



## Geometric Morphometric Analysis on Sexual Dimorphism of Guppy *Poecilia reticulata* in Lake Sebu, South Cotabato, Philippines

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### Abstract

*Poecilia reticulata* is an introduced species in the Philippines which is a native species of Brazil, Guyana, Venezuela, and the Caribbean Islands. It is commonly known as guppy (Peter, 1860). The principal objective of this study is to describe the variations in morphology of the species as well as identify its sexually dimorphic traits using Geometric Morphometric Analysis. A total of 60 (30 males and 30 females) specimens were analysed and fourteen (14) landmark points were digitized in each sample. Relative Warp Analysis (RWA) (Rohlf 2008) was used and scores were subjected to Discriminant Function Analysis (DFA) and Multivariate Analysis of Variance (MANOVA) and Canonical Variate Analysis (CVA) of PAST software (Hammer et al., 2009) to determine the substantial variance between body shapes and sexual dimorphism of *P. reticulata*. The result in the DFA showed substantial variance between body shape of the sexes with females exhibiting wider dentary, along with bloated abdomen. On the other hand, males exhibit more elongated jaw region, posterior anal and ventral caudal fin. The CVA scatterplots also illustrated the sexual dimorphism in the body shape of *P. reticulata*. that shows no overlapping of plots which remarkably discriminated (separated) the male from female. With the results derived, sexual dimorphism of *P. reticulata* with the variation of its morphological structure is determined through the Application of Geometric Morphometrics.

**Keywords:** Geometric morphometrics, *poecilia reticulata*, relative warp analysis (rwa), sexual dimorphism.

### Introduction

GUPPIES, *Poecilia reticulata* (Peter, 1860), commonly known as million fish, or more likely to be called as rainbow fish, belongs to the family Poeciliidae (livebearer). It has about 200 species coming from 16 genera. Guppies are neotropical species which are natives of Northwestern South America, and was then introduced in many countries in Asia and other continents. They are typically small, brackish or fresh-water dwellers in which males have maximum lengths of 3.5 cm., and females having 6 cm. Females are pale olive-colored with transparent fins and are typically larger than males<sup>1</sup> (figure-1a). Males on the other hand are polychromatic, which means they have a variation of color combinations especially in their sides and fins. These colors are usually red, orange, yellow, blue, and green with white and black patches. With these variable colorations, no two males are identical in terms of pigment patterns. Figure-1b shows the image of *P. reticulata* taken from Lake Sebu, South Cotabato, Philippines.

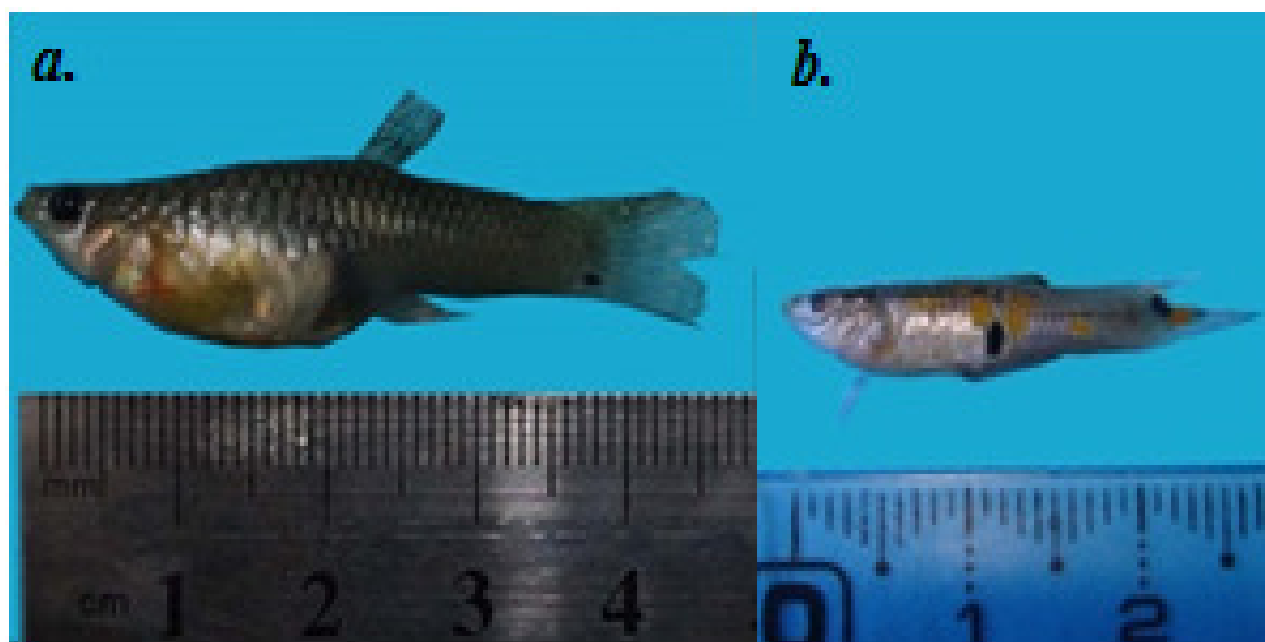
Wild type males are more colorful and have elongated finnage, and this is taken to extremes in the selectively bred aquarium varieties. The male fish also possesses a gonopodium, which is a modification of the anal fin used in reproduction and is stick like in appearance. Females are larger, plumper and generally

