

Describing the Body Shape of *Oreochromis niloticus* (Linnaeus, 1758) Populations in Bukidnon, Philippines Using Relative Warp Analysis

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ABSTRACT

Samples of *Oreochromis niloticus* were collected from the two selected lakes in Bukidnon, Philippines namely Lake Apo and Pulangui Lake and were subjected to geometric morphometric analysis to describe the body shape variations.

Significant degrees of variations were detected both within and between populations which were elucidated by multivariate analysis of variance (MANOVA), canonical variate analysis (CVA) and discriminant function analysis (FDA). Sexual dimorphism was clearly detected between the two sexes in both populations, thus shape variation can be accounted to different evolutionary role between sexes. Additionally, geographic isolation and varying level of genepool could be the factors in the variation observed in populations between the two lakes. Thus, the present study demonstrated the capability of geometric morphometrics analysis to distinguish and describe the variations in body shape existing within and between populations of *O. niloticus*. Further studies on the genetics of shape variations are recommended to advance the knowledge and understanding about the divergence in the species which would greatly contribute to its conservation and to aquaculture management strategies.

Keywords: Canonical variate analysis, geometric morphometrics, Lake Apo, Pulangui Lake, relative warps, sexual dimorphism

INTRODUCTION

Cichlid are fishes that are native to Africa and the Middle East, but are now globally cultured and currently produced and consumed in nearly 100 countries (Fitzsimmons, 2000). This group constitutes about 4% of the total aquaculture production worldwide (Nyingi et al., 2009). One of these species is *Oreochromis niloticus* (Linnaeus 1758), which has been introduced in the Philippines in 1970s for aquaculture purposes particularly because of its fast growth (Guerrero 2002) and the fact that it can be easily reproduced in ranged of culture environment such as freshwater and brackish water ponds, cages and pens, small farm reservoirs, r rice-fish integrated systems and, although negligible, even marine fish cages (BFAR 2008).

Bukidnon is a province located in North Central Mindanao, a landlocked plateau with landscape dotted with various lakes. Lakes are confined bodies of water and populations of freshwater fish living in lakes are mainly confined to the limits of its environment and are isolated from other freshwater fish populations of the same species. Population of fish when exposed to varying environmental conditions, like all organism, are capable of making adaptations

enhancing their chances of survival. These locally adaptive morphological changes is the main source of variations and what makes them somewhat different from other fish population of the same species (Turan et al., 2006). On the contrary, hybrids may also evolve from extensive intra-breeding (El Serafy et al., 2007).

In this study, landmark-based geometric morphometric analysis was employed to investigate and describe the body shape variation that occurs within and between populations of *O. niloticus* which may be caused by environmental isolation. Bookstein (1991) defined geometric morphometrics as a statistical study of biological shapes and shape variations among different populations. This method allows the analysis of morphological variation in a more comprehensive manner and provides a clearer view of deformations when using thin plate spline (Bookstein, 1989; Zelditch et al., 2004). It permits the study of shape and size, offering powerful analytical and graphical tools for the quantification and visualization of morphological variation. The efficiency of geometric morphometric had already been proven in several studies in the analysis and description of variation occurring within and between populations (Nacua, Durado Marcus et al., 1996). It's utility to distinguish form changes and sexual dimorphism is also widely reported (Nauca et al., 2012; Durado et al., 2012; Fabrigar et al., 2018).

FRAMEWORK

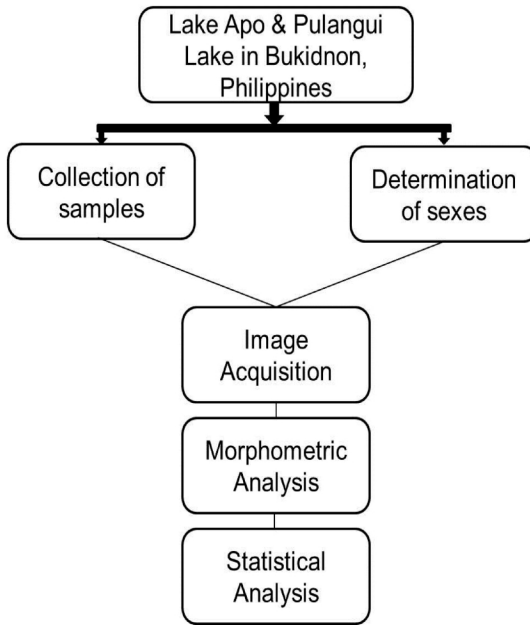


Figure 1. Describing the body shape of *Oreochromis niloticus* (Linnaeus, 1758) populations in Bukidnon, Philippines using relative warp analysis.

