www.abiosci.com

ISSN: 2348-1927

RESEARCH ARTICLE

Annals of Biological Sciences
2014, 2 (4):1-10

Qualitative and Quantitative Analysis of Scale Shape Variation between Different Body Regions of Redbreast Wrasse (*Cheilinus fasciatus*)

Queenilyn B. Albutra, Mark Anthony J. Torres and Cesar G. Demayo

Department of Biological Sciences, College of Science and Mathematics, MSU-Iligan Institute of Technology, Iligan City, Lanao del Norte, 9200, Philippines

Correspondence: queenilyn_albutra@yahoo.com

(Received: 29/07/14) *(Accepted:18/11/14)*

ABSTRACT

Scale morphology became important in fish systematic and phylogeny after the introduction and development of scanning electron microscopy and image analysis software. In this study we examined the morphology of the scales of the redbreast wrasse, Cheilinus fasciatus using image analysis and the applications of elliptic Fourier (EFA) method to understand variations in scales within regions of the fish. Four types of scales of the fish were observed - cycloid, cosmoid, ganoid, placoid, leptoid and ctenoid types. To quantitatively determine shape variation in the scales in different body regions, geometric morphometric analysis using EFA was done. The Fourier coefficients were analyzed statistically using the Kruskal-Wallis test. The results of the multivariate statistical analysis revealed that there is shape variation in the scales obtained from the different regions. Scales obtained from regions BC and J regions of the fish were the most variable shapes. The result of multivariate analysis is consistent with the result in the morphological observation of the scales. Variation in the scale shape might be contributed to the swimming mode of the fish.

Keywords: Cheilinus fasciatus; scale morphology; geometric morphometrics; Elliptic Fourier Analysis; Kruskal-Wallis test

INTRODUCTION

Fishes have protective structure in their skin that protects them from scrapes, parasites and other external injuries. Scales are part of organism's integumentary system and they are categorized based on their shape and to the class of animals. The type of scales a fish possesses can often reflect the behaviours and lifestyle of the species. The huge popular of bony fishes, including the *Cheilinus fasciatus,* is generally described as having a round or cycloid type of scales that have a smooth outer edge [11].

Detailed qualitative and quantitative descriptions however have not been reported in literatures. The redbreast wrasse, *Cheilinus fasciatus*, belongs to the family Labridae that inhabits the reefs across the Indian and Pacific Ocean. It feeds mainly on crustaceans and mollusks and is often seen resting on the seabed. Its head is greenish-blue, followed by a distinctive red-orange band followed by black and white stripes. Adults generally have a more pronounced red band than juveniles [5]. Wrasses are sexually dimorphic. Many species are capable of changing sex. Juveniles are a mix of males and females (known as Initial Phase or IP individuals) but the largest adults become territory-holding (Terminal Phase or TP) males [15]. The fish therefore is an interesting species to study in its morphological structure especially the scales which is considered very important in taxonomic studies.

During the late 19th century and the 1st half of the 20th century, the importance of scale morphology in systematic studies, e.g. Williamson 1851; Baudelot 1873; Timms 1905; Cockerell 1910, 1915; Chu 1935; Lagler 1947; Kobayashi 1951, 1953, 1955; McCully 1961, significantly increase with the enormous advances in light microscopy [9]. Years after, scale morphology became more important in the study of fish systematic and phylogeny, e.g. De Lamater & Courtenay 1973, 1974; Hughes 1981; Lippitsch 1990, 1992; Roberts 1993, after the introduction and development of scanning electron microscope [9].

The introduction of recent methods in shape analysis such as geometric morphometrics (GM) has greatly persuaded the growth of systematics and evolutionary relationships of organisms. Geometric morphology is a field concerned with studying variation and change in the shape of organisms or objects [16] and are useful in analyzing the fossil record. It is believed that morphometrics can measure a trait of evolutionary importance and can assume something of their ontogeny or evolutionary relationship by identifying changes in the shape of organisms [17]. While there are several methods for extracting data from shapes which include measurement of lengths and angles, landmark and outline analysis, each of these have their own benefits and weaknesses. We however in this study used outlined analysis in the form of Elliptic Fourier Analysis, a geometric morphometric method. While Elliptic Fourier Descriptors (EFDs) can be powerful tools for the analysis of biological shapes, they are not easy to apply, since several complex procedures are involved, such as image processing, contour recording, derivation of the descriptors and multivariate analysis of the descriptors [8]. The methodology however is made easier with the development of image analysis software such as the SHAPE software developed by Iwata and Ukai (2002) [8] which was used in this study.

