters for fish identification and to give an interpretation for the surface scale ornamentation in terms of functional approaches.

2. Materials and methods
2.1. Specimen collection
In the present work, 253 scales from ten specimens of *A. bifasciatus* (170 - 350 mm Total length (TL)) were examined to elucidate their scale characteristics. These specimens were collected from the Southern Red Sea, Egypt during the period January 2015 to December 2015.

2.2. Scale preparation and measurements
The scales were gently removed from the left side of the body from the following positions on the body (Fig. 1 a):
a- Region A, below the anterior part of the dorsal fin (BDFS).
b- Region B, post-operculum (POS).
c- Region C, below the lateral line, between the pectoral and pelvic fins (BLLS).
d- Region D, caudal peduncle directly above the lateral line (CPS).

e- Region E, anterior lateral line scales (ALLS)
f- Region F, middle lateral line scales (MLLS)
g- Region G, posterior lateral line scales (PLLS) from caudal peduncle region.

The scales of the first four regions (A, B, C and D) were used for morphometric measurements and radii counts. While scales forming the lateral line were examined to show the lateral line pattern, shape and characters of the lateral line canal.

Examined scales were cleaned carefully to remove the adhering tissues debris without damage in the scale surface. Then they were immersed in a solution of 10% ammonia for 24-36 h. to soften adhering tissues and to clean them. Cleaned scales were dried on a filter paper and kept between two glass slides.

Fig. 1b shows the structure of a sectioned scale, types of radii and the morphometric measurements considered. The primary, secondary and tertiary radii were counted to reveal intraspecific variations. The morphometric measurements were treated in terms of indices (L1/L, L2/L, L1/L2 and W/L) where L, L1, L2 and W are scale length, rostral field length, caudal field length and scale width, respectively.

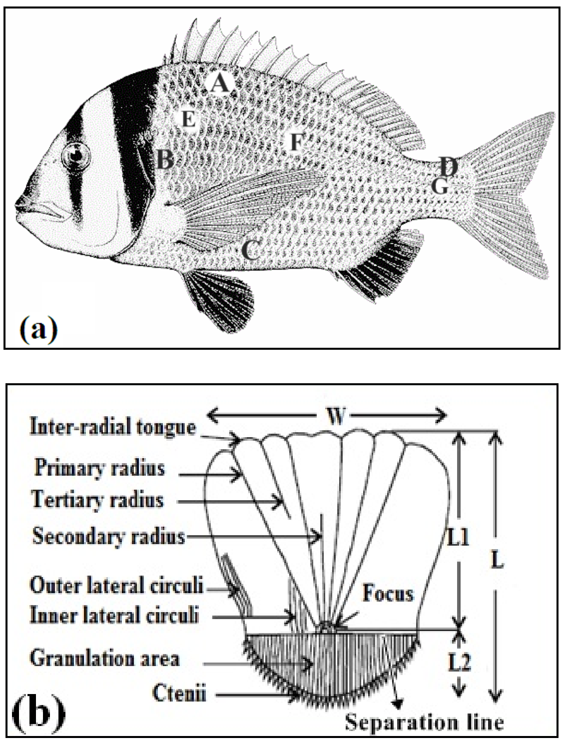

Fig 1: (a): Schematic drawing of *A. bifasciatus* showing the body regions for scales collection. (A= BDFS, B = POS, C = BLLS, D = CPS, E = ALLS, F = MLLS and G = PLLS) and **(b):** A diagrammatic structure of scale showing the different regions, terms and morphometric measurements.

2.3. Microscopic study

Scanning Electron Microscopy (SEM) was used to study the morphology and microstructures of the scales in the rostral, lateral, and caudal regions. The cleaned and dried scales that are used for Scanning Electron Microscope (SEM) examination were mounted 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.

2.4. Statistical analysis

Basic statistics of scale characteristics were estimated. To clarify intraspecific variation of *A. bifasciatus*, ANOVA was applied on the morphometric indices of scales using SPSS package, release 16.0.0 [19].