This study was conducted to determine and describe the variations that exist in the body shape of *Oreochromis niloticus* populations. Collection of specimen were accomplished in two selected lakes of Bukidnon province in the Philippines which are identified as suited sites based on available literatures. The specimen were then sorted by determining the sex and then were prepared for data acquisition. Data acquisition was performed on the specimen by taking photographs of samples in uniform orientation, distance and angle, these are achieved using a tripod and an immovable stage from which the specimen are placed. The images acquired were then digitized and the data generated were subjected to morphometric analysis to create various diagrams. The resulting

scores in morphometric analysis were further examined to determine statistical significance using statistical software.

OBJECTIVES OF THE STUDY

This study determined the variations in the body shape existing within and between populations of *O. niloticus* sampled from the two lakes in Bukidnon, Philippines.

MATERIALS AND METHODS

Study site. The study was conducted in two selected lakes in Bukidnon: Lake Apo and Pulangui Lake from October to November 2016. Lake Apo ($7^{\circ}52'45''\text{N}125^{\circ}0'21''\text{E}$) is a crater type lake located in Barangay Guinoyoran, Valencia City. This is the smallest lake in Bukidnon with an area of 24 ha (59 acres) and it is being fed by surrounding small creeks and rivers and is isolated from other big rivers. It has an altitude of 640 masl and average depth of 17 m. On the other hand, Pulangui Lake ($7^{\circ}48'31''\text{N}125^{\circ}2'19''\text{E}$) is an artificial lake located Barangay Tubigon Maramag and is the largest out of four lakes of Bukidnon with an area of 1,985 ha (4,910 acres) and is the main water source for the province, both drinking and for irrigation. The lake is fed by its four major tributary rivers, the Manupali river, Muleta river, Bobonawan river and Tigwa river and drains into the Mindanao river. It has an elevation of 340 masl (Fig. 2).



Figure 2. Map of the Philippines showing the study sites in Bukidnon province: Lake Apo and Pulangi Lake.

Collection of samples and determination of sexes. Samples of *O. niloticus* with 30 males and 30 females were collected in each site. A total of 120 individual were used in the analysis. The sexes were determined through examination of the genital papilla located immediately behind the anus of the gonads (Popma and Masser, 1999).

Image acquisition. A DSLR (Cannon EOS 1200D) camera was used to capture the image of the specimens. These samples were mounted on an improvised platform to make the camera stable and to allow uniform focus. The samples were placed in a standard position with fins teased so as to show their natural position when swimming.

Morphometric and statistical analysis. Geometric morphometric analysis was performed based on the phenotypic aspect to determine the morphological differences within and between populations from different areas with distinct environmental conditions. This method enables the precise and detailed analysis of shape change and shape variation in organism based on the positions of homologous anatomical landmarks or shape outlines (Rolf, 1993). This

method allows generating graphic presentation of results for visual display and comparison of shape changes based on measured distances, angles and ratios.

Fifteen body landmarks from the left body side of the fish (equivalent to 15 X and 15 Y Cartesian coordinates) were digitized on the images (Fig. 3) using the TpsDig version 2.12 (Rohlf 2010). The landmarks chosen for this study followed Moushomi and Saha (2015) and Ndiwa et al. (2016). These landmarks were selected to provide a comprehensive summary of the morphology of the fish specimens. The standard length sizes ranged from 184–220 mm.

