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Describing variations in scales between sexes of the yellowstriped goatfish, *Upeneus vittatus* (Forskål, 1775) (Perciformes: Mullidae)

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ABSTRACT

Scales have numerous hidden details in their sculptural design that contributes effectively to fish identification and classification. A traditional approach has been made to study the scale morphology of the yellow striped goatfish *Upeneus vittatus* (Forskål, 1775) using a micron cam attached to a Gateway computer and a stereomicroscope in tandem with a 14 megapixel Kodak easyshare Z 1485 IS digital camera in which digitized images were processed using Corel Paint Photoshop 2x program.

To eliminate subjective human error, scale variation in scale images were subjected to Elliptic Fourier Analysis using 77 Fourier descriptors and Principal Component Analysis to discriminate variation between scale shapes within the male and female *U. vittatus* and to use the data generated to established variation between male and female species.

Scanned images (1200dpi) were binarized to generate chain codes using SHAPE 1.3 version. The data were analyzed using Principal Component Analysis conducted using the PAST software ver. 1.8.

Result of the study revealed that there is a high significance in the variation of shapes within male and female individuals of *U. vittatus* and that a significant variation in scale shapes was observed between male and female species.

This study demonstrated that scale characteristics can provide useful taxonomic information on the morphological differences between sexes of *U. vittatus* and that elliptic Fourier analysis and principal Component analysis are good tools to discriminate variation in scale shapes.

Keywords: Image analysis, elliptic Fourier analysis, principal component, scale variation, Fourier descriptors, chain codes.

INTRODUCTION

Many studies have been made regarding fishes. Most of these studies focused on fish economics, their ecological importance and systematics. In the study of fish identification and classification, scale morphology is one of the tools used by fish taxonomists. The use of scale size, shapes and number can be traced back to the first half of the 19th century when Agassiz (1883-1884) used it in fish taxonomy for the first time (Reza *et al.*, 2009; Jawad and Jufaili, 2007). Even fishery biologist also used scale to tell the age of fishes (Hickman *et al*., 1993).

Scales have numerous hidden details in their sculptural design that contributes effectively to fish identification and classification. Circuli, radii, ctenii, lateral line canal and other structures associated with scales have been used authentically for classification (Kaur and Dua, 2004; Dicenzo and Sellers, 1998; Hollander, 1986; Hughes, 1981).

This study was conducted to morphologically describe the scales of male and female yellow striped goatfish *Upeneus vittatus* (Forskål, 1775) known for its distinctive black stripes on its tail and the presence of a pair of barbells just below the symphysis of their lower jaw (Fig. 1). This commercially important demersal species are mostly found in sandy or muddy sand bottoms (Prabha and Manjulatha, 2008).

Shape analysis of the scales made use of methods of Geometric Morphonometrics (GM) such as elliptic Fourier analysis. Principal component analysis was also used to establish scale shape variation between body regions of the fish and to use these differences to describe scale variation between sexes. Sexual dimorphism in scale structures might be useful for taxonomic studies since there has been a clamor that an intensive analysis of variations within taxonomic units and between sexes should be done (Lagler, 1947).

Qualitative descriptions of the fish scale were patterned after that of Lippitsch (1992) and Kuusipalo (1998).

Fig. 1: A photograph of an unsexed goat fish, *U. vittatus* showing its pinstriped tail and lateral lines (image resolution 480dpi).

MATERIALS AND METHODS

Collecting and Preparation of Samples

Adult male and female *U. vittatus* weighing 250g each and has a length of 150 mm were used. Scales were removed from the six (6) different fish body regions as illustrated in the Atlas of Common Squamatological Material in Coastal British Columbia and an Assessment of the Utility of Various Scale Types in Paleofisheries Recontruction (Patterson *et al.*, 2002; Casteel, 1976) with the use of a flat end forceps. Thirty (30) scales were taken from each body region except in some regions which the number did not reach 30 (Fig. 2). Scales removed from the different body parts were separately stored in a plastic container and labeled according to body regions and sex. Scales were then allowed to air dry before they were soaked in a very dilute liquid detergent for thirty (30) minutes after which they were rinsed in tap water and left to dry in their separate containers before they were mounted on slides.

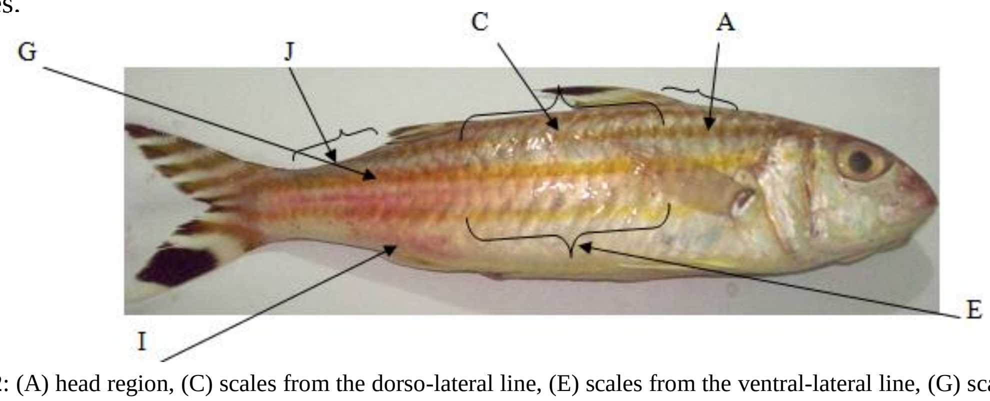

Fig. 2: (A) head region, (C) scales from the dorso-lateral line, (E) scales from the ventral-lateral line, (G) scales on the posterior above the dorso-lateral line, (I) scales below the ventral lateral line posteriorly located (480dpi).