MATERIALS AND METHODS

The fish scales were obtained from the fresh redbreast wrasses (*Cheilinus fasciatus*). Scales were removed from different body regions of the fish and were labelled from A to J (Fig. 1). Scales were then soaked to a dilute solution of detergent to soften the tissues attached. The scales were then cleaned by removing the tissues attached to the surface of the scales. Extra precaution was made in cleaning to prevent damage on the scales. After cleaning, the scales were allowed to air dry. Once dried, these were mounted between the two glass slides with an adhesive tape put in the edges of the slides to make it stable. Extreme precaution was made to make sure that the scales did not curl or fracture during its positioning to the slides. Images of the scales were taken by scanning.

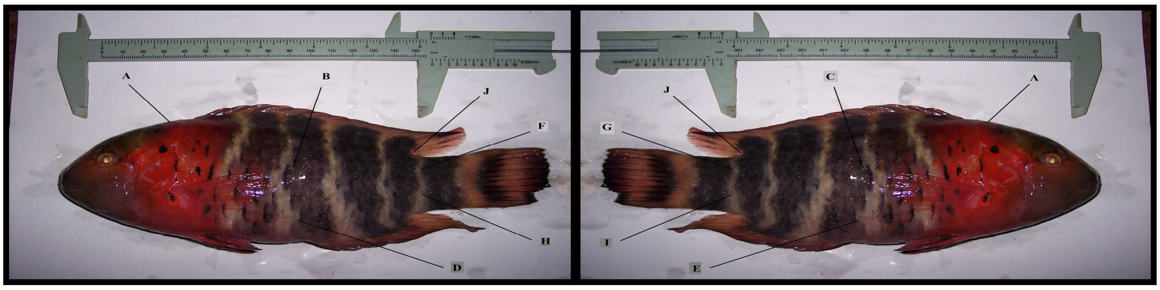

Fig.1. Body Regions of the *Cheilinus fasciatus* to where the scales were obtained.

After the scales were mounted and observed under the microscope, descriptions of the scales per region were made and variations between the scales in each region were noted. However, in the morphological description and multivariate analysis of the scale's shape, region B was fused with region C as well as the region D and E, region F and G, and region H and I. This was done since the two body regions of the fish were symmetrical. Thus, from the original 10 regions, the number of scales to describe and analyze reduces to 6. The descriptions were made based on the scale's size, over-all shape, focus, circuli and radii. Each scale is divided into 4 fields, namely: the anterior field, which is rooted in the skin and covered by the former scale; the posterior field, which covers the anterior field of the latter scale and contains coloration; and the 2 lateral fields.

The scanned images were converted into bitmap images since the SHAPE software that was used in this study can only handle full color (24 bit) bitmap images. One of the programs in the SHAPE software is the ChainCoder program. It extracts the contour of objects from an image file and records them as chaincode (Freeman, 1974). Chaincode is a coding system for describing geometrical information about contours in numbers from 0 to 7.

ChainCoder converts a full color image to a binary (black and white color) image, reduces noise, traces the contours of objects and describes the contour information as chaincode. ChainCoder outputs a chaincode file, which is analyzed by the program Chc2Nef [8].

In the Chc2Nef program, it calculates the normalized EFDs from the chain code information. The normalized EFDs are calculated in accordance with the procedures suggested by Kuhl and Giardina (1982) [12]. Chc2Nef can perform two types of normalization. One is based on the first harmonic ellipse that corresponds to the first Fourier approximation to the contour information. The size and orientation of the contour is standardized in accordance with the size and alignment of the major axis of the ellipse. The starting point for tracing the contour is also standardized with respect to the major axis. The other type of normalization is based on the point of the contour farthest from the center (i.e. the longest radius). This normalization is performed in accordance with the direction and absolute size of the vector from the center to the farthest point. However, in this study, normalization based on the longest radius was performed over the other [8].