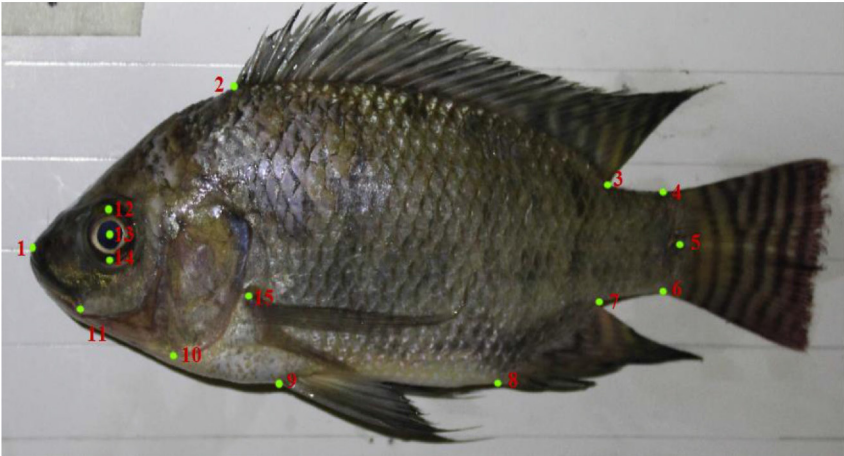


Figure 3. Image of *O. niloticus* showing the 15 homologous anatomical landmarks

Image of *O. niloticus*, digitized (1) snout tip; (2) and (3) anterior and posterior insertion of the dorsal fin; (4) and (6) dorsal and ventral region of the caudal peduncle where there is the greatest curvature; (5) posterior most body extremity; (7) and (8) posterior and anterior insertion of the anal fin; (9) insertion of the pelvic fin; (10) juncture of the ventral edge of operculum with the ventral outline; (11) posterior extremity of premaxillar; 12 and 14 superior and inferior margin of the eye; 13 center of the eye; 15 insertion of pectoral fin.

The geometric configurations composed of x and y coordinates from digitized landmarks transformed first into shape variables before executing the statistical analysis of shape variation. Since the images contain shape and non-shape variables resulting from differences in position and orientation of the

specimens during image acquisition, Generalized Procrustes Analysis (GPA) were performed using MorphJ software (Klingenberg, 2011). The Principal Component Analysis (PCA) also known as Relative warp Analysis was also performed using the same software. The generated relative warps were then used to determine the different body shape variation exhibited by the samples. Using the Paleontological Statistics (PAST) software (Hammer et al., 2001), the PCA scores were subjected to Multivariate Analysis (MANOVA) and were further supported by Canonical Variate Analysis (CVA) and Discriminant Function Analysis (DFA) to further analyze and elucidate the variations existing between the two sexes and also between the different geographical location from which the specimens were collected.

RESULTS AND DISCUSSION

Geometric morphometric analysis was employed to analyze and describe the body shape variation that exists within and between populations of *O. niloticus*. There are numerous factors that may contribute to variation in the species population; one that leads to several consequences is geographical isolation that results in limited gene pool and varying selection pressures. Sexual dimorphism is also one of the main sources of shape variation within population. Dimorphism mainly arises from the different evolutionary role between sexes. These information would be vital in making comparison to other species of *Oreochromis* which are not studied yet.

The illustrations of body shape variation within the populations of *O. niloticus* from Lake Apo and Pulangui Lake is summarized in Figure 4a and 4b respectively. The relative warps scores were used to generate the boxplots for both sexes. Alongside are the deformation grids for both positive and negative extreme warps with expansion factor scale. The relative warp on the top is the mean body shape for the two populations. Consequently, Table 1 presents the shape description of these variations or shape change for both sexes in each population with their respective variance percentages.

The boxplot shown in Figure 4a and 4b compared the body shape of the two sexes together with the deformation grids with expansion scale changes in shape across variances. In Lake Apo, *O. niloticus* male showed a great variation (highest expansion) in the posterior insertion of the dorsal fin down region,

orbital region down to the ventral edge of the operculum (head region). The female showed variation from the pectoral fin region, pelvic fin region and slightly radiates to the caudal region (belly region). Additionally, the female exhibit dorsal fins that is longer than that of the males. On the other hand, males have lengthier region between snout and dorsal fin.

In Pulangui Lake, male and female *O. niloticus* shared the same trend in body shape compared to population in Lake Apo with the males having highest expansion in the head region and females in the belly region. Also, like in Lake Apo, females exhibit longer dorsal fins than males, while males have lengthier region between snout and dorsal fin.

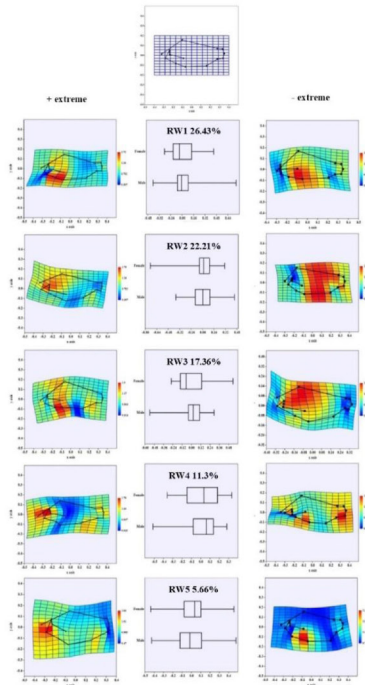


Figure 4a. Summary of landmark based on geometric morphometric analysis showing the boxplot and variation of the body shapes between sexes of *O. niloticus* Males and Females of Lake Apo as explained by each of the significant relative warps.

