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Application of geometric morphometrics for body shape analysis in *Selaroides leptolepis* population from Butuan Bay, Philippines

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Abstract
Genomic and environmental adaptation possibly develops to phenotypic plasticity. As expected, this results to the structural variation that affects symmetrical outlines of species. Systematized techniques such as geometric morphometrics were applied to detect the phenomenon while considering landmark points to identify the fluctuating asymmetry (FA). FA illustrates deviations from the original state of symmetry. It quantifies variations between the left and right side of bilateral structure hence the two sides are genetically alike. In this study morphological differences of *Selaroides leptolepis* were investigated. About 60 individuals consisting of 30 males and 30 females were collected and analyzed for fluctuating asymmetry. FA from both samples subjected to Procrustes ANOVA, Principal Component Analysis (PCA), Implied Deformation and Histogram to evaluate the asymmetrical differences using Symmetry and Asymmetry in Geometric Data (SAGE) software. The result shows highly significant FA ($P<0.0001$) on both male and female samples. It indicated morphological deviation and associated with genetic components and environmental causation. Thus, the advancement of utilizing modernized technique ensue to discriminate shape variations.

Keywords: Fluctuating asymmetry, marine fishes, morphological traits, landmarks

1. Introduction

Geometric Morphometrics (GM) has developed a rapidly expanding field of research in biology [1-3], nevertheless, in all biotic elements, changeability could be enormous even within one species or genus.GM is the most suitable method for quantitative comparison of two-dimensional biological shapes. Also, it is continually using landmarks and semi-landmarks coordinates utilized by various authors in recent years as a quantitative approach to studying systematic and developmental problems both in flora and fauna [4]. Significantly, this modern application analyzes the relative positions of anatomical landmarks and sets of points used to approximate curves and surfaces to quantify shape and size [5]. Further, several studies often used this method to describe the form and exponentially contributes to the field of biology [6- 9]. Whereas, Fluctuating Asymmetry (FA) is the subset of GM and define as variance in subtle differences between the left and the right sides in bilaterally symmetrical organisms and it provides a measure of how well an individual can buffer its development against internal genetic and external environmental stress during ontogeny [10, 11]. Further, this known to be the most commonly used approach to developmental stability is fluctuating asymmetry [12]. An important assumption in studies involving FA as a measure of developmental stability is that the subtle asymmetries should not have a heritable basis [13]. FA served as an indicator of environmental stress and had a better understanding of genetic and ecological effects [14]. Nevertheless, any change of nutritional components, increase or decrease, may result in decreased developmental stability [15]. The approach enables us to understand the geometry of the species throughout the analysis and, similar studies with Fourier and wavelet it provides graphical visualizations of the statistical findings that can support the biological explanation.

Distinctive measurement procedures (e.g., outline curvatures, wavelets, and Fourier) develop in various evaluations of the diversities among specimens [33, 34]. While studies widely utilized geometric morphometric analysis as a tool to investigate the body shape formation of fish species [35-39]. Further, the mechanism quantifies the shape difference of organisms from anatomical landmarks to the Cartesian coordinates, fusing an accurate statistical system with systematic processes for overlaying landmark outlines of all samples in a conventional coordinate structure [24, 32].
Moreover, the total fluctuating asymmetrey often measured in stressed environments comprises the additive or synergistic effects. While others correlate it as age/development related and therefore FA associated with stress, environmental or genetic factors. Thus, the study determines fluctuating asymmetry (FA) of *Selaroides leptolepis* population from Butuan Bay, Philippines, using Geometric Morphometric analysis.

2. Materials and Methods
2.1 Locale of the Study
The study area was located in Butuan Bay, Agusan del Norte in Caraga Region. (Figure 1). A Northeast section in Mindanao with a geographic coordinates (latitude of 9°.03' 60.00" N, and longitude of 125°21 '59.99" E). The fish sample collection was done in the month of September 2018 with the aid of local fisherman. Proper fish preservation techniques were applied to the collected samples and immediately brought to laboratory for further processes.