3. Results

3.1. General morphology of scales

The scales of *A. bifasciatus* are mainly of the sectioned type (i.e. with well-developed radii) on all parts of the body. The scales of *A. bifasciatus* show distinctive surface ornamentation which in its simplest case consists of circuli and grooves, forming nearly circular rings area center called focus. The scales on the post-operculum region (POS) were the largest ones in comparison with those of the other regions studied. In the rostral part of the scale, the circuli were partitioned by deep and narrow groves (radii) that run radially between the focus and anterior rim. The radii on the scales of *A. bifasciatus* can be categorized into three types depending on their origin and end on the scale including: Primary, secondary, tertiary (Fig. 1b).

3.2. Morphometrics and counts of radii

Table 1 shows the basic statistics of the scale morphometric indices (relative to scale length, L) from four body regions (A, B, C & D). This table reveals intraspecific variations in these scale morphometric characteristics of the four body regions. The indices L1/L and W/L showed significant difference between scales of the four body regions ($p<0.05$) whereas, L2/L and L1/L2 revealed highly significant difference ($p<0.01$). Such morphometric indices were size- free ($P>0.05$) (Table 2).

The percentages of occurrence and basic statistics of the primary, secondary and tertiary radii counts are given in Tables 3-5. Table 3 shows variation in the primary radii counts of the scales from the four regions (A, B, C & D). These counts were ranged between 7 -17. The secondary radii counts ranged between 0 – 1 (Table 4). The tertiary radii counts ranged between 0-5 (Table 5). Such radii counts were size- free ($P>0.05$) (Table 6).

Table 1: Basic statistics, Mean ± SD and (Range) of the morphometric indices of scales from four body regions of *A. bifasciatus* from the Southern Red Sea, Egypt.

Index	Region A (PDFS) Mean±SD (Range)	Region B (POS) Mean±SD (Range)	Region C (BLLS) Mean±SD (Range)	Region D (CPS) Mean±SD (Range)
	N= 62	N= 52	N= 81	N= 58
L1/L*	70.52±3.84 (62.5-77.78)	72.79±4.19 (64-81.25)	73.48±4.30 (62.14-81.81)	75.61±5.27 (60.87-85)
L2/L**	29.46±3.86 (22.22-37.50)	27.06±4.31 (18.75-36)	26.62±4.27 (18.18-37.86)	24.44±5.24 (15-39.13)

L1/L2**	243.69±45.68 (166.67-350)	278.11±59.56 (177.78-433.33)	285.56±61.72 (164.15-450)	326.97±86.27 (155.56-566.67)
W/L*	122.76±8.83 (96.15-143.33)	129.31±17.28 (76.92-177.63)	118.71±13.16 (89.47-153.57)	105.39±13.88 (81.82-130.30)

N: Number of scales.
* Differences are significant at 0.05 level.
** Differences are highly significant at 0.01 level.

Table 2: Correlation coefficient between total length (TL) and the morphometric indices of scales from four body regions of *A. bifasciatus* from the Southern Red Sea, Egypt.

Regions	Index	TL	L1/L	L2/L	L1/L2	W/L
Region A (PDFS)	TL					
	L1/L	0.202				
	L2/L	0.168	0.072			
	L1/L2	0.141	0.071	0.085		
	W/L	0.225	0.127	0.126	0.095	
Region B (POS)	TL					
	L1/L	0.388				
	L2/L	0.404	0.085			
	L1/L2	0.362	0.073	0.084		
	W/L	0.200	0.037	0.026	0.004	
Region C (BLLS)	TL					
	L1/L	0.021				
	L2/L	0.024	0.077			
	L1/L2	0.011	0.069	0.083		
	W/L	0.347	0.039	0.003	0.008	
Region D (CPS)	TL					
	L1/L	0.009				
	L2/L	0.017	0.097			
	L1/L2	0.001	0.069	0.072		
	W/L	0.279	0.393	0.403	0.359	

Table 3: Percentages of occurrence and basic statistics of the primary radii counts of scales from four body regions (A, B, C & D) of *A. bifasciatus* from Southern Red Sea, Egypt.