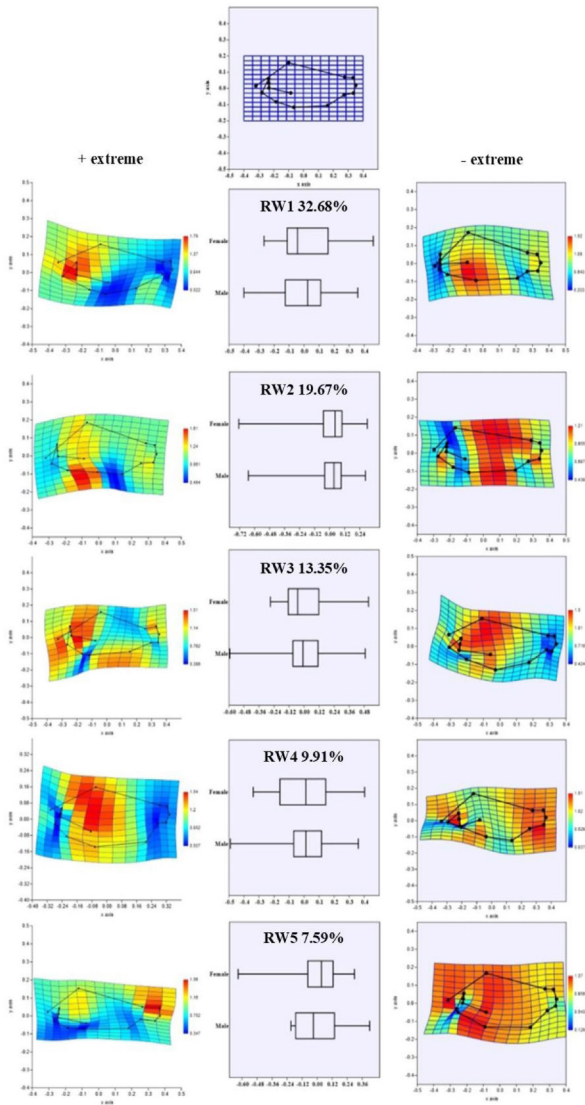


Figure 4b. Summary of landmark based geometric morphometric analysis showing the boxplot and variation of the body shapes between sexes of *O. niloticus* Males and Females of Pulangui Lake as explained by each of the significant relative warps.

Table 1

Variation in the body shapes of O. niloticus populations as explained by each of the significant relative warp and its corresponding percentage variance.

RW	Male	Female
	Lake Apo	
RW 1 26.43%	Variation from the pelvic (highest expansion) fin towards the back of the orbital region (slight expansion) near the dorsal fin. Mouth region has the highest contraction.	Variation from the region covered by ventral edge operculum, lateral fin, anterior insertion of anal fin to pelvic fin (highest expansion). Highest contraction observed in oral and orbital region
RW 2 22.21%	Variation from the orbital region towards the posterior insertion of dorsal fin (highest expansion). Contraction was observed in the region covered by the pectoral fin, posterior insertion of anal fin and the pelvic fin. Caudal fin region is also contracted.	Variation from dorsal fin region towards caudal fin and the belly region (highest expansion). Contraction in the orbital region towards the posterior insertion of dorsal fin.
RW 3 17.36%	Variation in the region covered from posterior insertion of dorsal fin, pectoral fin and pelvic fin towards the ventral edge operculum (highest expansion). Highest contraction is in the region between insertion of pelvic fin and anterior insertion of anal fin	Variation in the region covered from posterior half of dorsal fin, pectoral fin towards the orbital region (highest expansion). Highest contraction on caudal region and slight contraction on the oral region.
RW 4 11.3%	Variation in the oral and orbital region (highest expansion). Highest contraction from the region covered of dorsal fin, anterior insertion of anal fin, insertion of pectoral fin and the pelvic fin.	Variation in the caudal region and covered within region of pelvic fin, pectoral fin and ventral edge operculum (highest expansion). Slight expansion is observed in the orbital region and region between oral and posterior insertion of dorsal fin.
RW5 5.66%	Variation in the oral and orbital towards the chin region (highest expansion) and Slight expansion on the anal fin region. Contraction is highest in the caudal region and towards the dorsal fin region.	Variation in the region within pectoral fin, operculum and pelvic fin (highest expansion). Contraction is observed in the rest of the body region.

