

Applications of Relative Warp Analysis in Describing of Scale Shape Morphology Between Sexes of the Snakehead Fish *Channa striata*

Elani A. Requieron, Mark Anthony J. Torres and Cesar G. Demayo

Abstract— Variation in shape has always been an important means of distinguishing individuals. With the development of advanced methods in statistics, modeling and computer science, biological studies have become more quantitative. New methods in Geometric morphometrics like relative warp analysis for example have been very important in describing and discriminating within and between organisms and populations. This method was therefore used in describing the shape of scales of the snakehead fish *Channa striata*, an invasive fish species known to have shown variability in color distribution in the different regions of the body of the fish. Quantitative measures using Relative Warp Analysis (RWA) made use of the tpsRelW software version 1.46 developed by Rohlf in 2008. Results show variations in scale shapes within and between sexes of *C. striata*. Discriminant function analysis showed significant variation ($p < 0.05$) between male from females. Statistical analyses showed that these variations are attributed to the scale width index, location of lateral tips (up or down), focus location, variations in the center margins of the anterior and posterior regions and differences in the lateral field display. Canonical variate analysis (CVA) of the scales also showed variations from the different fish body regions (A, C, E, G, I, and J). This study clearly show that RWA is a good quantitative tool in describing shapes and in discriminating sexes within species.

Index Terms—Relative warp analysis, landmarks, canonical variate analysis.

I. INTRODUCTION

ALL manuscripts must be in English. These guidelines Variation in shape has always been an important means of distinguishing individuals [1]. Lepidology or the use of scale morphology and squamation for fish classification had its origin as early as 18th century [2] when Agassiz (1833-1843) begun using fish scales for taxonomy. Since then the importance of scale morphology in systematic studies earned great interests [2-7].

With the development of newer tools in imaging, statistics and computer science, studies of variations have become quantitative. Geometric morphometric methods for example are the best available alternative [8] to obtain size and shape components from any biological form or image [9]. Morphometric researches are now made easy by landmark-based analysis using Thin-Plate Spline (TPS) series.

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Landmark-based methods differ from others because they utilize biological equivalences between structures, matching or “homologous” landmarks [10].

Landmark analysis aims in recording the position of certain important landmarks or define the outline of the object in its simplest form, removing extraneous information such as size differences, rotation and location, thereby, facilitating comparison between different objects visualized via thin-plate splines. These visualizations are formed by calculating the mean location of landmarks, and drawing a rectilinear grid over them. This grid is then deformed (“stretched”) so as to keep the landmarks in the same grid square, while moving the points themselves to their locations in each specimen. We therefore use the method to investigate if scale morphology could be used to discriminate between male and female sex of the fish describing variations in scale shape changes that are confined to specific body regions of fish. Landmark-based such as relative warp analysis was applied to validate the use of truss network system. The results of the study will provide information about the nature of sexual dimorphism in the organism being essential for understanding the ecology, behaviour and life history of the species, as well as for making morphological comparisons within and between sexes and individuals. Furthermore, the greater the morphological differences that will be observed between the sexes, the greater the competition is between individuals’ of the same sex to attract members of the opposite sex ensuring the continuity of the species. A good example of this organism is the snakehead, *Channa striata*.

C. striata is a species of snakehead fish. It is commonly known as snakehead murrel, common snakehead, chevron snakehead or striped snakehead and locally known as “Haloan.” *C. striata* (Fig. 1) appears cylindrical with elongated body; head flattened and snake-like (figure 1f). It can grow upto 1 meter in length. The male fish (figure 1a) shows a more slender body than female ones (figure 1b). It has single dorsal fin with 37-46 rays that covers most of the entire length of the body (figure 1d). Its anal fin (figure 1e) with 23-29 rays appears more than half the length of the dorsal fin. Rounded caudal fin of diphycercal type (figure 1c) and thoracic type of pectoral fin (figure 1d) with 15-17 rays and 6 pelvic fin rays can be observed spinous soft type (figure 1h). The large superior mouth consists of 4-7 canine teeth behind a single row of villiform teeth. Upper-jaw teeth shape is

primarily multicuspid. Protruding lower jaw is evident in this species.