Regions	N	7	8	9	10	11	12	13	14	15	16	17	Mean±SD
A (BDFS)	62	0	0	0	1.61	4.84	27.42	33.87	24.19	4.84	1.61	1.61	13.5±2.45*
B (POS)	52	0	5.77	11.54	7.69	13.46	7.69	11.54	15.38	9.61	11.54	5.77	12.5±3.02*
C (BLLS)	81	2.47	6.17	8.64	13.58	18.52	14.81	18.52	13.58	0	3.70	0	11.11±2.93*
D (CPS)	58	1.72	7.89	20.69	25.86	15.52	13.97	6.89	8.62	0	0	0	10.5±2.45*

*Differences are highly significant at 0.01 level.

Table 4: Percentages of occurrence and basic statistics of the secondary radii counts of scales from four body regions (A, B, C & D) of *A. bifasciatus* from Southern Red Sea, Egypt.

Regions	N	0	1	Mean±SD
A (BDFS)	62	70.97	29.03	0.49±0.28
B (POS)	52	75	25	0.50±0.35
C (BLLS)	81	76.54	23.46	0.49±0.37
D (CPS)	58	60.35	39.65	0.49±0.48

*Differences are highly significant at 0.01 level.

Table 5: Percentages of occurrence and basic statistics of the tertiary radii counts of scales from four body regions (A, B, C & D) of *A. bifasciatus* from Southern Red Sea, Egypt.

Regions	N	0	1	2	3	4	5	Mean±SD
A (BDFS)	62	53.23	40.32	6.45	0	0	0	1±1*
B (POS)	52	42.31	21.15	34.62	0	0	1.92	2±2.1*
C (BLLS)	81	34.57	39.51	22.22	3.70	0	0	1.5±1.29*
D (CPS)	58	55.35	32.14	12.5	1.78	1.78	0	2±1.58*

*Differences are highly significant at 0.01 level.

Table 6: Correlation coefficient between total length (TL) and the radii counts of scales from four body regions of *A. bifasciatus* from the Southern Red Sea, Egypt.

Regions	Index	TL	Primary radii	Secondary radii	Tertiary radii
Region A (PDFS)	TL				
	Primary radii	0.310			
	Secondary radii	0.283	0.232		
	Tertiary radii	-0.243	-0.141	-0.155	

Region B (POS)	TL				
	Primary radii	0.246			
	Secondary radii	-0.087	0.103		
	Tertiary radii	0.302	-0.112	-0.048	
Region C (BLLS)	TL				
	Primary radii	-0.087			
	Secondary radii	-0.004	-0.042		
	Tertiary radii	0.348	-0.260	-0.075	
Region D (CPS)	TL				
	Primary radii	0.346			
	Secondary radii	-0.111	0.097		
	Tertiary radii	0.391	0.220	0.068	

3.3. Scanning electron microscopic studies
3.3.1. Rostral field
In the inter-radial space, the rostral rims of the scales in seven regions form tongue-like projections that are free of circuli near the rim (Fig. 2a).These projections may be convex or straight. The 1st inter-radial circulus was straight or slightly convex. The radial grooves in the rostral field of the scales in seven regions appeared as a deep irregular groove (Fig. 2b).

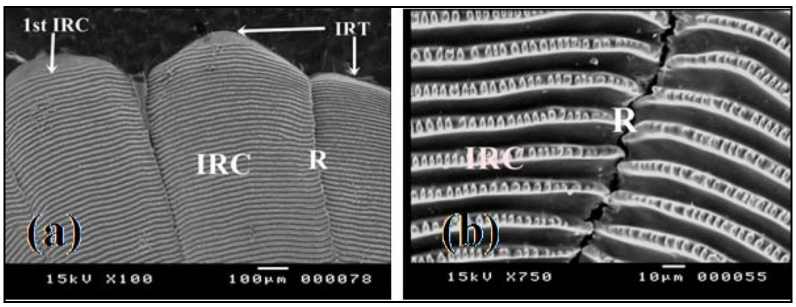

Fig 2: Scanning electron micrographs show (a) the Inter-radial Tongues (IRT), 1st inter-radial circulus (IRC) and Radii (R) in the rostral field of the scales recorded in *A. bifasciatus*; (b) Deep radial grooves(R) with irregular split.