Table 1 Continued

Pulangui Lake		
RW1 32.68%	Variation from the oral region, orbital region towards the posterior insertion of dorsal fin (highest expansion). Contraction is observed in the region within ventral edge of operculum towards the caudal region.	Variation in region within pectoral fin, pelvic fin and ventral edge of edge of operculum (highest expansion), slight expansion radiating towards posterior dorsal fin insertion and anterior anal fin insertion. Contraction is highest at the oral and orbital region.
RW2 19.67%	Variation in the region enclosed by regions between pectoral fin, pelvic fin and ventral edge of operculum (highest expansion). Slight expansion in region of back of the orbital region towards the posterior insertion of dorsal fin. Contraction is highest at region between pectoral fin and anal fin.	Variation in the region from the back of posterior insertion of dorsal fin down to pelvic fin towards the caudal region (highest expansion) and slight expansion in the chin region. Highest contraction is observed between oral, orbital and posterior insertion of dorsal fin
RW 3 13.35%	Variation in the region of oral, orbital pectoral radiating towards dorsal fin posterior insertion (highest expansion). Slight expansion in region between pelvic fin and anterior insertion of anal fin. Highest contraction in the region between ventral edge of the operculum and pelvic fin and slight contraction in the rest of the dorsal fin.	Variation in the front of the orbital region radiating towards more than half of dorsal fin (highest expansion). Slight expansion in region within ventral edge of operculum towards pectoral fin and pelvic fin. Highest contraction is on the caudal region.
RW4 9.91%	Variation in the back of orbital region towards half of the dorsal fin until the pectoral fin (highest expansion). Slight expansion on the region between ventral edge of operculum and pelvic fin. Highest contraction on the oral, orbital and caudal region.	Variation in the orbital region and lower caudal fin region (highest expansion). Slight expansion between oral region towards dorsal fin, half of dorsal fin towards caudal region and between ventral edge of operculum downward to pelvic fin. Highest contraction on oral region downward to the ventral edge of the operculum.
RW 5 7.5%	Variation on the upper caudal region (highest expansion), slight expansion in back of orbital region radiating towards the dorsal fin. Highest contraction in front of orbital and region between pectoral fin and pelvic fin. Slight contraction is observed	Variation in the oral region, dorsal fin region, and pelvic fin region, ventral edge of operculum, and pectoral region (highest expansion), slight expansion radiates towards the rest of the body from middle of dorsal fin to the caudal region. Contraction is highest in the lower orbital region.

The comparison of body shape differences between sexes in each population was emphasized using statistical tool such as MANOVA, CVA and DFA. The results of MANOVA and DFA for each population are summarized in Tables 2 and 3. MANOVA revealed that the body shape variation in Lake Apo is not significant with p-value of 0.55 while Pulangui Lake population has a significant body shape variation with p-value of 0.001. This difference of significance in body shape variation can be attributed to population isolation and varying degree of gene pool for each lake with Pulangui Lake having many tributary rivers resulting to higher gene pool compared to Lake Apo.

To support these findings, CVA and DFA plots were generated to show whether there are significant differences in the body shapes of males and females for each population (see Figure 5a, 5b, 5c and 5d). Figure 5 b and d summarized the CVA plots that showed variations between the two sexes and accounts for 88.33% of the variance for Lake Apo and 95% for Pulangui Lake. While Figures 5 b and c showed the DFA plots of the pooled scores of both males and females *O. niloticus* from each population. A minimal overlap of some of their morphological characteristics is a further emphasis on the difference between the two sexes as shown by DFA plots.

Table 2

Summary of the MANOVA results of two different populations of O. niloticus.

	Lake Apo	Pulangui Lake
Wilks' lambda	0.3471	0.2287
Pillai trace	0.653	0.7713
P-Values	0.5556-0.5546	0.001019-0.001017
Eigenvalue1	1.881	3.372
Eigenvalue2	9.85E-05	3.88E-05

Table 3

Summary of the DFA results of two different populations of O. niloticus.