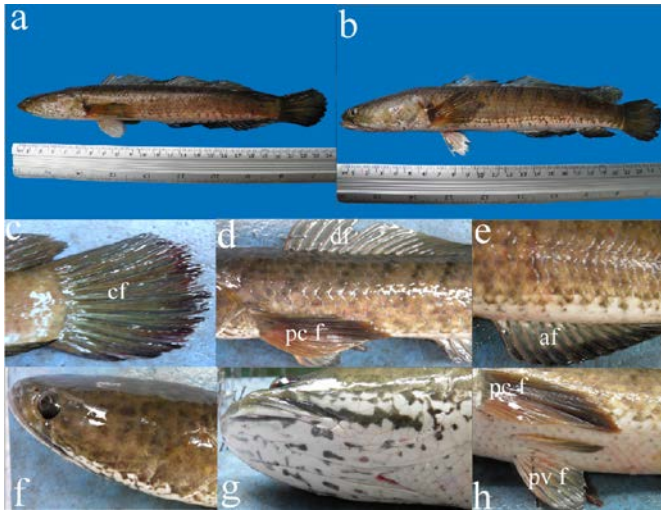


Figure 1. (a) male and (b) female *C. striata*. Fish parts (c) caudal fin (cf), (d) dorsal fin (df), pectoral fin (pc f), (e) anal fin (af), (f) snake head, (g) ventral surface of snake head, (h) pectoral fin (pc f), pelvic fin (pv f) pinned down.

II.METHODOLOGY

A total of 180 scales per sex (30 scales per body region) were processed and analyzed Sex classification was initially identified based on external morphology (female-stouter abdomen; male-sharper abdomen) and later confirmed by dissection thru direct examination of the gonads. Male fishes had whitish soft textured gonads whereas female ones had yellowish coarsely textured gonads with visible lumps of eggs.

Scales were removed carefully from 6 different fish body region (see figure 2) using a flat end forceps [11, 12]. This regions are: (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) scale below the ventral lateral line posteriorly located, and (J) above the caudal peduncle. At least 30 scales were taken from each region with few exceptions. Scales were soaked separately per region in clear empty vials with dilute liquid detergent for 30 minutes. To obtain cleaner scales, it was highly recommended that they should be soaked for not more than 20 hours otherwise discoloration begins (absorption of color from liquid detergent). They were rinsed gently with tap water and left to dry but with minimal moisture left preventing the scales from curling before mounting on slides.

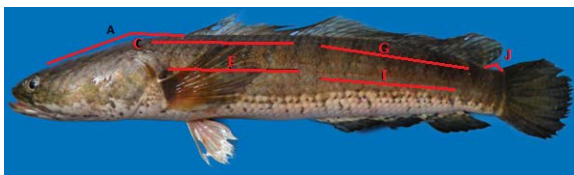


Figure 2. Six body regions (A, C, E, G, I, and J) of *C. striata* showing the area where scales were removed.

Moistened scales were mounted and arranged based on size per region on a standard size glass slide. Depending on scale size each slides contains at least 6 scales per slide. They were then covered with adjacent clear glass slide and secured with invisible tape at 3 edges leaving 1 edge untapped to allow excess water to evaporate before fully covering it after 24 hrs. Prepared slides with mounted scales were then ready for photography.

Prepared slides with mounted scales were photographed using Veho usb microscope with 400x magnification. Digital images of scales captured were then transferred and recorded in the computer for image processing. For consistency, elevated crown view of fish scale with anterior area (or embedded portion) on top while posterior area (or exposed part) at the bottom were photographed all throughout.