3.3.2. Inter-radial circuli, grooves and denticles
The intercircular grooves in the rostral field were narrow (Fig. 3a). The interradial circuli bear strong small denticles or tooth-like structures with thick free ends oriented backwardly, some denticles were based on vesicle like base.

3.3.3. Outer lateral circuli, grooves and denticles
The outer lateral circuli were low and bear weak denticles (Fig. 3b). The grooves between circuli were wide and flat.

3.3.4. Inner lateral circuli, grooves and denticles
Inner lateral circuli were thick and separated by V-like grooves (Fig.3c). These circuli bear thick and wide denticles with rounded tops.

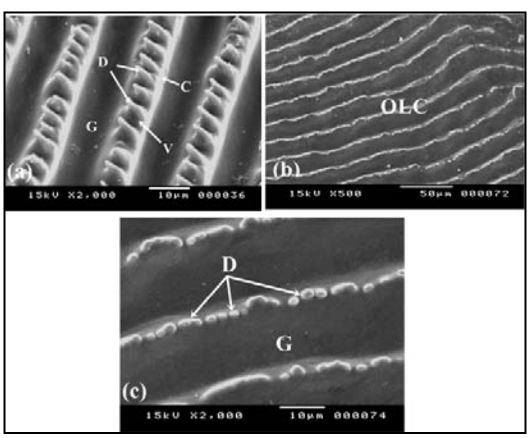

Fig 3: Scanning electron micrographs show (a): The inter-radial circuli; (b): The most outer lateral circuli and (c): The inner lateral circuli recorded in scales of *A. bifasciatus*. Denticles (D), vesicle-like bases (V), groove (G), inter- radial circuli (C) and outer lateral circuli (OLC).

3.3.5. The focus region:
This region was characterized by unique pattern of horny plates- like structures surrounded by rows of tubular ridges in scales and noncircular found (Fig. 4a&b).

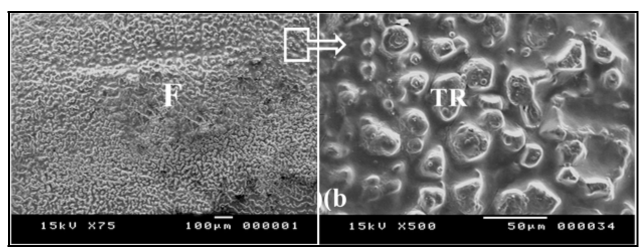

Fig 4 (a & b): Scanning electron micrographs show: The focus region with horny plates –like structures surrounded by rows of tubercular ridges recorded in scales of *A. bifasciatus*. The focus region (F) and tubercular ridges (TR).

3.3.6. Caudal field
The granulation area in caudal field has no circuli but contains bear shape segments and conical shape centii extended posteriorly in the ctenoid scales of seven regions (Fig. 5a).

3.3.7. Lateral line canal
The lateral line canal is divided into a rostral wide tube extend anteriorly to some extent end and do not reach the anterior margin. In caudal field, there were three pores extend from the center of the scale toward the posterior margin having V-like shape (Fig. 5b).

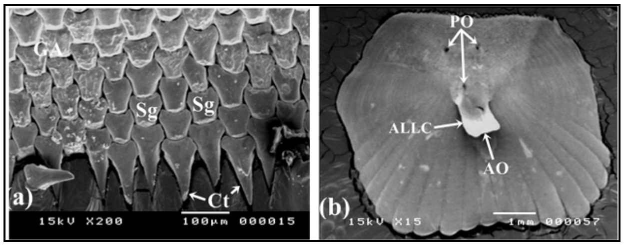

Fig 5(a & b): Scanning electron micrographs show (a): The forms of ctenii and segments in the granulation area of scales of *A. bifasciatus* and (b) The characteristics of the lateral line canal: a wide anterior lateral line canal with anterior opening and three posterior opening. Ctenii (Ct), segments (Sg), granulation area (GA), anterior lateral line canal (ALLC), anterior opening (AO) and posterior opening (PO).