Mounting of Scale Materials

Scales for mounting were soaked in freshwater for a few seconds before they were mounted on the slides to avoid curling and breaking of scales. Pairs of laboratory slides (1" x 3") were used in the process. Each slide contains 5 to 10 scales depending upon the size. To avoid problem with scales with varying thickness mounted together, the slides were pressed hard against each other and was taped together using an Armak invisible tape at the opposing side while the other opposing side were left untapped to allow remaining water residue to evaporate. They were left for 24 hours in that position to ensure dryness before examination after which the untapped end were secured before examination.

Photography of Preserved Fish Scales

The images of the fish scales were viewed using a macron cam attached to a gateway computer and were immediately saved. The digital images of the scales were then processed using Corel Paintshop2x. As for the detailed structures of the scales such as the ctenii and canaliculi which was not very clear with the macron cam, a stereoscope was used at its lowest magnification (2X) and photographs were taken using a Kodak easyshare Z 1485 IS digital camera with a resolution of 14 megapixels.

Morphological Analysis of Scale Shapes and Lateral Line Scale Shape

Scale shapes and shapes of the lateral line scale identification has been patterned after the work of Jawad (2005).

Image and Contour Recording

The scales were uniformly scanned at 1200 dpi using Plustek *Optic Slim 2000* scanner. The 24-bitmap images generated were processed into gray scale binary images using Shape Version 1.3 software after which a one step noise filter was applied. Noise filter was done to erase grainy marks (Iwata and Ukai, 2006) on the surface of the scales. After noise reduction, contours of the scales were extracted via edge detection and the contour was stored as chain codes (Dalayap *et al.*, 2008). Normalized Elliptic Fourier Descriptors (EFD) obtained from the chaincodes were calculated using Elliptic Fourier transformation as suggested by Kuhl and Giardina (1982). Normalization of data obtained from chain codes used the first harmonic ellipse as a basis which corresponds to the first Fourier approximation and utilized the 20 harmonics number to be calculated as suggested by Iwata and Ukai (2006).

Elliptic Fourier analysis

To verify and further strengthen the result of the initial scale data generated during the early part of the study, scales were subjected to Elliptic Fourier Analysis (EFA) to help visualize the pattern of shape variation using SHAPE 1.3 version software. Coefficient descriptors generated were then subjected to Principal Component Analysis (PCA) because scale shapes of *U. vittatus* vary considerably from region to region and PCA reduced data dimensionality. PCA scores explained variation in scale shapes and variations were visualized when PCA scores were used to reconstruct contour of the scales using Paleontological Statistics (PAST) software (Hammer et.al., 2001) where principal scores generated were used for the analysis. Differences in scale shapes between body regions and sexes were analyzed using a non-parametric Kruskal-Wallis test. Box- and whiskers and scatter plots were used to visualize the distribution of shape variation using the principal component scores.

The schematic diagram in Fig. (3) summarizes how the analysis was done in the study.

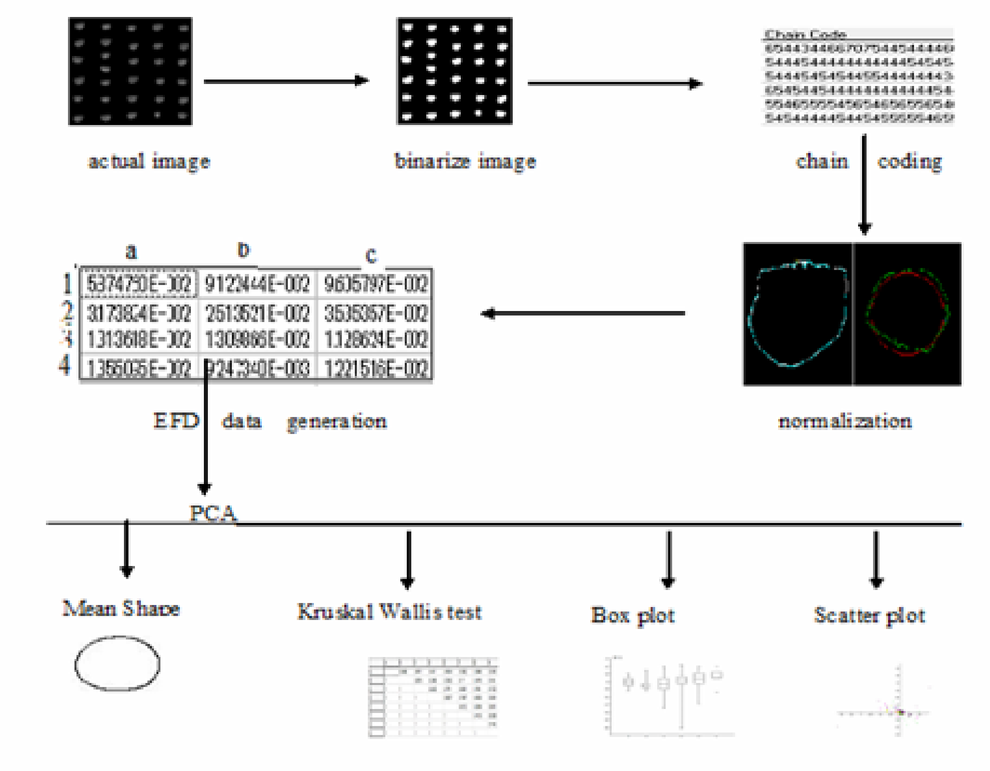

Fig. 3: Schematic diagram of the flow of analysis.