exhibit dull coloration. Although, aquarium bred varieties may show some quite intense coloration in the caudal fin. Guppies are known to be widely distributed throughout the country. The researchers have found profuse population of the fish in Lake Sebu. The municipality of Lake Sebu is a part of the South Cotabato Province located in its south western part. Lake Sebu is a natural tectonic lake that lies 700 m above sea level. The lake has a water area of 354 hectares with a maximum depth of 57 m at the center and 5 m on the shoreline (figure-2). It is the largest lake among the four lake systems found near the municipality and is one of the bodies of water supplying important irrigation to the provinces of Sultan Kudarat and South Cotabato. Fish kill is annually occurring since 1960's and usually on the month of September to December (Lake Management Plan of Lake Sebu, 2010). For this reason, Lake Sebu is identified as a stressed environment because of the occurrence of events like fish kills and its consistent exposures to human activities especially by hybrid fish growing processes.

The researchers would like to distinguish whether the lake condition affects the morphological structure as well as the sexual selection of *P. reticulata* since guppies are considered to be invasive and as good bioindicator of the physical and chemical stressors of its habitat



**Figure-1.a.**  
**Poecilia reticulata (Froese and Daniel, 2010)**



**Figure-1b**  
**Photographed using Nikkon camera of the Female (a) and Male (b) Poecilia reticulata**

In addition, sexual dimorphism of *P. reticulata* can be attributed morphologically to the shape of its body that is influenced by sexual selection. In fact the aquatic environment is recognized as a powerful force in modeling the morphology of an organism during ontogeny. Thus, shape variation between the sexes could also be explained by ecological or niche selection. But in terms of scientific and technological methods of explaining shape variances, geometric morphometrics can be applied.

Geometric morphometrics is a method that uses a set of landmarks to explain shape. Landmark is a two or three-dimensional point defined by a tightly labeled set of rules. Since guppies are remarkable to have distinct and marked difference in sexes, the study re-examines and quantifies sexual shape dimorphism in *Poecilia reticulata* using Geometric Morphometrics.