	Lake Apo	Pulangui Lake
<i>P</i> -Value	0.008137	0.00343
Correctly classified (%)	88.33% (11.67% overlap)	95% (5% overlap)

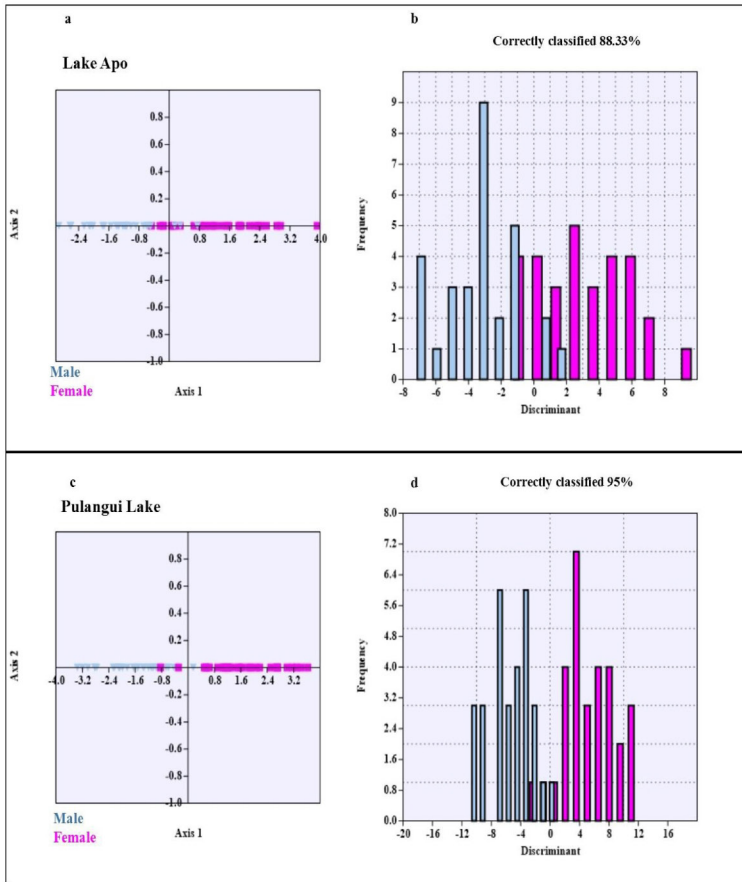


Figure 5. CFA and DFA plots of the relative scores of *O. niloticus* population

Analyses were made and boxplot were generated from the pooled relative warps to see if there are variations existing within sexes in both populations. Figure 5 illustrated the summary of relative warps with corresponding variances between populations of *O. niloticus* Male (Fig. 6a) and Female (Fig. 6b). It generally showed that the pooled male population exhibit variations in terms of the expansion of the head region and a slight expansion to the anal fin region, contraction is highest in the pectoral fin region towards the mid-section of the body. On the other hand, females exhibit variation in terms of expansion in mid-section of the body, expansion is pronounced in the chin region radiating towards the belly region. Highest contraction occurred in the head region and scattered peripherally.

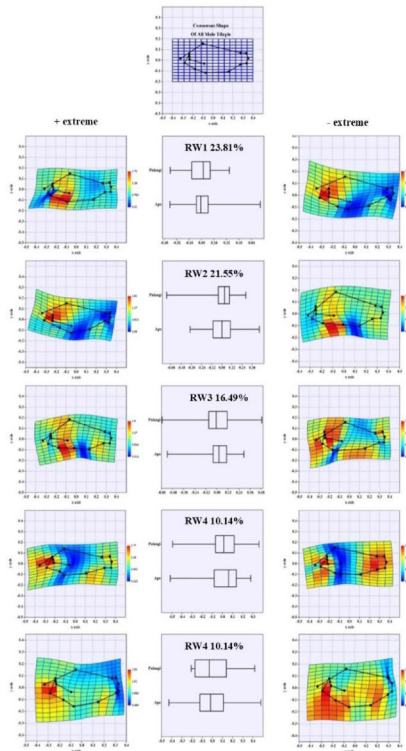


Figure 6a. Summary of landmark based geometric morphometric analysis showing the boxplot and variation of the body shapes within sexes between populations of *O. niloticus* explained by each of the significant relative warps of males.