RESULTS AND DISCUSSION

General Morphology of the Scales

The study revealed that all the scales found in the different regions of the fish body have a focus, a radius, a circuli, a lateral field, a posterior field, an anterior field and a ctenii found at the posterior field of the scale (Fig. 4). This is also true for both sexes of fish.

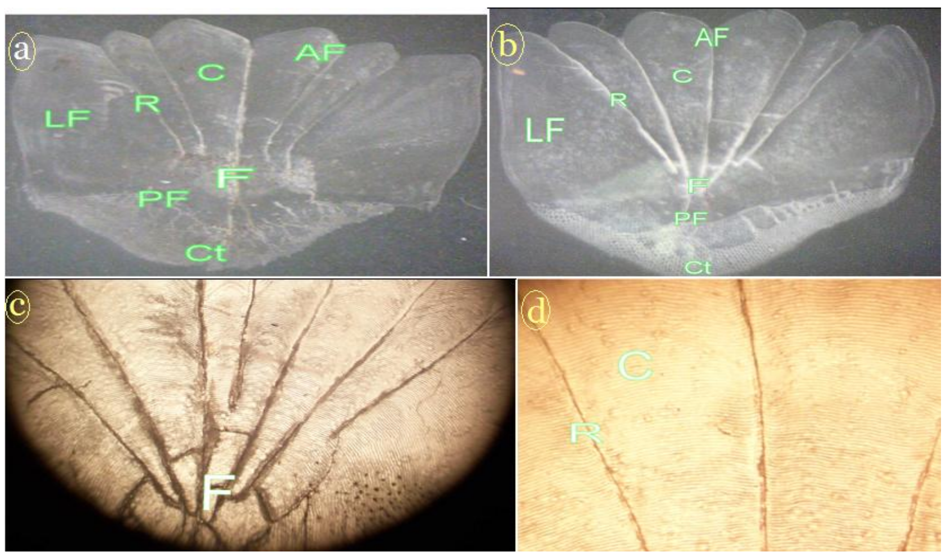

Fig. 4: A macrophotograph of a ctenoid scale of ***Upeneus vittatus*** showing anterior field (AF), posterior field (PF), lateral field (LF), ctenii (Ct), radius (R), focus (F), circuli (C) of (a) male (image dimensions 625 x 266 pixels); a female (b) (image dimensions 523 x 479 pixels; (c) a fish scale showing its focus (image dimensions 2592 x 1456 pixels); more details in (c) (image dimensions 765 x 544 pixels) and (d).

The presence of ctenii at the posterior field of all scales strengthened the claim that these scales are ctenoids. An Illustration of the ctenii of the fish is shown in Fig. (5).

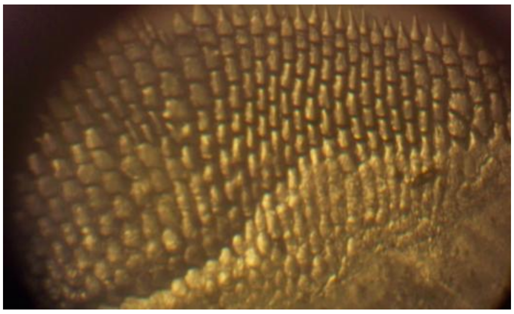

Fig. 5: Stereomicrophotograph showing the ctenii at the posterior field of *U. vittatus* scale (Photograph dimensions 2251 x 1456 pixels).

Both male and female fish have varying scale shapes. Scale shapes are illustrated in Fig. (6).

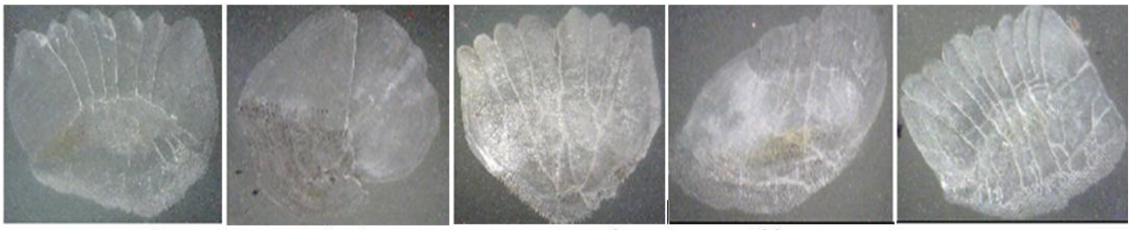

Fig. 6: Shapes of body scales generally found in male and female *U. vittatus* (image resolution: 96 dpi).

There are several types of scale shapes. There is elliptic, oblong, pentagonal, rectangular, square, triangular and cycloid (Jawad, 2005) but in the case of *U. vittatus*, only five types were manifested. They are only cycloid, oblong, square, triangular and rectangular shapes. Variation in scale shapes do occur within body regions in some species of fish (Jawad, 2005) and this was also observed in both sexes of *Upeneus vittatus*.