Landmarking

The landmarks selected are located on seven (7) homologous points of scale found common to all scales of the species being observed (figure 2). To ensure and satisfy statistical needs, at least 30 scales per each of the six (6) regions of scales were observed. Landmarks 1 and 3 are the left and right ventro- and dorso-lateral tips of the anterior portion of the scale, respectively. Landmark 2 is in the center of the anterior edge of the scale; landmarks 4 and 6 are the left and right boundary between the anterior area with circuli and the posterior area covered by ctenii, respectively; landmark 5 is the focus of the scale; and landmark 7 is located at the tip of the posterior portion of the scale [1] (figure 3).

Morphometrics

In order to best represent the external shape of the scales, converted tps file of scales (digital images) were digitized using 7 homologous points using the software tpsDig2 version 2 [13]. This extracts x and y coordinates of landmarks on the images. Since the x and y coordinates processed would vary from image to image, these must first be standardized in order to remove biases in size, shape and rotation using Procrustes superimposition method. This method translates the centroid of the shapes to (0,0) [9]. This is then warped with the landmark coordinates showing the positive and negative deformation from the consensus scale shape as depicted in the grid square. The differences in spline shape were then used to describe the scale shape differences between sexes of each species. The relative warp scores recorded were converted in MS Excel worksheet and were then used in analyzing intraspecific variation in scales at different body regions (6) of each species of freshwater fishes found using the Paleontological /Statistics Analysis (PAST) software [14]. The relative warp scores were used to generate values that will constitute the multivariate shape data sets.

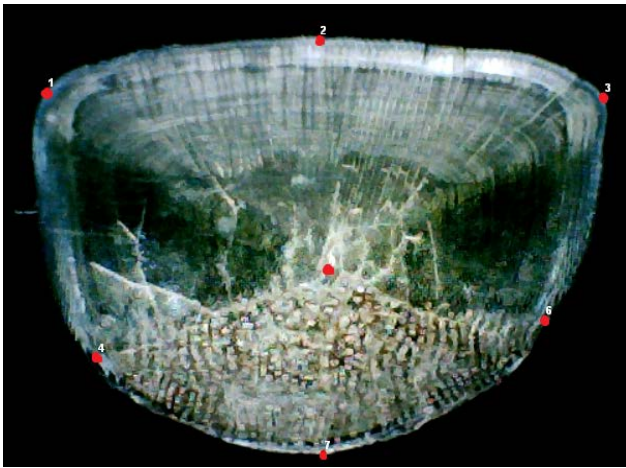


Figure 3. Landmarks used for digitizing image of ray finned freshwater fishes: (1) left lateral tip, (2) center of anterior region, (3) right lateral tip, (4) left boundary between anterior and posterior region, (5) focus, (6) right boundary, and (7) center in the posterior region.

Discriminant Function Analysis (DFA) was used to best discriminate (separate) scale shape difference between sexes. Equality of the means of the compared groups was tested using Hotelling's t-squared showing a p value. The level of significance for the p value is ≤ 0.05 . Multivariate Analysis (MANOVA) was used for the analysis of these relative warp scores for the different body regions where the scale was taken. Canonical Variate Analysis (CVA) was performed to assess the variation between the six body regions where the scales were taken.

III. RESULTS AND DISCUSSION

Quantitative analysis of scale morphology of female *C. striata* reveals 90.91% variation from the first five relative warps (Fig. 4). RW1 accounts for 48.08% describing for the extent of variations in scale width (RW1), RW2 accounts for 16.91% describing variations due to the location of lateral tips in the anterior region, RW3 accounts for 11.68% describing variations of more pointed posterior tip forming a more concise area, RW4 accounts for 8.59% due to differences in right boundary location between anterior and posterior region and RW5 accounts for 5.65% due to higher location of focus and more pointed center of the anterior region.

In male *C. striata*, RW1 accounts for 35.20% for variations in scale width. Wider male scales were found in the positive coordinates while slender scales in the adjacent side. RW2 accounts for 14.86% due to the location of focus. Positive scores reveals lower focus showing more area of scales embedded in the fish body while those focus anteriorly located provides less area embedded and hence more exposed part of scales. RW3 accounts for 12.07% observed to affect the anterior margins of the scales. The rest of the 92.98% difference (RW4 to RW7) was due to focus location and variation in the location of lateral tips in the anterior region as shown in the plots of extreme shape configurations in the seven relative warps (Figure 5).