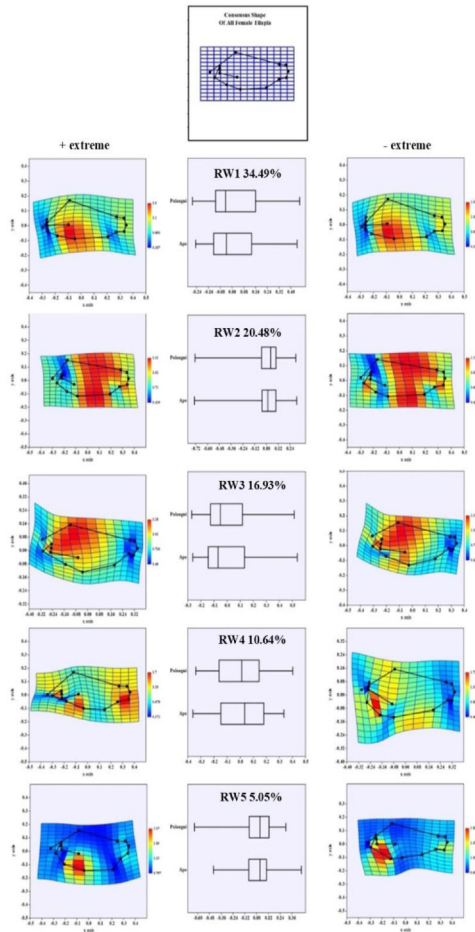


Figure 6b. Summary of landmark based geometric morphometric analysis showing the boxplot and variation of the body shapes between populations of *O. niloticus* explained by each of the significant relative warps of females.

Previously generated relative warps within sexes between populations were subjected to CVA and DFA and their corresponding plots were also created. Figures 7a and 7c showed the CVAs for each sex and Figures 7b and 7d showed the DFAs also for each sex. Based on the CVA plots in each sex, it is apparent that there are significant differences in the morphological attributes between sexes for each population with only a miniscule to no overlap between them, thus each population are separated from each other.

The results from DFA also reinforce the result obtained in CVA since there is only 1.67% overlap in male population while the female has no overlap in their morphological attributes. The MANOVA and DFA between the two populations are shown in Tables 4 and 5.

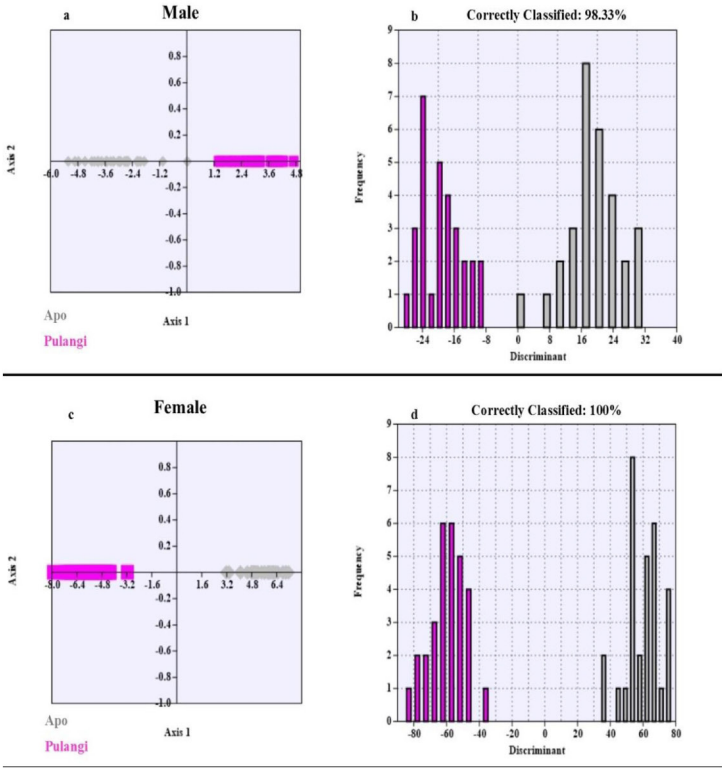


Figure 7. CVA and DFA plots of the relative scores of *O. niloticus*. Male (a, b) and Female (c, d). CVA (a, c). DFA (b, d).