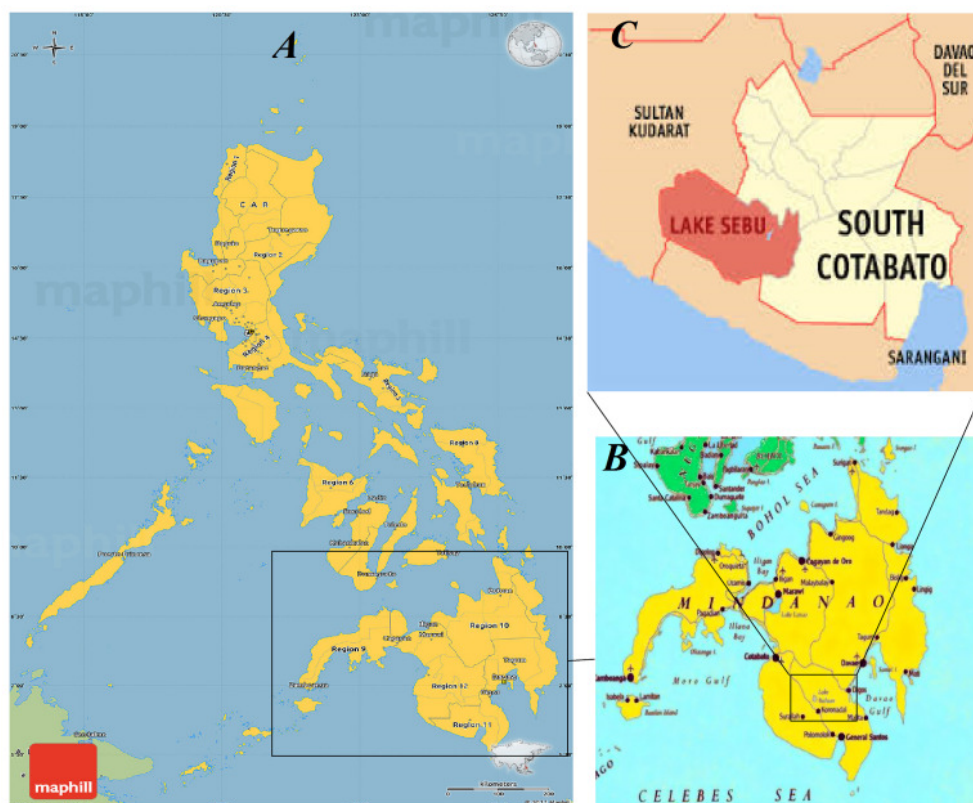


Figure-2

Map of the Philippines (a), Mindanao (b) and Lake Sebu South Cotabato (c), where the sampling was done

In this study, visual representations summarizing the variation among specimen using Relative Warp Analysis (RWA), substantial variance between body shapes of the sexes and sexual dimorphism of *P. reticulata* by the Discriminant Function Analysis (DFA), Multivariate Analysis of Variance (MANOVA) and Canonical Variate Analysis (CVA) were used.

## Material and Methods

**Fish Sampling and Study Area:** A total of 60 (30 males and 30 females) individuals were collected from Udong Creek – one of the contributory creeks of Lake Sebu. They were placed in a styropor box filled with ice for the preservation of the color as well as the body shape. Sorting of males from females was made through the identification of their observable physical attributes. With the use of high-definition camera (Nikon D3000), concerning the minute size of the fish, guppies were imaged for clear modification of the primary portions. Each sample was captured thrice for precise illustrations. Biometric measures followed to determine the length, size, and other biometric data of the specimen.

**Digitization of Fish Specimen:** A total of fourteen (14) landmarks (14 x and 14 y Cartesian coordinates) were selected to provide a summary of the morphology of the fishes. The landmarks were digitized on both sides of each specimen image

using the Tps Dig ver. 2.10<sup>2</sup> (figure-3), and was then subjected to Relative Warp Analysis to obtain scores that will be used in the PAST software in order to test the difference in sexes. This provides plots that show the distribution of two sexes along the two canonical variate axes.

**Morphometrics:** To have a clearer representation of the body shape, converted tps file of fishes (digital images) were digitized using 14 homologous points using the software tps Dig 2 version 2. Through this, x and y coordinates of landmarks were extracted on the images. The images were then warped with the landmark coordinates showing the positive and negative deformation from the consensus body shape as described in the grid square. The relative warp scores recorded were documented in MS Excel worksheet and were initiated as a source in analyzing intraspecific variation in body shapes of the species using the Paleontological /Statistics Analysis (PAST) software. Discriminant Function Analysis (DFA) was used to have a distinct body shape difference between sexes of *P. reticulata*. Using the Discriminant Hotelling's t-square which shows a p value, the equivalence among the compared sexes was checked. The level of significance for the p value is < 0.05. Accordingly, Multivariate Analysis of Variance (MANOVA) and Canonical Variate Analysis (CVA) was also used to further visualize differences between sexes.