Table 1**:** Variation in the type of scales, scale shape, scale size, and shape of the posterior margin of male and female yellow striped goatfish, *U. vittatus.*

Body Regions	Type of Scale	Scale Shape	Scale Size	Shape of the Posterior margin group of ctenii
Male Fish				
Region A	ctenoid	square	small	triangular, tongue-like, square, lower lateral line scale
Region C	ctenoid	triangular	moderate	tongue-like, square
Region E	ctenoid	square	moderate	tongue-like, square
Region G	ctenoid	square and oblong	small to moderate	tongue-like, square
Region I	ctenoid	square and triangular	small to moderate	triangular, square, lower lateral line scale
Region J	ctenoid	triangular	small	triangular
Female Fish				
Region A	ctenoid	square and cycloid	small	triangular, tongue-like, lower lateral line scale
Region C	ctenoid	square	moderate	triangular, tongue-like, square
Region E	ctenoid	square	moderate	triangular, tongue-like
Region G	ctenoid	square and triangular	small to moderate	triangular, square, tongue-like,
Region I	ctenoid	square and triangular	small to moderate	triangular, square, lateral line scale, tongue like
Region J	ctenoid	triangular	small	triangular

Within body regions of the fish, males have square shapes in the head unlike females that have square and cycloid shape. Triangular shapes in scale are above the lateral line in males while females still possess square scales in this region. Both sexes have uniformed square scales in region E but variations in shape within a region occur in region G for both sexes. Both have common shape in region J. Oblong scale shape is only present among the males while cycloid scale is in females. This can be considered as a key point in the differentiation of the two sexes of *U. vittatus*.

The scales also showed variation in the shape of the posterior margin group of ctenii in both sexes of *U. vittatus.* Variations are shown in Fig. (7) (image resolution is 96 dpi).

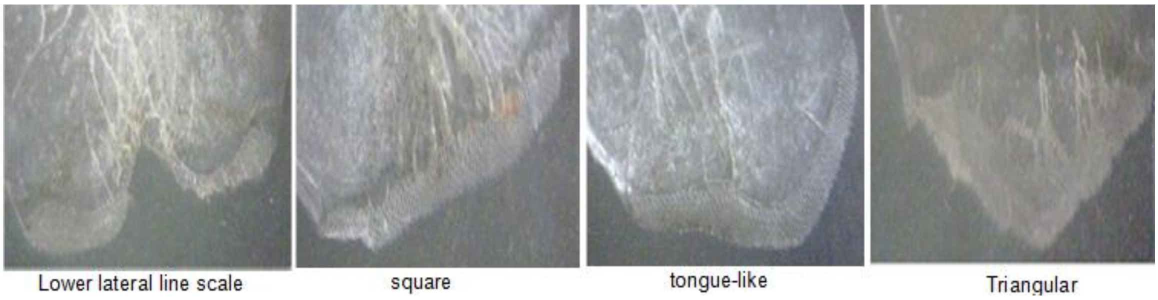

Fig. 7: Ctenoid scales of *Upeneus vittatus* showing variations in the posterior margin of the scale where ctenii are grouped.

Table 2: Variation in focus position, type of radii, number of radii and circuli appearance of male and female goat fishes, *U. vittatus*.

BODY REGIONS	FOCUS POSITION	TYPE OF RADII	CIRCULI APPEARANCE
Male Fish			
Region A	centrally located	mostly primary; very few secondary	distinct; disrupted
Region C	most center; few posterior	mostly primary; very few secondary	distinct; disrupted
Region E	most center; few posterior	mostly primary; very few secondary	distinct; disrupted
Region G	most center; few posterior	mostly primary; very few secondary	distinct; disrupted
Region I	most center; few posterior	mostly primary; very few secondary	distinct; disrupted
Region J	posterior	all primary	distinct; disrupted
Female Fish			
Region A	centrally located	mostly primary; very few secondary	distinct; disrupted
Region C	most center; few posterior	mostly primary; very few secondary	distinct; disrupted
Region E	most center; few posterior	mostly primary; very few secondary	distinct; disrupted
Region G	most center; few posterior	mostly primary; very few secondary	distinct; disrupted
Region I	most center; few posterior	mostly primary; very few secondary	distinct; disrupted
Region J	posterior	all primary	distinct; disrupted

The focus is the part of the scale that developed first during ontogenesis. The position of the focus on the scale remains the same throughout the life of the individual species (Jawad, 2005; Liu and Shen, 1991). The scale of both male and female species of *U. vittatus* removed from the different regions showed that most of its scale has centrally located focus. Arrangement of focus between regions follows a pattern. From the head, focus are all centrally located while the middle body regions have a mixture of centrally located focus with few of them located at the posterior field while the J region have focus which are all located on the lower posterior field. The position of the focus for both sexes of the fish in every region is the same. Posterior location of the focus is due probably to lateral scale growth rather than a combination of anterior and posterior scale growth (Jawad, 2005; Roberts, 1993). No apparent differences exist between sexes in terms of focus position.

From the focus, lines of growth start to appear and develop into ridges. These are the circuli or the lines of growth. They are plenty on the anterior part of the scale. The circuli of *U. vittatus* when examined closely using a stereomicroscope and photographed with a 14 megapixels Kodak Easyshare z 1485 IS digicam and digitized using Corel Paint Photoshop 2x program showed that circuli in the scales of both male and female species of *U. vittatus* have manifested a disrupted circuli. These disruptions might be due to the exposure of the fish to environmental and biological factors such as spawning, stunted growth or abundance of food. The condition of circuli on the surface of the scale in between radii is also a morphological point of comparison although in these cases both sexes of fish demonstrate the same type of circuli. Circuli observed here are those above the focus only and did not reach the first ridge due to optical limitations. A close observation on the circuli of both sexes revealed that *U. vittatus* species have disrupted circuli (Fig. 8).

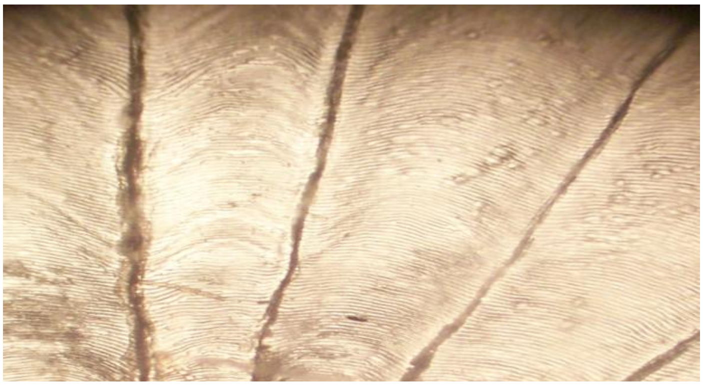

Fig. 8: A disrupted circuli in *U. vittatus* in between radii.