4. Discussion

Many authors have been studied the stability of surface structure and surface ornamentation of the rostral and caudal field of scales [10, 12, 14, 15, 3, 4, 16, 18, 5, 6]. These studies investigated the potential of scale and squamation characters of some teleosts species revealing a wide spectrum of unexplained useful characters. In the present work, it was noted that, in spite of the fact that *A. bifasciatus* is reef-associated and inhabit shallow coastal waters mainly aroreefs and were subjected to various environmental factors, their size –free scale characters and ornamentation were stable. Hence, one can conclude that the impacts of such factors were omitted and size-free fixed scale characters are expressions of their divergent evolution. Moreover, these structures were not controlled by the environmental factors. It was concluded that the intraspecific variations were considered to be genetically controlled. The genetically fixed scale characters would not appear to be subjected to strong selection pressures in spite of their functional significance as referred by [20, 21]. These findings were emphasized by results of [22, 25]. The results of the present work reflected the stability of the surface structures of rostral and caudal field of scales of *A. bifasciatus*. This stability may provide useful criteria for systematic purposes in other teleosts.

In the present study, intraspecific variations were reflected on the bases of quantitative scale characters in terms of the size-free morphometric indices of the scales from different body regions. [23, 9, 3, 11, 26, 12, 4] used similar morphometric characteristics of scales for identification of some teleosts.

The presence of primary, secondary and tertiary radii is considered as growth phenomenon. It was weakly influenced by genetic factors [27]. The better nutritive conditions of the fish may be correlated with the higher number of radii [28]. Radii represent the line of scale flexibility [16]. There was no significant correlation between number of radii and fish size and consequently were size free for *A. bifasciatus* in the present work. The patterns of distribution of such size-free counts in scales of *A. bifasciatus* revealed highly significant intraspecific differences in primary and tertiary radii counts. Ganzon *et al*. [18] stated that the environment is recognized as a powerful force in modeling the morphology of an organism during ontogeny. The epidermal cover of *A. bifasciatus*, like other swimming fishes undergo friction forces due to water flow in coral reefs habitats. So, one can speculate that the interradial circuli with their denticles and those of lateral fields may play an important role to offer a resistance to these frictional forces by mechanical anchoring. This statement could be generalized for reef fishes.

The present study revealed also that the shape of the first interradial circuli was convex or straight and all denticles on the interradial circuli were oriented posteriorly towards the scalar focus, while those found the inner lateral circuli are slightly oriented towards the scale center. This means that the denticles have multi directions and hence, may be involved in the mechanical anchoring of the scale into the covering dermis to prevent the movement or detachment of the scale. Jawad [10] stated that small-sized processes located on the circuli cannot anchor the scale in the dermis as securely as can the well-developed denticles but may be anchored in the surrounding tissue by the bundles of the collagen fibers concerting the upper part of the scale to the overlaying dermis [29]. These denticles are not homologous to breeding tubercles and contact organs [30].

The pattern of granulation of the caudal field including shape and size of segments and ctenii and overall caudal field of the scales of *A. bifasciatus* were constant with fish size. Such findings emphasized on the importance of caudal field of scales as a taxonomic characters not only at the level of species or genera but also at family level [31, 23, 24, 9, 11, 12, 4].

The anterior opening of the lateral line canal of *A. bifasciatus* studied is hidden by an evelike extension cantilevered over it. The anterior opening is wider than the posterior one. The cantilevered anterior extension of the canal may help in direction of water motion speed and direction [32]. A wide range of various structural patterns of lateral line canals was recorded in different teleost species by many authors [e.g., 23, 24; 11; 12, 4, 5]. Its form was ranged from a simple perforation to a long canal with or without simple to highly complex cantilevered extensions acting as covers for the anterior opening.

Variations in the shape and position of the anterior and posterior openings of the lateral line canal reflect its importance to differentiate between some fish species belonging to the same genus or different genera. Also, it may be important in identification of groups (orders, families, genera and species) especially when combined with other equally impressive characters of scale structure [31, 12, 4].

5. Conclusion

The quantitative scale characters (morphometric indices and radii counts) displayed a lot of intraspecific variations in *A. bifasciatus* studied. Application of scanning electron microscope to reveal morphology, ultrastructure and surface ornamentation of fish scale had facilitated its utility to distinguish the taxonomic groups over a continuum ranging from higher taxa to species.

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