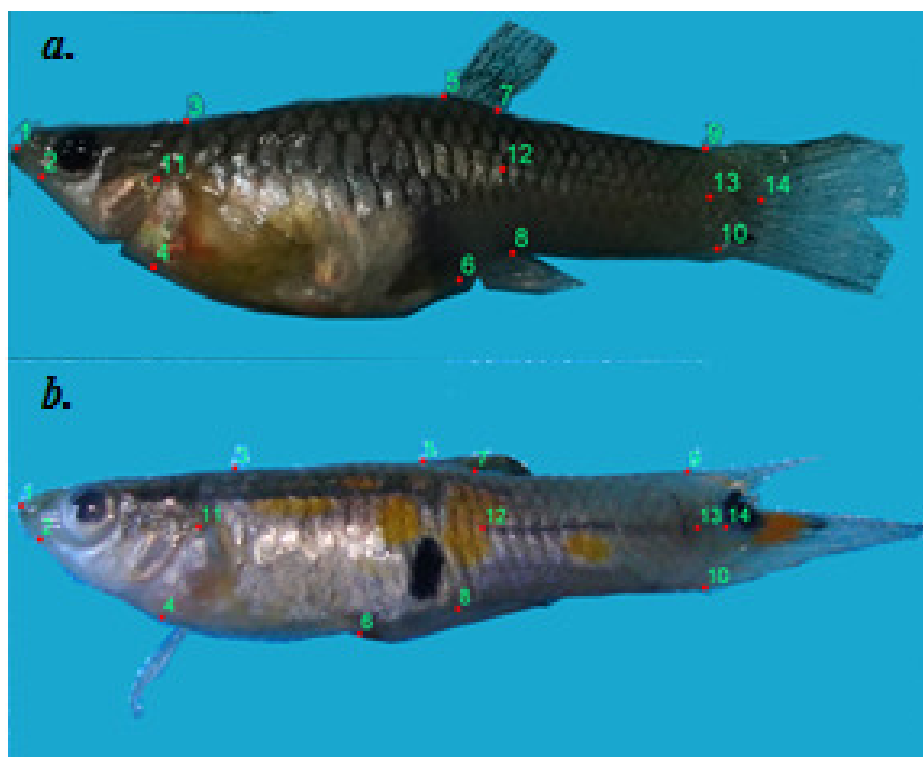


Figure-3

**Landmarks' description of the Guppy, *Poecilia reticulata* (a. female, b. male):** (1) snout tip; (2) posterior extremity of premaxillar; (3) dorsal occiput; (4) insertion of operculum on the lateral profile; (5) and (7) posterior and anterior insertion of the dorsal fin; (6) and (8) anterior and posterior insertion of the anal fin; (9) and (10) dorsal and ventral insertion of the caudal fin; (11) point of maximum extension of operculum on the lateral profile; (12) and (13) anterior and posterior region of the lateral line from the middle part of the body to the caudal peduncle ; (14) posterior most body extremity.