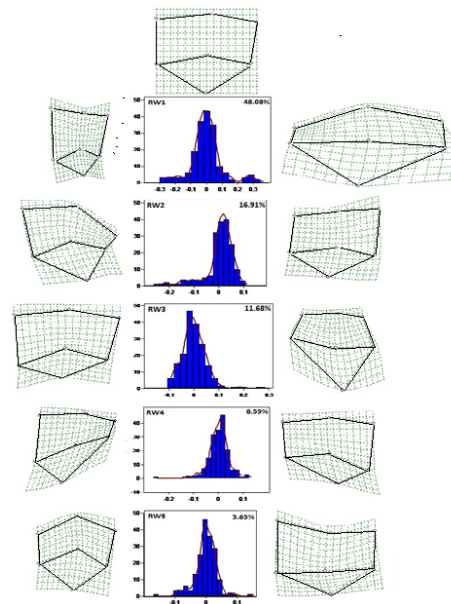


Figure 4. Summary of the Geometric Morphometric Analysis of scales from 6 fish body regions showing the consensus morphology (uppermost panel) and the variation in scale shape of female *C. striata* found in Bayug, Mandulog River, produced by the first 5 and 7 relative warps respectively explaining more than 5% of the variance {(a) RW1=48.08%, RW2=16.91%, RW3=11.68%, RW4=8.59%, RW5=5.65%};

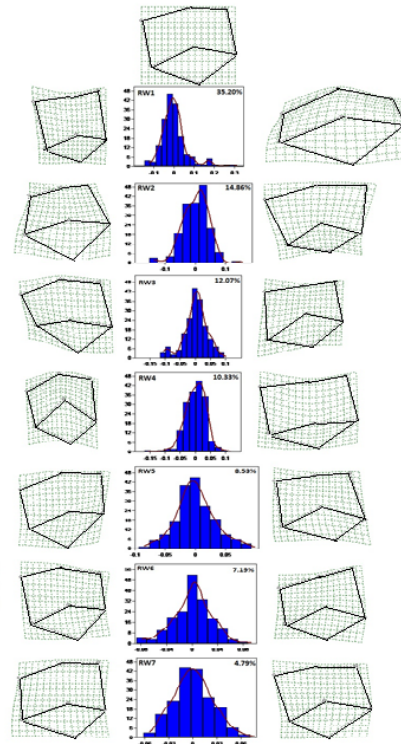


Figure 5. Summary of the Geometric Morphometric Analysis of scales from 6 fish body regions showing the consensus morphology (uppermost panel) and the variation in scale shape of male *C. striata* found in Bayug, Mandulog River, produced by the first 5 and 7 relative warps respectively explaining more than 5% of the variance {RW1=35.20%, RW2=14.86%, RW3=12.07%, RW4=10.33%, RW5=8.53%, RW6=7.19%, RW7=4.79% }

Relative warp scores of male and female *C. striata* was subjected to PAST using MANOVA to provide a better image to visualize the differences and distribution of variation between sexes. CV Analysis also revealed a significant variation of scales (n=180 male, n= 180 female) taken from the six different fish body region of male (Table 1) and female (Table 2). *C. striata* namely: region A (n=30), region C (n=30), region E (n=30), region G (n=30), region I (n=30), region J (n=30).