In the anterior and lateral parts of the scale where the ctenii are mostly located, they are enclosed by deep narrow grooves that run radially to the focus. They are called radii. The scales of *U. vittatus* have well developed radii. Both primary and secondary radii are present in the scales except in region J. The number of primary radii is higher than the secondary radii which exist in only very few scales and the number of secondary radii per scale vary from 2 to 4 only. Again, a trend occurs here. A combination of primary and secondary radii are only present from the head (region A) to body regions A, C, E, G, H but it is absent in the J region (Table 2). This trend might be useful in taxonomy. There is no consistency in the number of radii within regions. These radii divided the anterior part of the scale into several sections which give rise to the "teethlike" structures of the scale. The number of the crest of the teeth-like structures varies, their shapes also varies. The edges of the lateral field also vary from each other. These contribute to the unique architectural design of each scale. The radii formation is considered to be related to the accommodation power of the large surface area of the anterior and lateral parts of the scale in the lesser parts as these two parts of the scale are overlapped by the posterior part of the preceding scale. The higher number of radii is correlated with the better nutritive conditions of the fish (Johal *et al.,* 2006; Tandon and Johal, 1966). Radii also represent scale flexibility (Esmaeili and Gholami, 2007). No variation between sexes has been observed in terms of the types of radii.

Elliptic Fourier and Principal Component Analysis

Scale shapes were converted into chain codes and subjected to elliptic Fourier analysis obtaining 77 normalized elliptic Fourier coefficients. Scale outlines were then subjected to principal component analysis to measure degree of variation between male and female scales of *U. vittatus* (Fig. 9).

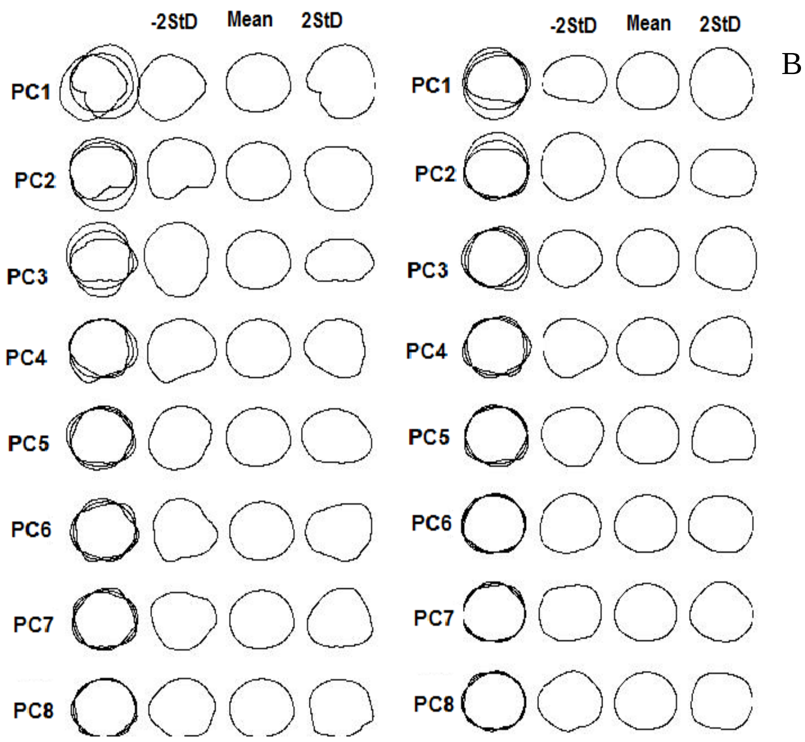

Fig. 9: Reconstructed scale outline of male and female scales of *U. vittatus* (A. male, B. female) illustrating the eight effective principal component.

The shapes of the scales assumed different contours but these variations have been narrowed down into principal components. Principal component analysis have described eight effective principal components for both male and female *U. vittatus* which accounts for 93.45% variation in scale shapes in males and 90.94% variation in female shapes. In table (3), Eigen values and the proportion of variances of the effective principal components of both sexes of fish are given.

Table 3: Eigen values and the variances of the effective principal components.

MALE FISH				FEMALE FISH			
Principal Component	Eigen values	Proportion (%)	Cumulative (%)	Principal Component	Eigen values	Proportion (%)	Cumulative (%)
1	4.0719E-002	39.2968	39.2968	1	1.2824E-002	34.0335	34.0335
2	2.3909E-002	23.0745	62.3713	2	1.0120E-002	26.7530	60.7865
3	1.4008E-002	13.5188	75.8901	3	5.5579E-003	14.6927	75.4792
4	7.5593E-003	7.2953	83.1854	4	2.1336E-003	5.6404	81.1196
5	4.0681E-003	3.9260	87.1115	5	1.5161E-003	4.0080	85.1275
6	3.2621E-003	3.1482	90.2596	6	9.0681E-004	2.3972	87.5247
7	1.7321E-003	1.6716	91.9313.	7	7.4435E-004	1.9677	89.4924
8	1.5739E-003	1.5190	93.4503	8	5.4738E-004	1.4470	90.9395

In male scales, Principal Component 1 (PC1) accounts for the variation in length-width ratio (34%), PC2 (23%) accounts for the variation in the posterior margin of the scale. Shape of the posterior margin ranges from tongue-like, square triangular and the lateral line scale; PC3 for the variation (13.52%) in the shape of the anterior field and the posterior field of the scale; variations here can be attributed to the scalloped shape of the anterior field brought by the presence of radii in varying number and distances from one tooth to another tooth while PC4 describes the variation (7.29%) in the lateral aspect of the scale. The remaining principal components accounts for the remaining 10% variation.