## Result and Discussion

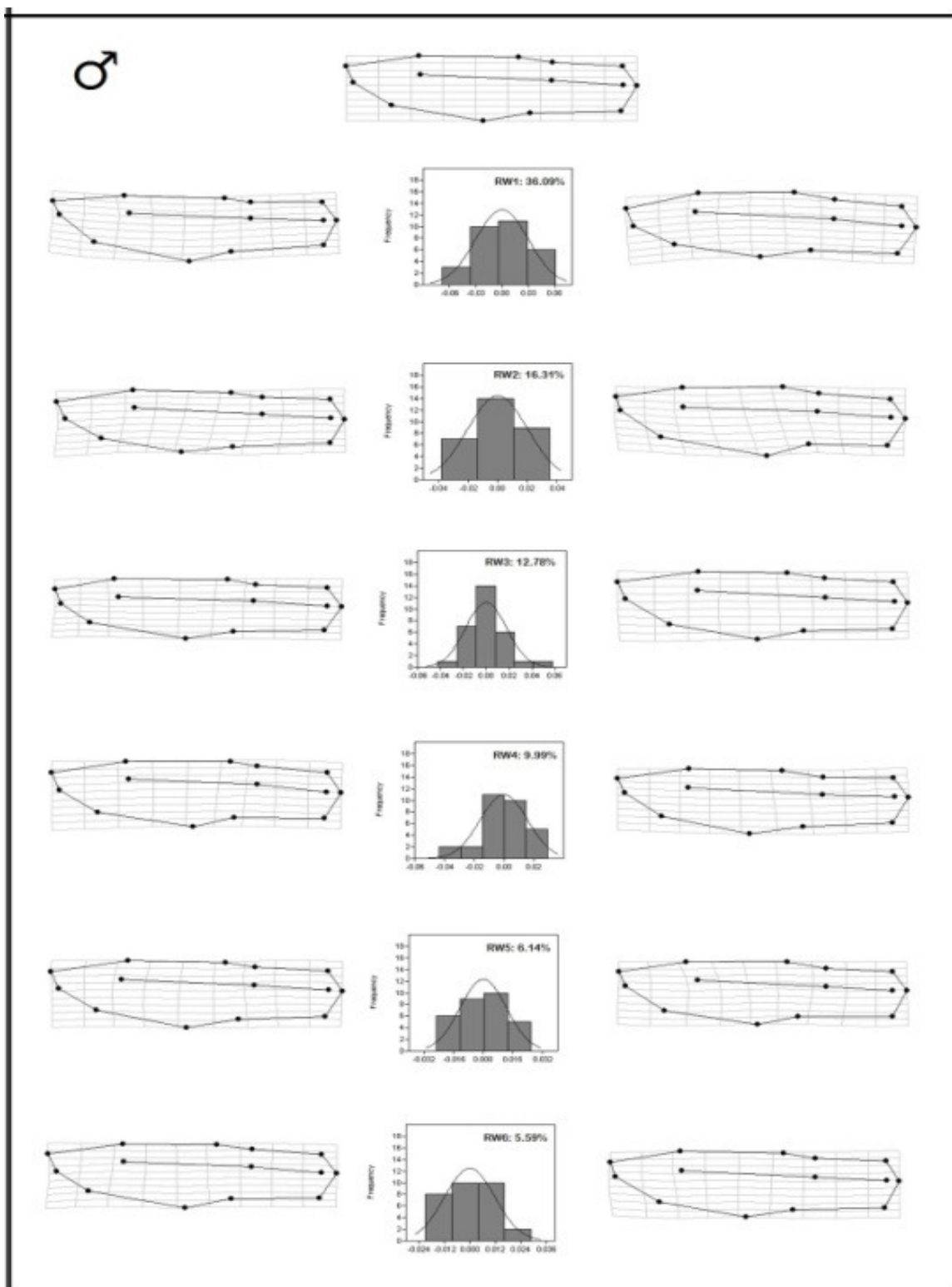
Patterns of intra-specific differences in body shapes within each sex were summarized via frequency histograms of the Relative Warp Scores. Quantitative analysis of body shape morphology of male and female *P. reticulata* shows variation from the first six relative warps explaining the variance of five percent and more. In male (figure-4), illustrates 86.9% total variation with RW1 that accounts for 36.09% describing for the broadness of the lateral profile to the anal fin, RW2 accounts for 16.31% describing differences in the body depth including the head, RW3 accounts for 12.78% showing variations of the extended jaw, RW4 accounts for 9.99% due to differences in the posterior region including the dorsal fin base, RW5 accounts for 6.14% curvature in the body depth specifically in the lateral profile that includes the anal fin base and RW6 accounts for 5.59% differences in the head region including the mouth.

In female (figure-5), RW1 accounts for 36.38% for variations in the tail and head regions where focus location occurred, RW2 accounts for 16.75% due to the variation in the head region including the lateral portion of the operculum, RW3 accounts for 14.09% observed for the variation in snout tip to the posterior extremity of the premaxillar that would affect the

lateral profile in the insertion of operculum. RW4 accounts for 7.32%, RW5 accounts for 6.45% depicting variance due to focus location in the snout region and RW6 accounts for 4.74% variation in the head region. Totaling, 85.73% variation was shown in the quantitative analysis of body shape morphology of the female *P. reticulata*.

Relative Warp scores of male and female *P. reticulata* was subjected to Discriminant Function Analysis (DFA) via Discriminant Hotelling's t-square using PAST software. Male's body shape is relatively different to those of the females as what the value has shown,  $p < 0.05$ , which is highly significant (figure-6).