Table 2. Male *C. striata* CVA Scatter scores at 6 body regions

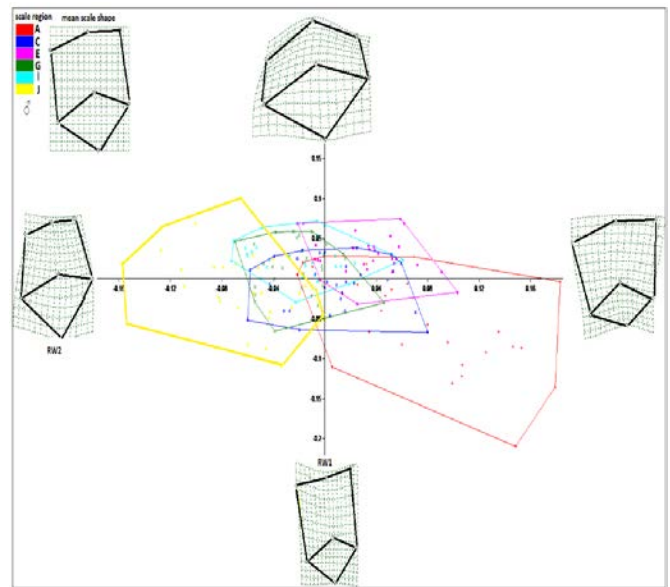
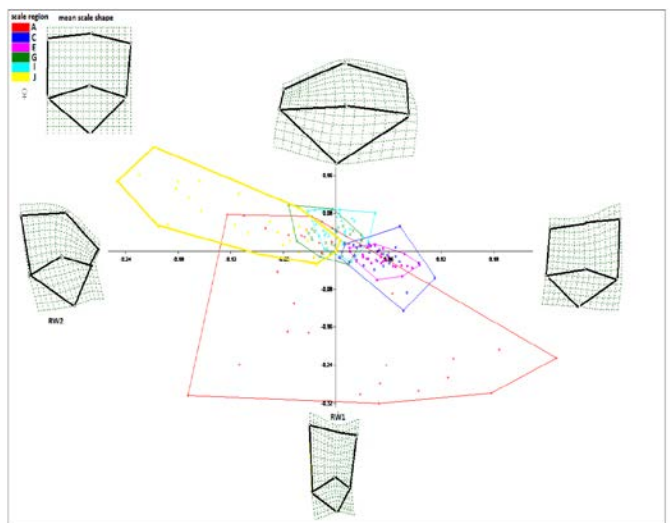
male	A	C	E	G	I	J
A	0	0.00096	1.64E-06	4.84E-07	1.73E-08	4.97E-11
C	0.014402	0	1.73E-06	0.001328	1.78E-06	4.90E-07
E	2.46E-05	2.60E-05	0	1.19E-09	8.24E-07	5.55E-14
G	7.26E-06	0.019918	1.78E-08	0	0.003296	4.76E-05
I	2.60E-07	2.67E-05	1.24E-05	0.049445	0	3.85E-06
J	7.46E-10	7.34E-06	8.32E-13	0.000714	5.77E-05	0

Table 3. Female *C. striata* CVA Scatter scores at 6 body regions

female	A	C	E	G	I	J
A	0	3.38E-05	1.02E-05	0.000206	7.06E-07	8.07E-06
C	0.00051	0	9.32E-07	3.93E-10	1.50E-12	2.23E-17
E	0.00015	1.40E-05	0	1.54E-14	6.18E-17	1.83E-19
G	0.00310	5.89E-09	2.32E-13	0	3.78E-07	1.82E-09
I	1.06E-05	2.24E-11	9.27E-16	5.67E-06	0	2.77E-11
J	0.00012	3.35E-16	2.74E-18	2.73E-08	4.16E-10	0

Figure 7 shows the CVA Scatter plot variation distributed along six body regions of male *C. striata* along the first two relative warps. It is evident that shapes and sizes vary between regions. Region A tends to have shapes drawn to positive from the reference point in the RW2 axis while some scales scores can be seen near the negative axis of RW1. Regions C, E, G, and I have scale scores nearest the mean shape. Region J presents bigger convex hulls in RW2 axis. This scales possessed slightly narrower scales with variation in the lateral tips of the anterior region. Male “Haloan” scales have overlapping convex hulls indicating similar shapes in region C, E, G, and I. Regions A and J shows bigger convex hulls at opposing sides. Region A scale scores tends to have wider scales than the rest of the region since they are located at the head of the fish. While region J have scales that are narrower in shapes since they originated at the dorsolateral side of caudal peduncle. This region is believed to be the origin of every new scales produced in fish body. It has been observed that development of scales localizes on the first scale appearing in the caudal peduncle in teleosts [15].