In female scales, PC1 accounts 34% variation in the length–width ratio of the scale, PC 2 (26.75%) for the variation in the anterior aspect of the scale; PC 3 for the variation (14.69%) in the posterior aspect of the scale and PC 4 (5.64%), the lateral shapes of the scale. Variability in scale shape is defined by the effective principal component. The rest of the effective principal components have described a lower degree of scale variation in both sexes. As cited by Dalayap, et al. (2008), "it must be emphasized that while shape trends along principal component axes frequently corresponds with observed morphology, they do not represent the actual appearance". This is basically true because what appear in the principal component analysis are reconstructed contours or shapes that illustrates pattern of variability in shapes due to the reduction in the number of variables. These reconstructed contours are the effect of the principal components.

The mean shapes of both male and female individuals of *U. vittatus* are asymmetrically ovoid in shape. It looks like an egg shape but has no symmetry (Fig. 9A and B.).

Using the principal component scores of the male and female scales, a scatter plot was constructed to determine the scale shape distribution. Scale shape distribution along the first and second principal component of the elliptic Fourier coefficients help in determining the extent of similarities and differences among the scale shapes within the male or female *U. vittatus* species.

In males (Fig. 10), the distribution of scales shapes is generally oriented toward the central axis of the first two principal components as indicated by the cloud formation along the central axis. This is an indication of close association or strong similarity in shape among the scales from varying body regions of the fish which lead most likely near the mean shape. However, distribution of scales in region A shows a relative distance from the mean shape which implies shape variability. Regions E and G showed a considerable variation in their shape as scales from these regions lie on top of the PC2 axis and are separated by the OC_2 axis towards the PC1 axis. Region E scales lie on the -2Std side of the PC1 axis and deviate towards the 2StD side of the axis. These distances and distribution of scales implies variation among the scales within the male species.

In females (Fig. 11), distribution of scale shapes is a little bit wider than in male. Scales are not so thickly clouded in the central axis compared to male though close distances are maintained in some scales while other scales still have overlapping shapes. Like in males, scales of region A have a wider distribution than the rest of the scales in female fish though because they can be found in all fields and distantly situated from the rest of the scales. Region I and E display greater variation in shape as emphasized by the location of their position along the PC1 and PC2 axis. This distribution in the scatter plot implies that scales' close distance and overlapping leads to communality of some shape characteristics and lesser variation while those that are not closely distant showed variations in shapes.

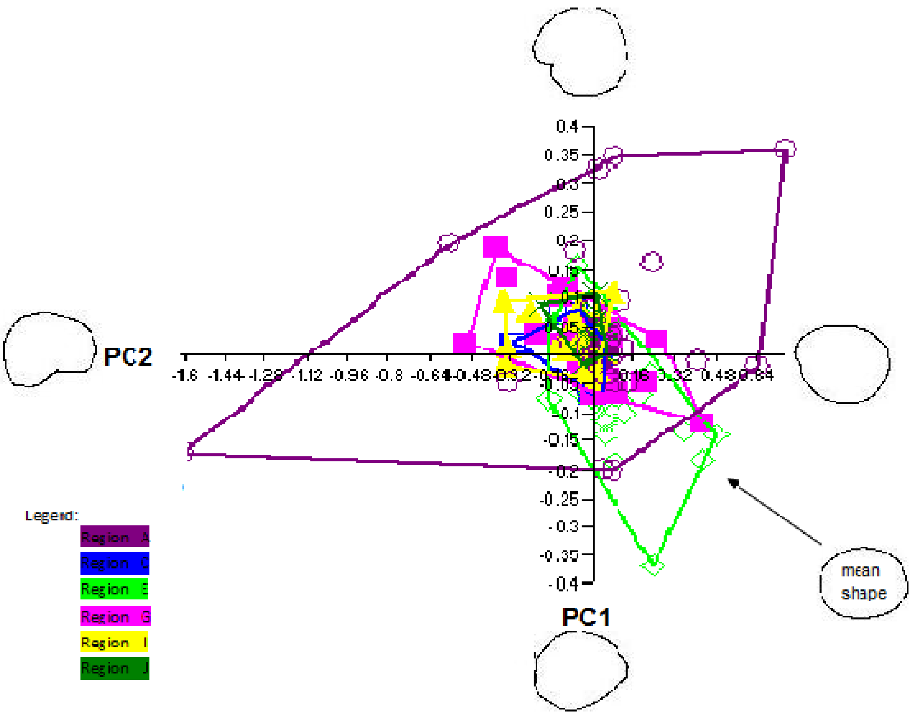

Fig. 10: A scatter plot indicating the direction of distribution of male scales along the first two principal components of the Elliptic Fourier coefficients. A clouding can be observed around the central axis.