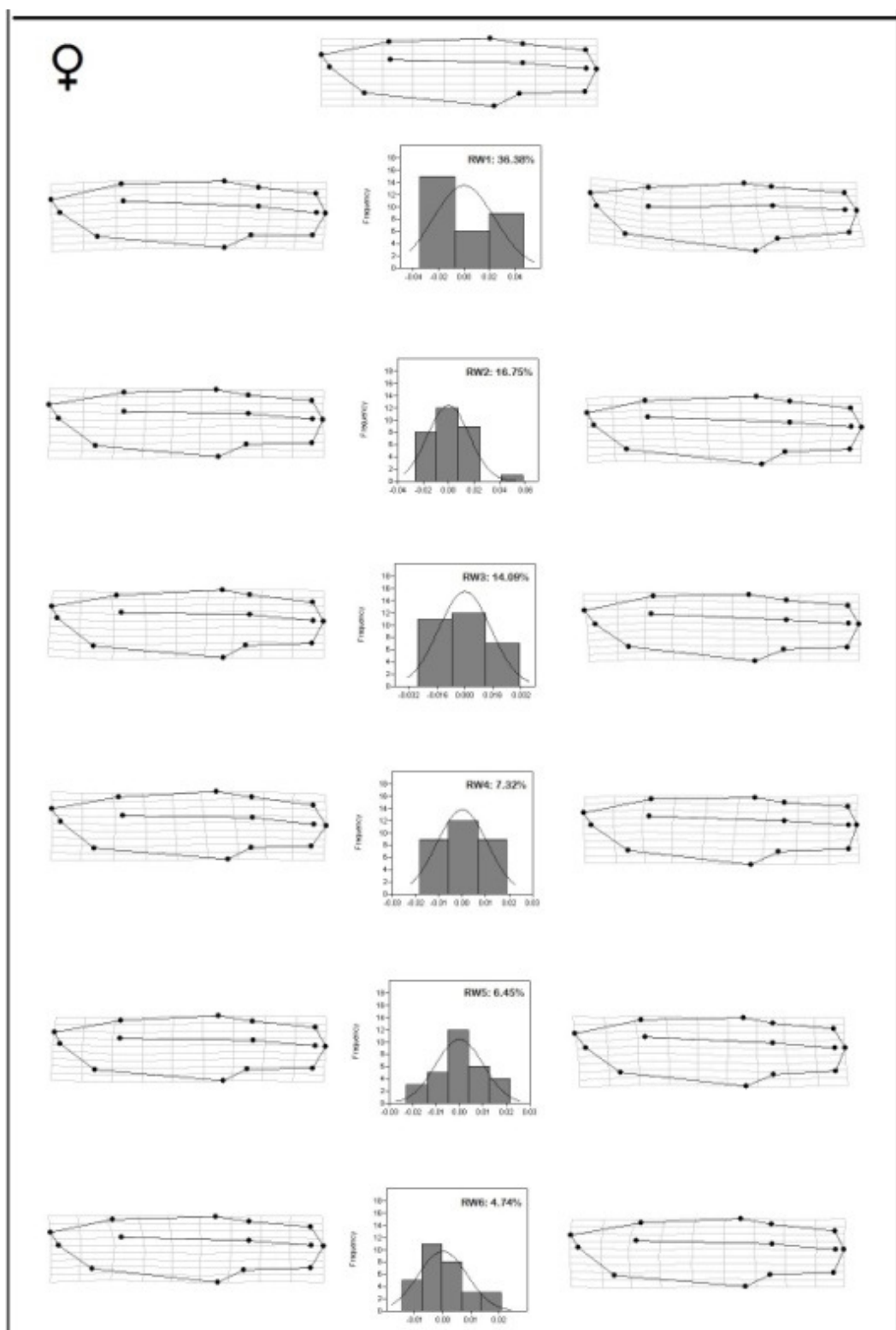
The CVA scatterplots also illustrated the sexual dimorphism in the body shape of *P. reticulata*. Figure 7 shows no overlapping of plots which may imply that the landmarks used, remarkably discriminated (separated) male from female. It shows significant differences in body shapes of the two sexes based on the distribution of the samples (Wilks' lambda = 0.04969; Pillai trace = 0.9503; P-Value = 1.006E-32). The first axis explains much of the variation between the sexes and accounts for nearly 100% of the variance (Eigenvalues for CV1 and CV2 are 19.12 and 1.42E-16, respectively).



**Figure-4**

Summary of the Geometric Morphometric Analysis of the fish's shape showing the consensus morphology (topmost panel) and the variation in body shape of male *P. reticulata* produced by the 6 relative warps respectively explaining more than 5% of the variance RW1=36.09%,RW2=16.31 %,RW3=12.78%,RW4=9.99%,RW5=6.14%, RW6=5.59%





**Figure-5**

Summary of the Geometric Morphometric Analysis of the fish's shape showing the consensus morphology (topmost panel) and the variation in body shape of female *P. reticulata* produced by the 6 relative warps respectively explaining more than 5% of the variance RW1=36.38%,RW2=16.75%,RW3=14.09%,RW4=7.32%,RW5=6.45%, RW6=4.74%.