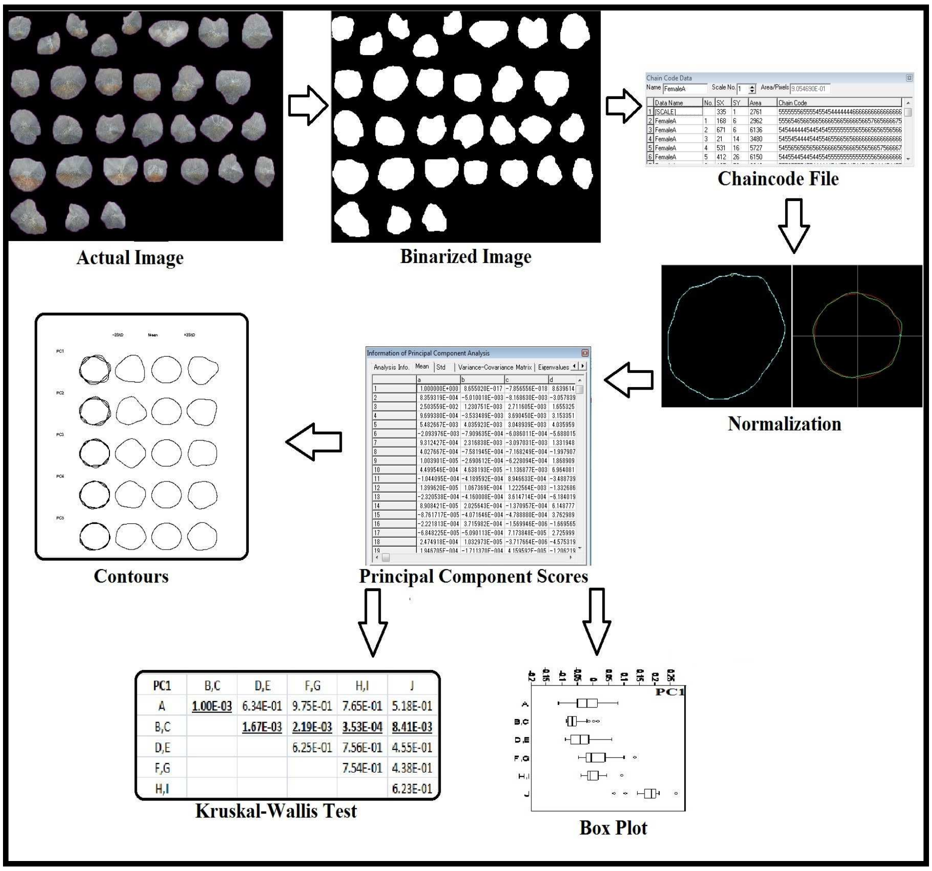

Fig.2. Schematic diagram of the scale shape analysis.

PrinComp program performs a principal component analysis of the normalized EFDs derived by Chc2Nef. When a contour shape is described in the first 20 harmonics of Fourier coefficients, the number of normalized EFD coefficients becomes large (77 or 80). However, principal component analysis can efficiently summarize the information contained in these coefficients. The principal component analysis conducted by PrinComp is based on the variance covariance matrix of the coefficients and not on the correlation matrix, because coefficients with small variance and covariance values are generally not important for explaining the observed morphological variations. The principal component scores can be used as observed values of morphological features in subsequent analysis and in the case of this study, morphological analysis of the shapes of fish scales. This program also allows visualizing the variation in the shape of the scales as explained by each component [8].

To verify if the shape variation of the scales explained by each principal component is statistically significant, the Kruskal-Wallis test was performed using the PAST software as the platform. The Kruskal-Wallis test is non parametric version of one way anova, that is, it does not make any assumption on the nature of the underlying distributions (except continuity). As many other non parametric tests, it will not use the values of the observations directly, but will first convert these values into ranks once these observations are merged into a single sample and instead of comparing sample means, it compares sample means of ranks. The Kruskal-Wallis procedure tests the null hypothesis that *k* samples from possibly different populations actually originate from similar populations, at least as far as their central tendencies, or medians, are concerned. The graphical comparison such as box plot was used to visually see the distribution of the different groups. There are a number of options available in the box plot that was utilized in order to examine the groups and these include the means, median and error bars [7]. Figure 2 below shows the schematic diagram of the whole process done in this study.

RESULTS AND DISCUSSION

Morphological Description

The scale's over-all shape, as shown on figure 3, varies from square shaped, circular, oblong, heart shaped, fan shaped, rectangular and D-shaped. The anterior field of the scales, as shown figure 4, varies from being straight, lobate, rounded, curved, pointed, wavy and oval. The margin of the anterior edge, shown in figure 5, also varies from being undulate, crenate and smooth. For the posterior field (figure 6), it varies from being oval, rounded, pointed, curved and elongated. The focus of the scales varies from being distinct to indistinct (figure 7) and with respect to its location; it is located either in the central field, posterior field, posterio-lateral field and lateral field (figure 8). The circuli are curved and are concentric along the outline of the scale. It runs continuously between the four fields except when disrupted by the radii (figure 9A and 9B). However, in most of the scales, the circuli becomes indistinct in the posterior field (figure 9C). Radii are present mostly posterior field than in the anterior field while only few are present in the lateral fields (figure 10).

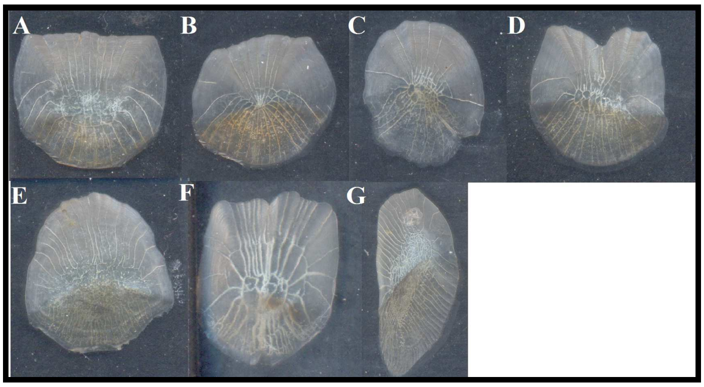

Fig.3.Various over-all shapes of the scales from being square-shaped(A), circular(B), oblong(C), heart-shaped(D), fan-shaped(E), rectangular(F) and D-shaped(G).