Table 4

Summary of the MANOVA results for O. niloticus males and females between two populations

	Male	Female
Wilks' lambda	0.08319	0.02895
Pillai trace	0.9169	0.9712
<i>P</i> -Values	3.96E-09-3.91E-09	1.889E-15-1.757E-15
Eigenvalue1	11.02	33.53
Eigenvalue2	7.396E-05	0.0001504

Table 5

Summary of the DFA results for O. niloticus males and female between two populations

	Male		Female	
	<i>P</i> -Value	Correctly Classified (%)	<i>P</i> -Value	Correctly Classified (%)
Lake Apo vs Pulangui Lake	1.026E-08	98.33	4.663E-15	100

The data from MANOVA revealed the comparison of male and female from each population demonstrated that there are differences existing between the two sexes. Thus, sexual dimorphism has been detected and exists in the two populations of *O. niloticus*. Wilks' lambda and Pillai trace highlights this finding. Populations or groups can be tested using tools such as DFA and CVA if they can be well separated from each other or blend into each other into a continuum (Hammer, 2002).

In animal taxonomy, sexual dimorphism is considered prevalent and is one of the most interesting sources of phenotypic variation among organisms and considered as a very important area of study in the field of evolutionary biology (Benitez et al., 2011). According to Hendrick and Temeles (1989), there are three main adaptive mechanisms favoring the evolution of sexual dimorphism. These are sexual selection, dimorphic niches and food competition. Sexual selection usually applies on males when females exhibit nonrandom mating pattern or when mate competition favors certain male traits. Dimorphic niche hypothesis, however suggest that selection acts typically on females because of

reproductive constraints (Hedrick and Temeles, 1989). In addition, ecological selection acts differently on both sexes and can influence sexual dimorphism by favoring both dimorphic niches and as a result, also the dimorphic structures (Hedrick and Temeles, 1989).

A number of studies showed that the different reproductive roles, niche divergence between sexes, preference of one sex for specific traits of the other sex and competition within group of the same sex can influence the variation in external structure (Slatkin, 1984; Shine, 1989; Anderson, 1994). In comparison to other fish species, few studies have shown sexual dimorphism in dorsal and anal fin being pointed particularly in the mature males and rounder in females in *Sarotherodon galilaeus* Linnaeus and *O. aureus* Steindachner (Chervinski, 1965), pelvic fins reaching or passing the anus in males but not in females in *Tilapia zillii* Gervais, *S. galilaeus* and *O. aureus* (Chervinski, 1983), a thicker and continuous dorsal fin in mature males and notched dorsal fin in females of *O. aureus* (Fishelson, 1966), and a thicker lip in the upper jaw in mature males of *O. mossambicus* (Seitz, 1949).

This observation showed that males and females do not differ only in terms of their reproductive organs but also in external structures that are not related or liked to reproduction (Darwin, 1874; Andersson, 1994). It has been observed in this study that the variations that exist in the populations of *O. niloticus* are mainly the expansion or contraction of body regions specifically the head and belly region and mostly varying length of dorsal, ventral and caudal fins. Therefore, it is probable that the causes of these variations are attributed to one or few of the factors mentioned earlier.

In the CVA and DFA plots generated from the pooled data of two populations (see Fig. 6), it denotes a clear difference between the two populations. This observation is further supported by the results from MANOVA as vouched by the Wilks' lambda, Pillai trace and the *P*-Values (Table 4 and Table 5). These findings indicate that geographical separation could be a contributing factor to the populations' distinction from each other since there is no interaction or migrations between these populations (Turan et al., 2004). Isolation also present a population to varying selection pressures, an important prerequisite for allopatric speciation. These isolated populations could become morphologically and genetically differentiated through adaptive and non-adaptive processes (Grant et al., 2000) ultimately leading to formation of distinct gene pools.

Since, the ability of the fish stock or populations to adjust or evolve as separate biological entities is limited by the flow of genes among populations. The disparity between populations observed in *O. niloticus* in this study could be attributed to the populations' response to their present environment and to the present selection pressure at play in each geographic location they are situated. One example of selection pressure that could have contributed to their differentiation is artificial selection by farmer and interbreeding of variety due to over domestication throughout the decades (De Silva, 1997). Thus, the phenotypic plasticity of fish enabled them to react and adapt to environmental change by changing their morphology, reproduction, or survival that alleviate the effect of such environmental stress (Stearns, 1983).

CONCLUSIONS

Results from MANOVA, CVA and DFA revealed significant differences between sexes of *O. niloticus* in each population as well as significant differences in sexes between populations. Therefore, sexual dimorphism was observed and exists in both populations. Environmental and geographical isolation greatly contributes to the observed distinction between populations of *O. niloticus*, since isolation permits little to no interaction between populations causing limited flow of genes between populations. However, further studies on the genetics of shape variations are recommended to advance the understanding about the diversity of the species which would greatly contribute to its conservation and to aquaculture management strategies.

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