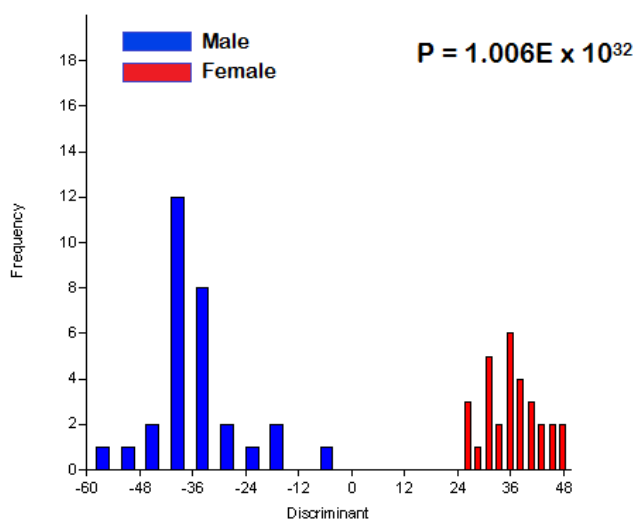


Figure-6

Graph of Frequency Distribution of the Discriminant Scores (DFA) of the relative warp scores of highly significant body shape between male and female *P. reticulata* ( $p = 1.006E \times 10^{32}$ )

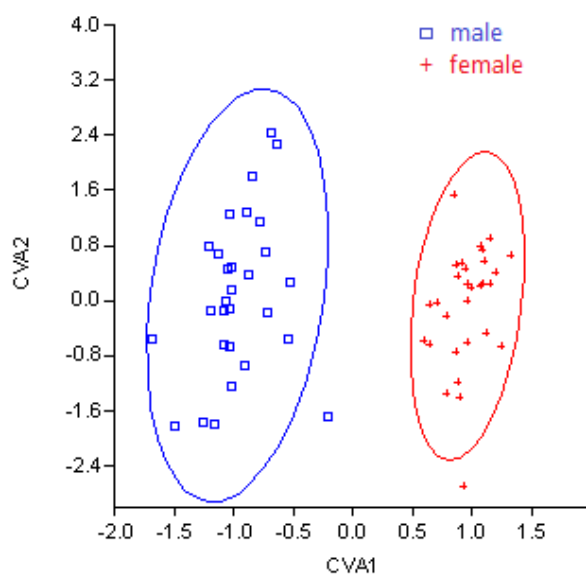


Figure-7

Distribution of the two sexes of *P. reticulata* along the first two canonical variate axes

The researchers took water samples on the lake and tested the physico-chemical properties of the water. Three (3) sample areas were examined to obtain the level of transparency of the water, oxygen, pH, and temperature (table-2). Phosphorus and Nitrate Analysis (table-3) together with the Coliform Test (table-4) were analysed at the laboratory.

Results in table-2 showed that the lake is unsustainable enough

for aquatic life to propagate since fish growth and activity usually require 5-6 mg/L of dissolved oxygen, dissolved oxygen levels below 3 mg/L are stressful to most aquatic organisms and levels below 2 mg/L will not support fish at all.

Phosphorus and Nitrate Analysis showed result in Table III that levels of phosphate and nitrate are high and elevating thus causes destruction of lake ecosystems<sup>3</sup> and has heavily impact in the overall health of the water and its inhabitants<sup>4</sup>, since phosphate level should not exceed 0.05 mg/l and unpolluted waters generally have nitrate levels below 1mg/l.

In table-4, showed results from the coliform test conducted in two (2) sample areas under three (3) trials. Positive scores indicate high population count of *E. coli* whereas negative scores denote low population count of the bacteria. Thus, numerous counts of bacteria were observed due the occurrences of fish kill and the wastes of the residents near the area.

The study on body shapes has a great role in many biological studies. Body shapes could be affected by many factors such as exposure to different rates of water flow that result to the change in the body's plasticity, the foraging ecology, physiological variables, differences in habitats in the intensity of sexual selection, and diet abundance.