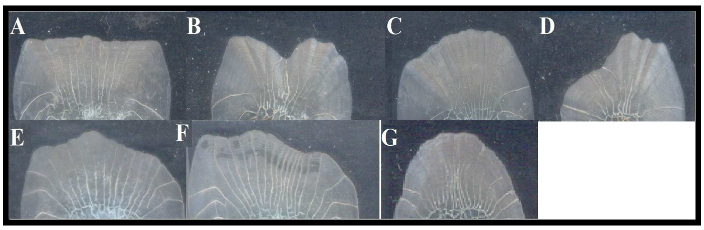

Fig.4.Various shapes of the anterior field; straight(A), lobate(B), rounded(C), curved(D), pointed(E), wavy(F) and oval(G).

Fig.5.Various margin of the anterior edge; undulate(A), crenate(B) and smooth(C).

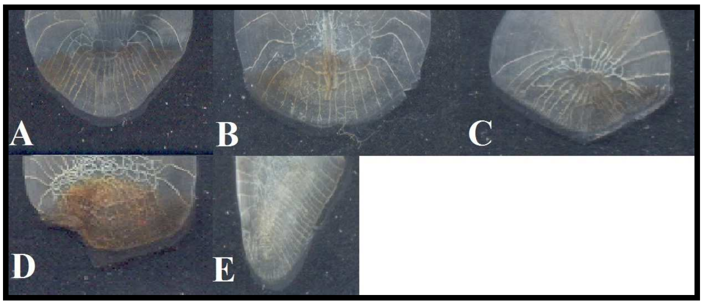

Fig.6.Various shapes of the posterior field; oval(A), rounded(B), pointed(C), curved(D), elongated(E).

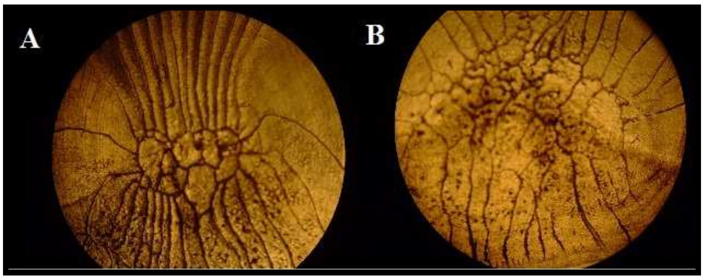

Fig.7.Part of *C. fasciatus* scale; showing the distinct focus(A) and indistinct focus(B)

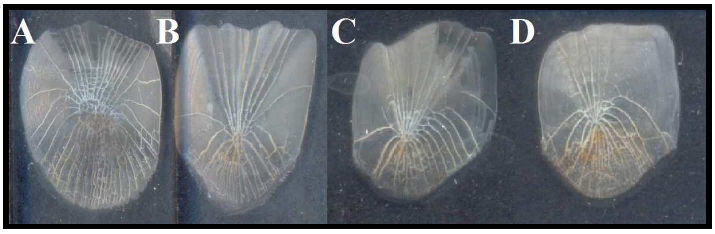

Fig.8.Various location of the focus; central field(A), posterior field(B), posterio-lateral field(C) and lateral field(D).

Fig.9.Part of *Cheilinus fasciatus* scale; showing the distinct circuli in the anterior field(A) and lateral field(B) and indistinct circuli in the posterior field(C).

Fig.10.Part of *Cheilinus fasciatus* scale; showing the radii in the anterior field(A), posterior field(B) and lateral field(C).

The scales in region A (figure 11) are generally moderate to large in size. The scale's over-all shape varies from circular; oval with irregular lobes in the outer edges; circular with bilobed anterior field; and square shaped with rounded posterior field. The outer edges of the scale are smooth except in the anterior edge which is slightly scalloped. The focus varies from being distinct to indistinct with markings inside and is slightly offset towards the posterior or lateral field. The circuli are curved and are concentric along the outline of the scale. It runs continuously between the four fields except when disrupted by the radii. However, in some of the scales, the circuli becomes indistinct in the posterior field. Radii are present mostly in the anterior and posterior field while only few are present in the lateral fields.

In regions BC and DE (figure 12), all the morphological characteristics of the scales were almost similar. The scales are large in size and shaped like a square with circular or oval posterior field. The outer edges are smooth except in the anterior field which is slightly scalloped. Focus varies from distinct to indistinct and is slightly offset along the posterior field. The circuli are curved and are concentric along the outline of the scale. It runs continuously between the four fields except when disrupted by the radii. Radii are present mostly in the anterior field and posterior field while minimal are present in the lateral fields.