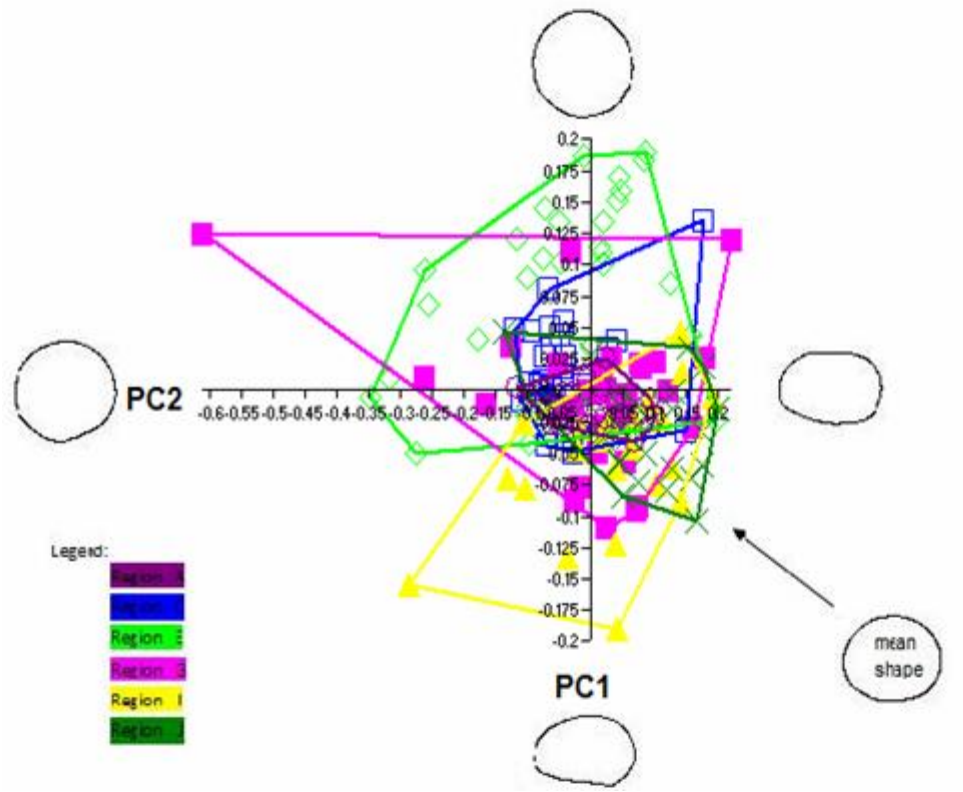

Fig. 11. Direction of distribution of female scales along the first two principal components of the elliptic Fourier coefficients.

Kruskal-Wallis test result (Table 4) have revealed that among the eight identified effective principal component in the male species of *U. vittatus*, PC1, PC3, PC7 and PC8 display a highly significant variation in scale shape within the different body regions of the fish. This implies that out of the 93.45% variation express by the component scores, 56% of these variations are highly significant. PC7 and PC8 have a very low proportion in shape variations but are highly significant.

Table 4: Results of the Kruskal-Wallis test of the 8 principal components of elliptic Fourier coefficients resulting from the shape outlines of the male fish.

COMPONENT	KRUSKAL-WALLIS VALUE	P-VALUE ≤ (.0001)	REMARKS
PC1	33.23	0.000003386	highly significant
PC2	16.03	0.006756	not significant
PC3	46.07	8.771E-09	highly significant
PC4	19.86	0.001327	not significant
PC5	9.929	0.007721	not significant
PC6	16.5	0.005555	not significant
PC7	29.09	0.00002225	highly significant
PC8	41.88	6.241E-08	highly significant

Box plots were constructed to visualize the differences in the distribution of the first eight effective principal components in the shapes of male (Fig. 12) and female scales (Fig. 13) of U. *vittatus*.

In the box and whisker plots illustrating the distribution of male scale shape variation in Fig. (12), presence of small boxes with thick lines can be observed prominently in some boxes particularly in PC1. This small box with thick lines indicates that these regions contain a very high number of variations within a very small segment of the sample (http://i.ehow.com/images/a04/f5/8/read-interpret-box-plot).

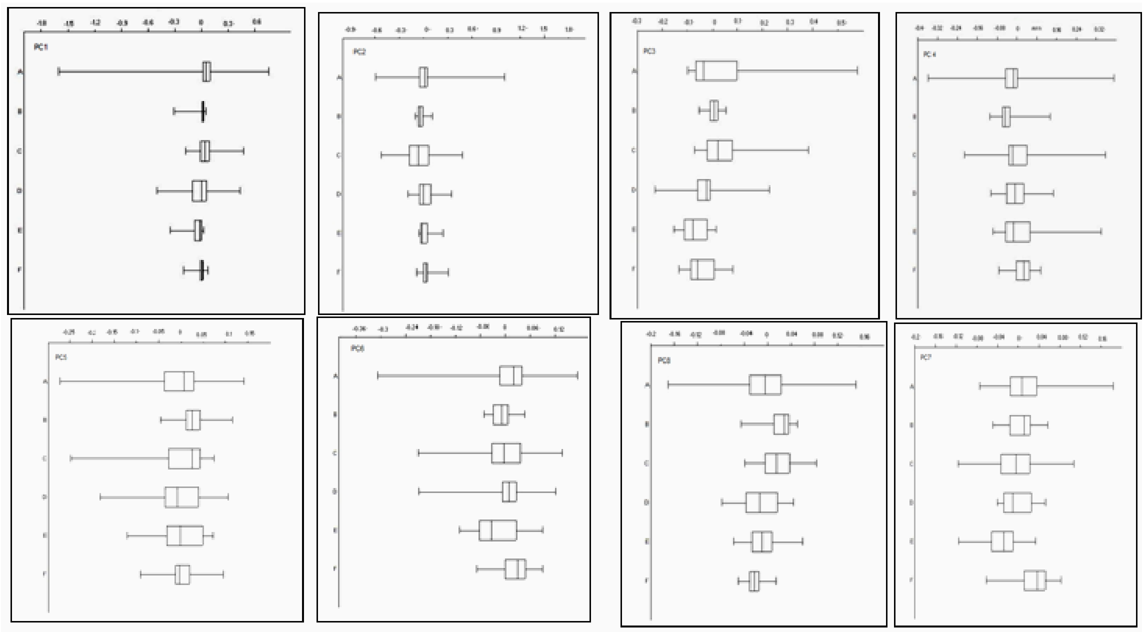

Fig. 12: Box plots showing the variation in the distribution of the first eight principal components in male scales of *U. vittatus* based on 77 elliptic Fourier coefficients.