However, based on the results gathered from the relative warp analysis down to the physico-chemical analysis of the lake, the researchers have determined that there is no significant effect to the morphological structure of guppies despite on how stressed the environment was, since guppies have this ability to remarkably tolerate the physical and chemical effects of its habitat. According also to a study in the 'Behavioural Ecology of the Trinidadian Guppy, *Poecilia reticulata*, as an Invasive Species' conducted by Amy E. Deacon, Ph. D., Guppies as an invasive aquatic species are more likely to be characterized by traits including rapid individual and population growth, early maturity, short lifespans, high fecundity, small offspring size and extensive dispersal capacity leading to the explanation that Guppies tend to adapt highly rapider to its environment requiring no alterations in its morphological shape. Another from Chervinski, 1984 also suggested that Guppies were able to survive in high salinity levels, same as through with Chung, that Guppies display considerable thermal adaptability.

Geometric morphometrics is a very important tool in describing organisms. Through this, one can analyze an organism's mutations on shape, developmental changes in their form, covariance among ecological factors and shape. It can also be used to measure a trait which is of evolutionary significance to know the changes in shape, function, or to their evolutionary connection. Interpretation of body shape morphology variations between sexes of *P. reticulata* can also be a factor in determining sexual dimorphism of the said species other than its remarkable traits of differentiation.

**Table-1**  
**Variations observed in *P. Reticulata***

	% Variation	Female	% Variation	Male
<b>RW1</b>	36.38%	Marks the difference in the tail and head region. Samples with positive scores exhibit more extended posterior region and somewhat parallel snout tip to dorsal occiput compared to the samples having negative scores.	36.09%	States the variations in the head region as well as the lateral profile to the anal fin. Samples with negative scores display shorter mouth and body and have an extended jaw than the samples in positive scores.
<b>RW2</b>	16.75%	Indicates variation in the head region including the lateral portion of the operculum. It also shows difference in the anal fin of both samples having negative and positive scores.	16.31%	Shows differences in the body depth including the head. Samples with positive scores have elongated body, shorter snout tip to the premaxillar and exhibit extended jaw.
<b>RW3</b>	14.09%	Accounts for the difference in snout tip to the posterior extremity of the premaxillar that would affect the lateral profile in the insertion of operculum. Negative scores samples provide extended jaw than that of the positive ones.	12.78%	Presents the variation in the head region showing an extended jaw in the samples with positive scores due to focus location.
<b>RW 4</b>	7.32%	Observes variation in the tail region. Samples with negative scores have constriction in caudal fin and shorter dorsal fin.	9.99%	Includes variance in the posterior region including the dorsal fin base. Samples with positive scores have extended dorsal fin than the samples with negative scores.
<b>RW 5</b>	6.45%	Depicts variance due to the focus location in the snout region. Shows longer snout tip in the positive scores samples. Samples with negative scores exhibit the opposite morphology.	6.14%	Displays curvature in the body depth specifically in the lateral profile that includes the anal fin base. Samples with negative scores have longer body depth and anal fin than that of the negative ones.
<b>RW 6</b>	4.74%	Illustrates variation in the head region where negative scores samples have shorter length between snout tip to dorsal occiput.	5.59%	Patterns the differences in the head region including the mouth. Samples with negative scores have larger mouth compared to samples with positive scores.

**Table 2**  
**Physico-Chemical properties of Lake Sebu**

TOP					
Waypoint	Depth(m)	Transparency	D.O.(mg/L)	pH	Temp. (°C)
<b>A</b>	25.7	0.7-0.2	1.6	7.4	26.9
<b>B</b>	6.9	0.5-0.3	3.8	7.5	27
<b>C</b>	13.4	0.4-0.2	3.8	7.5	26.6

**Table-3**  
**Phosphate-Nitrate analysis result**

Parameters	Concentration	Laboratory Sample No.			
		1	2	3	4
Phosphate	mg P-PO <sub>4</sub> /L	0.323	0.242	0.697	0.442
Nitrate	mg NO <sub>3</sub> /L	8.34	9.28	7.03	2.54

## Conclusion

The study has provided a clear manifestation on the sexual

dimorphism of *Poecilia reticulata* with the use of Geometric Morphometric analysis. Furthermore, Lake Sebu as a stressed environment has no substantial effect on the morphological structure of *P. reticulata*.

**Table-4**  
**Coliform Test Result**

Water Sample	Lactose Broth	Nutrient Agar	Eosine Methylene Blue
Sample A: t1	+	TNTC	+
Sample A: t2	+	TNTC	+
Sample A: t3	+	TNTC	+
Sample B: t1	-	-	-
Sample B: t2	+	TNTC	+
Sample B: t3	+	TNTC	+

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