In region FG (figure 13), the scales are also large and its shape varies from being square, circular and oval with smooth outer edges except the anterior field edge which is slightly scalloped. Focus varies from distinct to indistinct and is slightly offset towards the posterior field or the lateral field. The circuli are curved and are concentric along the outline of the scale. It runs continuously between the four fields except when disrupted by the radii. Radii are found mostly in the anterior and posterior field while few are present in the lateral field.

In region HI (figure 14), the size of the scales varies from moderate to large. The shape also varies from being oval to square with somewhat circular or oval posterior edge. The outer edges of the scale are smooth except in the anterior edge which is slightly scalloped and is sometimes loosely pointed in the anterio-lateral edge. Focus varies from distinct to indistinct and is loosely offset towards the posterior field or at the posterior-lateral field. The circuli are curved and are concentric along the outline of the scale. It runs continuously between the four fields except when disrupted by the radii. Radii are found mostly in the anterior and posterior field while only minimal number is present in the lateral field.

Lastly, in region J (figure 15), the scales are moderate in size and D-shaped with smooth outer edges. The posterior field is somewhat pointed compared to the anterior field which is more or less rounded. Focus is moderate in size to large and varies from distinct to indistinct with presence of hazy markings inside and is located slightly towards the convex lateral edge. The circuli are curved and are concentric along the outline of the scale. It runs continuously between the four fields except when disrupted by the radii. Radii are found all throughout the four fields however, the lateral field which is convex in shape tends to have lesser radii compared to the other three fields.

Fig.11.Various scales' shapes obtained from the region A of *C. fasciatus*

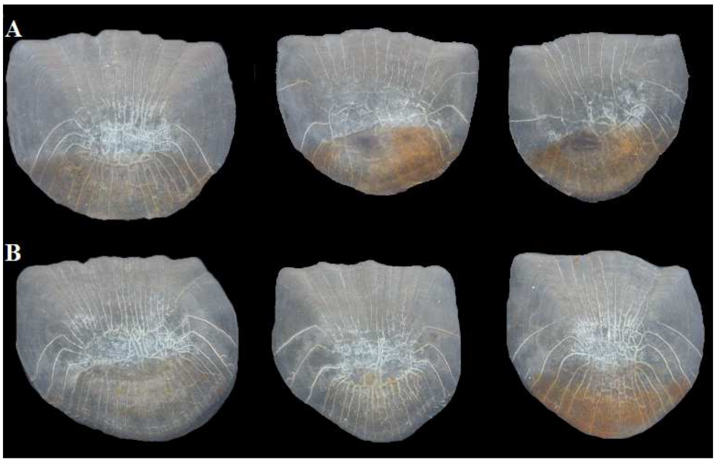

Fig.12.Various scales' shapes obtained from the region BC (A) and region DE (B) of *C. fasciatus*

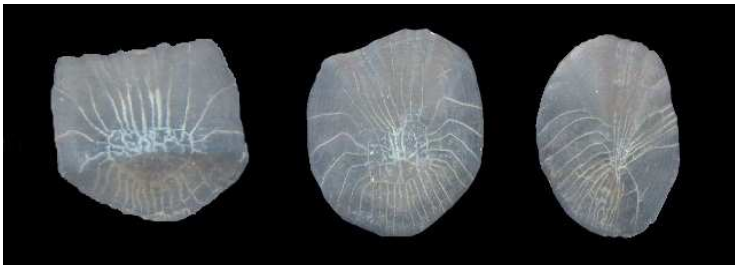

Fig.13.Various scales' shapes obtained from the region FG of *C. fasciatus*

Fig.14.Various scales' shapes obtained from the region HI of *C. fasciatus*

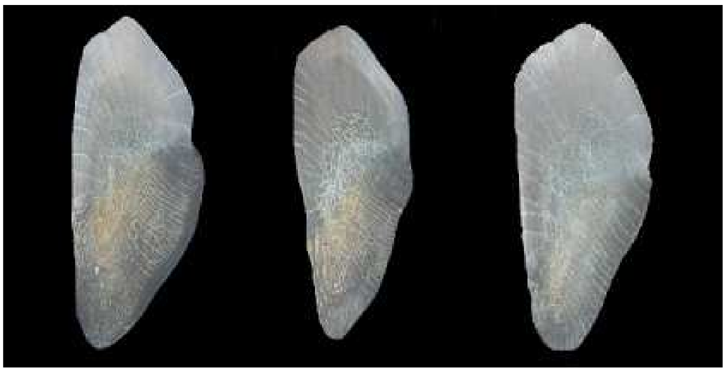

Fig.15.Various scales' shapes obtained from the region J of *C. fasciatus*

Multivariate and Statistical Analysis
Quantitative analysis of the shape of the scales based on the result of principal component analysis of the normalized EFDs shows that there was a variation in the shape of the scales between the different body regions (Table 1).