Figure 8 shows the female scatterplot of *C. striata* fish scales along the first two relative warps. It can be observed still that bigger convex hulls can be seen in region A and J. Region A has biggest variation from both sides of the axis. Region J tends to be drawn towards the negative side from the reference point. While the rest of fish body region where the scales were taken tends to have scale scores nearest the mean shapes. Regions C and E have similar scales while region G and I possess another group. This is due to the fact that such regions can be located adjacent to one another in the fish body regions.

Figure 7. CVA Scatterplot of the landmark coordinate data of male *C. striata* scales taken from the different fish body regions for the first two relative warp scores.Figure 8. CVA Scatterplot of the landmark coordinate data of female *C. striata* scales taken from the different fish body regions along the first two relative warp scores.

Discriminant function analysis of the RW scores show differences between sexes of *C. striata* (Fig. 6). Scale shape of male *C. striata* is significantly different from that of the female ($p < 0.05$) indicating sexual dimorphism in scale shapes.

Shape has a role in biological studies. Differences in shapes are produced due to various biological processes such as disease or injury, ontogenetic development and adaptation to local geographic factors. Differences in shapes signals different functional roles played [16]. The use of tools of geometric shape analysis has a tremendous advantage for it serves the purpose of visualization, interpretation and communication of results [16]. Hypothesis confirmation using statistical tests provides us with a powerful conclusion.

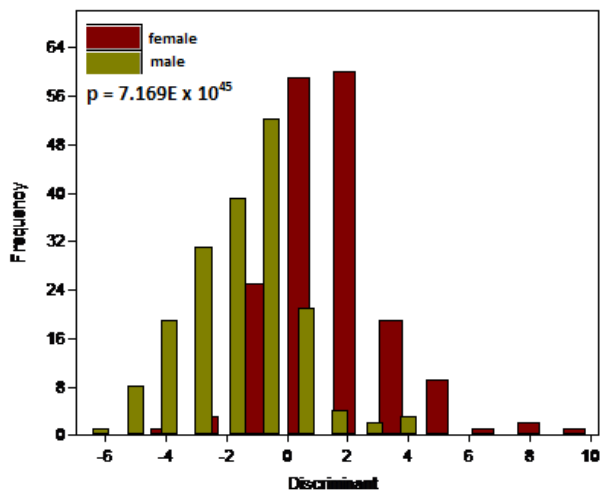


Figure 6. Graph of Frequency Distribution of the Discriminant Scores (DFA) of the relative warp scores of highly significant scale variation between male and female *C. striata* ($p = 7.169E \times 10^{45}$).

The use of these tools in squamatology have been started with the use of GM on the analysis of fish scales for identifying genera, species, and local populations within the Mugilidae [10]. Current studies used Elliptic Fourier Analysis in describing variations between sexes of the yellowstriped goatfish of male and female individual of *Upeneus vittatus* [17], and image analysis of yellow-tail parrotfish [18].

Understanding the scale morphological variations between sexes of the different species provides us with opportunity to validate their marked sexual dimorphism based on quantitative characteristics. The attempt to establish scale shape difference between sexes of *C. striata*, establishes the correspondence between form and function, and quantifies the description of characteristic traits used in the identification of species [19].

The analysis provides sufficient evidence that there is a significant variation between male from female scale shapes in *C. striata*. This study clearly shows that RWA is a good quantitative tool in describing and in discriminating shapes within species.

ACKNOWLEDGMENT

The senior would like to acknowledge the financial assistance from the Department of Science and Technology (DOST) for the scholarship grant and to Mr. Muhmin Michael Manting for the technical assistance.

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