In female scales, PC1, PC2, PC3 and PC5 Kruskal-Wallis test result showed that 77.88% of the variations in female scales are highly significant variations. Table (5) shows the results of the test.

Table 5: Results of the Kruskal-Wallis test of the 8 principal components of elliptic Fourier coefficients resulting from the shape outlines of the female fish.

COMPONENT	KRUSKAL-WALLIS VALUE	P-VALUE ≤ (.0001)	REMARKS
PC1	39.38	.000000199	highly significant
PC2	37.18	.0000005517	highly significant
PC3	35.23	.000001355	highly significant
PC4	22.0	.0005245	not significant
PC5	18.26	.002637	not significant
PC6	40.8	.0000001032	highly significant
PC7	4.04	.5437	not significant
PC8	5.687	.3378	not significant

Again, it can be observed from the value of PC6 (Table 3) that its percentage of variation is very low (2.39%) as compared to the other principal components PC5 (4.0%) whose p value (Table 5) is higher than the threshold value (P-value ≤ (.0001) and render its variation insignificance. This result is shown because these regions contain a very high number of variations within a very small segment of the sample as shown in the box plots illustrated in Fig. (13).

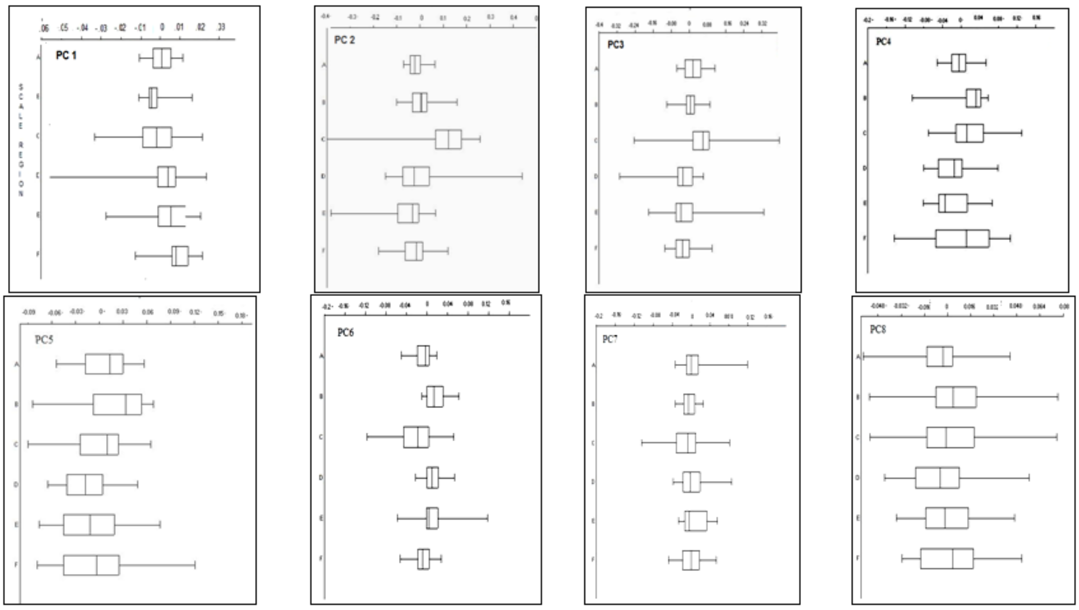

Fig. 13: Box plots showing the variation in the distribution of the first eight principal components in female scales of *Upeneus vittatus* based on 77 elliptic Fourier coefficients.

CONCLUSION AND RECOMMENDATION

Traditional identification of scale structures revealed the presence of focus, circuli, lateral line canals, radii, lateral fields, posterior fields, anterior field, and shapes of scales as the characteristics that have been observed in this work. Only the shape of the scale, position of the focus, type of circuli, type of radii and shapes of the lateral line have been considered here for point of analysis though these characters may constitute as criteria for differentiating sexes of *U. vittatus.*

The presence of disrupted circuli, the same type of radii existing in every body regions in both sexes of the fish cannot be used to establish sexual dimorphism in *U. vittatus* due to their similarities. The existence of an oblong scale shape unique to males only and that of a cycloid shape unique to females only is a good indicator of sexual dimorphism between the sexes of *U. vittatus.*

Though the shape of the lateral line scale shows also variations within body regions and between sexes, an intensive study should be made to establish a clear line in the differences on the morphology of the lateral scale line by using better optical equipments with higher resolution to differentiate other characteristics of the lateral line scales such as the arrangement of ctenii, position of the canal and its alignment. Other structures which are present in the scales which were not identified in this study should also be taken into consideration.

Elliptic Fourier Analysis have revealed seven effective principal components for both sexes of *U. vittatus* proving that objects with very irregular shapes such as the scales can be reconstructed to study its shape variation.

Furthermore, statistical analysis using Kruskal-Wallis test has also revealed that there is a significant scale variation among male scales and also within female scales and these variations quite differ in their manifestations in both sexes of *U. vittatus*. This signifies that scale morphology can be used as a tool for establishing sexual dimorphism in *U. vittatus*. This indicates that elliptic Fourier analysis and Principal Component analysis can be used to discriminate variation in scale shapes.

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