Table1. List of Effective Principal Components with its Corresponding Eigenvalue and %Variance

	Eigenvalue	Proportion(%)	Cumulative(%)
Prin1	7.02E-03	36.2639	36.2639
Prin2	4.11E-03	21.2638	57.5277
Prin3	2.31E-03	11.9181	69.4458
Prin4	1.29E-03	6.6507	76.0966
Prin5	1.06E-03	5.4573	81.5538
Prin6	6.72E-04	3.4722	85.026
Prin7	5.15E-04	2.6598	87.6859
Prin8	3.88E-04	2.0043	89.6902
Prin9	2.60E-04	1.3455	91.0357

A total of 76 principal components were generated during the analysis of the scale's shape, however; only 9 principal components, which accounts for 91.0357% of variation, were considered effective as shown in table 1. Every principal component represents a variable of the scales shape (length, width, shape of each field and etc.). PC1, which explains for the 36.2639% of variation, account for the variation in the over-all shape of the scales. PC2 (21.2638%) describes the variation in the length of the scales. PC3 (11.9181%) explains the variation in the shape of the anterior and posterior field. PC4 (6.6507%) is accountable for the variation in the margin of the posterior field while PC5 (5.4573%) describes the variation in the margin of anterior field. The remaining principal components explain for the rest of the scale shape's variation. Figure 16 below shows the different contours of the respective principal components which describe the variation of the scale's shape.

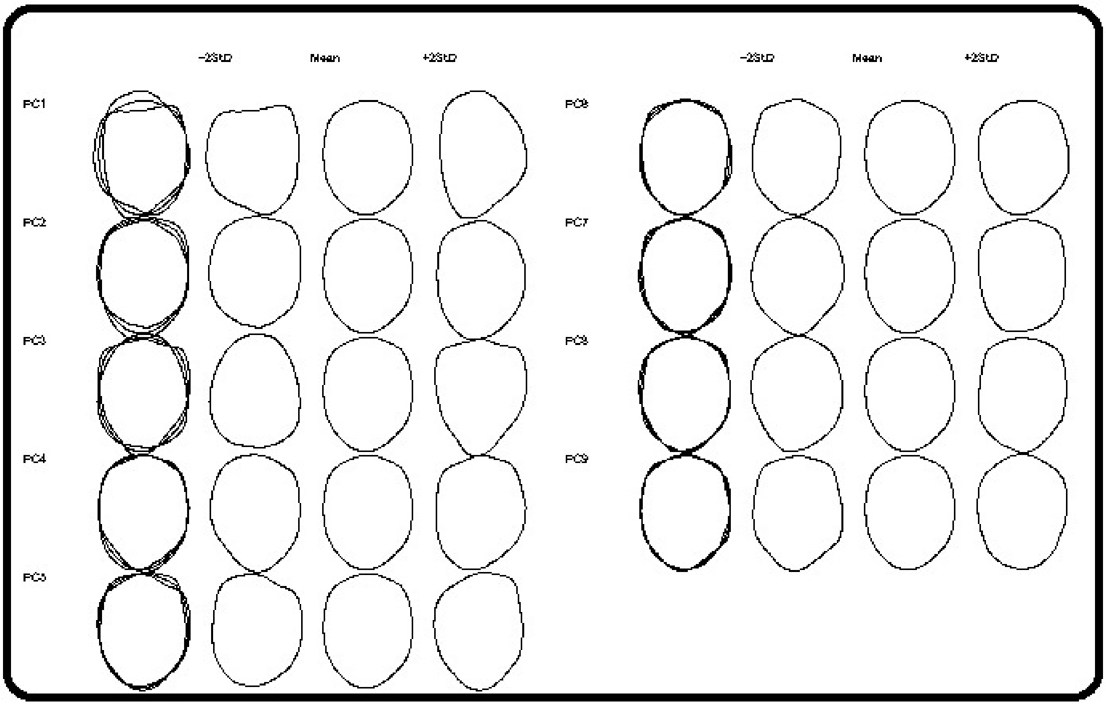

Fig.16.Reconstructed scale outline of the *C. fasciatus* scales

The result of Kruskal-Wallis test, as shown in table 2, showed that among the 9 effective principal components, only the PC1, PC2 and PC3 were considered extremely significant while others were considered not significant. This would imply that the over-all shape, length, shape of the anterior and posterior field of the scales were the most accountable factor for the variation of the scale's shape obtained from the different body regions of the fish.

Table2. Results of the Kruskal-Wallis Test of the 9 Effective Principal Components of Elliptic Fourier coefficients derived from the Scale's Outline of *C. fasciatus*

Component	KW	P-Value $\leq$0.0001	Remarks
1	80.08	8.082×10^{-16}	Extremely Significant
2	28.5	2.907×10^{-05}	Extremely Significant
3	31.8	6.506×10^{-6}	Extremely Significant
4	8.02	0.1551	Not Significant
5	7.14	0.2105	Not Significant
6	19.28	0.001702	Not Significant
7	15.69	0.007794	Not Significant
8	14.94	0.01064	Not Significant
9	19.16	0.001797	Not Significant

A box plot, as shown in figure 17, was used to visualize the distribution of the scale's shape in the different regions based on their principal component scores. Form the figure, it is clearly shown that region J has been separated from the rest of the region based on the first principal component. This would indicate that with respect to the over-all shape of the scales, region J was significantly different compared to the other regions.

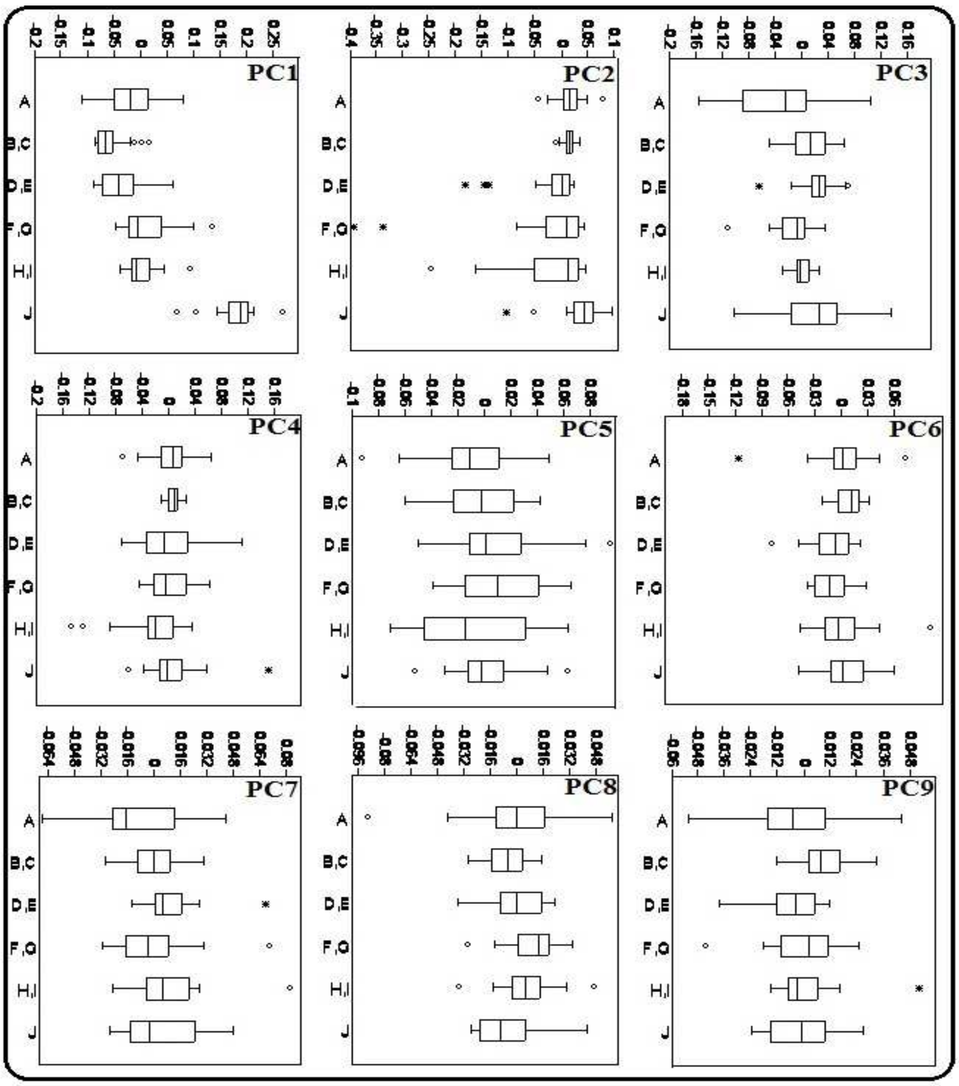

Fig.17.Box-and-whisker plot showing the distribution scale shape variations of the 9 effective components based on the principal scores

When look closely, region J was the only region leaning towards the positive side of the graph and when referred to figure 9, the positive side would correspond to the D-shape scales. This result was consistent with the result obtained the morphological description of the scales as shown earlier in this section. Looking at the physical appearance, it could be deduce that scales in region J differ from the others since it is the only region characterize with D-shape scales. The same situation was observed in the variation of scales based on the second principal component. With respect to the length of the scales, region J was also variable. The scales in the region were longer compared to the others. Also, looking at the scales appearance, it could really see the difference. For the plot of the remaining principal components, although variation in the location of boxes was observable, these were not as apparent as in the first two principal components. Some boxes were overlapping, indicating similarities in a certain characteristic of the scale as define by its principal component.

The variation of the scale's shape of *Cheilinus fasciatus* might be attributed to the location of the scales in the body of the fish. Scales in region BC, which are square-shaped, have the largest and the broadest of all the scales because the scales are situated in the lateral area of the fish which is the widest. On the other hand, the scales in region J, which are D-shaped, are narrow and elongated because they are situated in the dorsal part of the fish near the tail which is a narrow area. Although scales in region A were also obtained from the dorsal area, its scales were not narrow like in region F because the scales were obtained from the head part of the fish which is broader compared to the tail part. This would imply that the shape of the scale would adapt to the area where it is located.
The shape of the scales in regions B and C are more or less the same to the shape of the scales in regions D and E but differ in sizes. According to Alexander (1970) [1], the size and distribution of scales over a fish's body often, but not always, reflect the way it lives. Thus, fish that swims quickly, or that live in fast flowing waters tend to have small scales, while fish that swim slowly in slow moving waters tend to have larger scales. Larger, heavier scales supply more protection, but restrict movement while smaller, lighter scales offer less protection but allow for greater freedom of movement [1]. The scales of some fish decrease in size from the head towards the tail reflecting the need for greater flexibility towards the tail of the fish. This statement is consistent with the result of this study. Scales of regions D and E, which were obtained from the tail region, were the smallest scales among them all. Thus, the small scales in the tail region could provide the *Cheilinus fasciatus* the flexibility to glide smoothly as it swims.

CONCLUSION

Qualitative and quantitative analysis of *Cheilinus fasciatus* scales revealed that variation exist in the shape of the scales obtained from different body regions of the fish. Quantitative analysis of scales showed that the scales in region J has the most varied scale shape compared to the scales of other regions with respect to its over-all shape, length, and shape of anterior and posterior field of the scales. The result of qualitative analysis was supported by the result of quantitative analysis. Geometric Morphometrics analysis showed that the PC1, PC2 and PC3, which correspond to the over-all shape, length, and shape of anterior and posterior field of the scales respectively, account for the majority of variation in the scales. Quantitative results were visualized in the box plot and further strengthen by the result of Kruskal-Wallis test. Variation in the shape of the scales might be attributed to the location of the scales in the body of *Cheilinus fasciatus.*

REFERENCES

[1]Alexander, R.M. Alexander. Functional Design in Fishes. Hutchinson University Library, London. **1970.**
[2] B.A. Block, D. Booth and F.G. Carey. *J. Exp. Biol.*, **1992**, *166*:267-284.
[3] R. Dalayap, M.A. Torres, C.G. Demayo, A.A. Barrion, and A.T. Barrion. *Journal of Nature Studies*, **2008**, *7(1):* 175-182.
[4] H. Freeman. *Comp Surv.*, **1974**, *6*: 5797.
[5] R. Froese and D. Pauly."Cheilinus fasciatus" in FishBase. November 2006 version.
[6] Ø. Hammer, D.A.T. Harper and P.D. Ryan. *Palaeontologia Electronica*, **2001**, *4(1).*
[7] M. Hollander and D. A. Wolfe. Nonparametric Statistical Methods. Hoboken, NJ: John Wiley & Sons, Inc., **1999**.
[8] H. Iwata and Y. Ukai. *Journal of Heredity*, **2002**, *93*: 384-385.
[9] L, Jawad. *Tuhinga*, **2005**, *16*: 137-167.
[10] M.S. Johal, H.R. Esmaeili and M.L. Sharma. (2006). *Current Science*, **2006**, *91(11)*:1444-1446.
[11] K.V. Kardong. (1998). *Vertebrates: Comparative Anatomy, Function, Evolution* (second ed.). USA: McGraw-Hill, **1998**, pp. 747.

[12] F.P. Kuhl and C.R. Giardina. *Comput Graphics Image Process*, **1982,** *18*: 236–258.
[13] E. Lippitsch. (1992). *Journal of Fish Biology*, **1992,** *41*: 355-362.
[14] P.B. Moyle and J.J. Cech Jr. 2004. Fishes: An Introduction to Ichthyology, 5th Ed. Prentice-Hall, USA, **2004**, pp. 726.
[15] P.C. Wainwright, M.E. Alfaro, D.I. Bolnick and C.D. Hulsey. *Intergrative & comparative biology*, **2005,** *45*: 256–262.).
[16] M. Webster. Introduction to Geometric Morphometrics. **2006.**
[17] M. Zelditch, D. Swiderski, D.H. Sheets and W. Fink. Geometric Morphometrics for Biologists. Academic Press, **2004,** ISBN 0127784608.