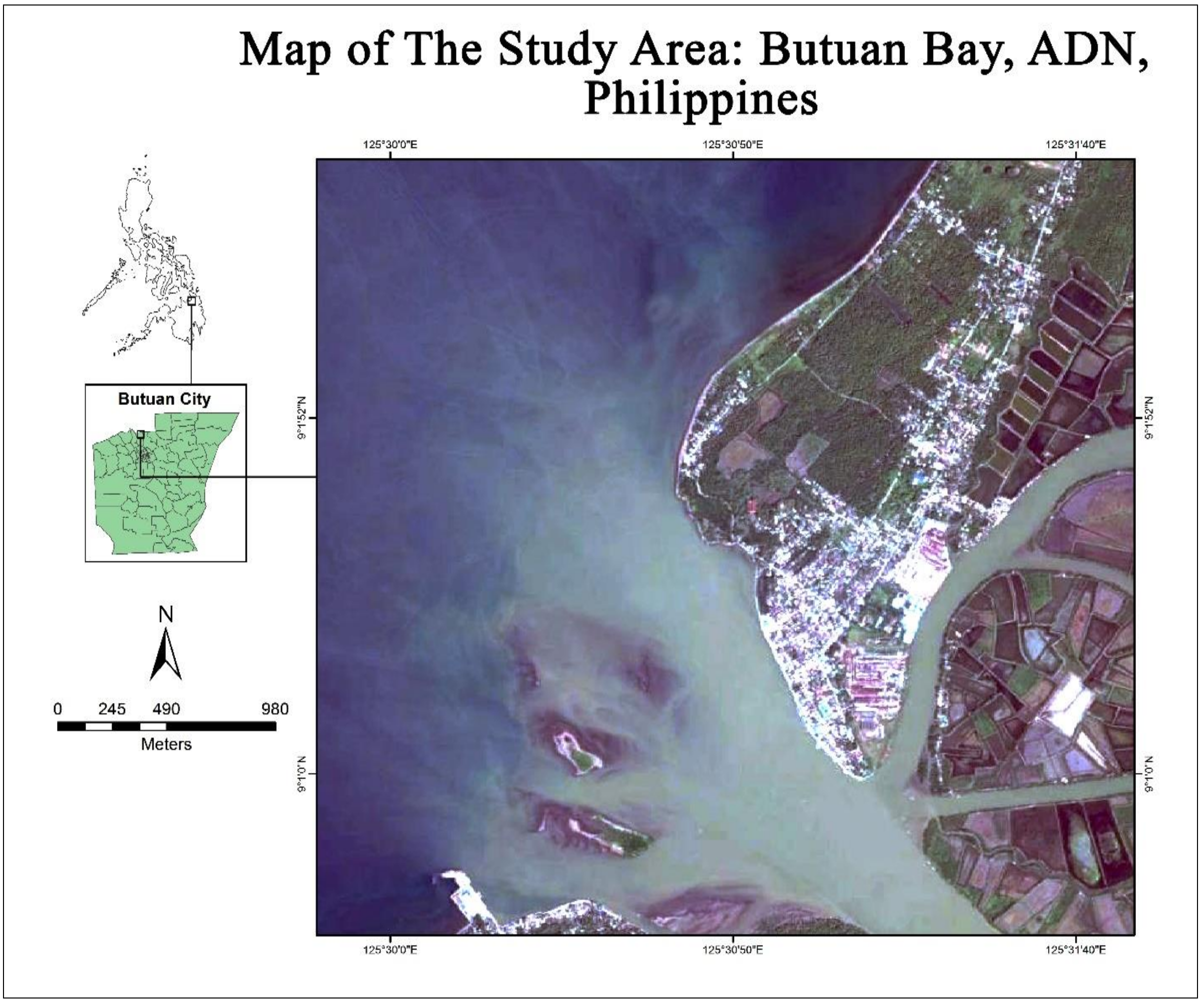

Fig 1: Map of the study area, Butuan Bay, Agusan del Norte, Philippines.

2.2 Processing of fish samples and sex determination
About sixty (60) individuals of *S. leptolepis* (Salay-Salay) was obtained of which 30 were males and 30 females. Individually, the fishes were positioned in the top of the Styrofoam where a pin used to stretch the fins. A 10% formaldehyde applied to its fins using a small paint brush to harden and let dry for an hour. Afterward, it was transferred to another Styrofoam and removed all the pins of the fish samples. Both the left and right lateral side of the fishes were photographed three times (tri-replicated) using a DSLR camera (Figure 2). While, sex determination was done using the dissecting materials, examining its genitalia. Sex had been identified through the presence of ovaries in a granular form yellow to orange color in females while in males a testes in a non-granular, smooth and whitish color.

2.3 Landmark Selection, Digitation and Shape Analysis
The photographed of the fish samples were separated according to its sexes while transported it to TPSutil to covert the file. The digitation process was followed utilizing TPSdig2 (Rohlf 2004). Sixteen (16) anatomical landmark points (Table 1) were undertaken to the samples. Further, shape analysis was also applied to the fish samples and were tri-replicated to lessen the measurement error. The bilateral (left-right) sides of the fish were digitized and where transferred to Symmetry and Asymmetry Geometric Data (SAGE) Software version 1.04 (Marquez, 2007) (Figure 3).

Fig 2: Actualized digitized sample and landmark points used to describe the body shape of *S. leptolepis*. **Above (Male) Below (Female).**

Table 1: Landmarks used to digitize the body shape of *S. leptolepis* (adopted from Jumawan et. al., 2016).

Landmark Points	Location
1	Snout tip
2	Posterior end of nuchal spine
3	Anterior insertion of dorsal fin
4	Posterior insertion of dorsal fin
5	Dorsal insertion of caudal fin
6	Midpoint or lateral line
7	Ventral insertion of caudal fin
8	Posterior insertion of anal fin
9	Anterior insertion of anal fin
10	Dorsal base of pelvic fin
11	Ventral end of lower jaw articulation
12	Posterior end of the premaxilla
13	Anterior margin through midline of orbit
14	Posterior margin through midline of orbit

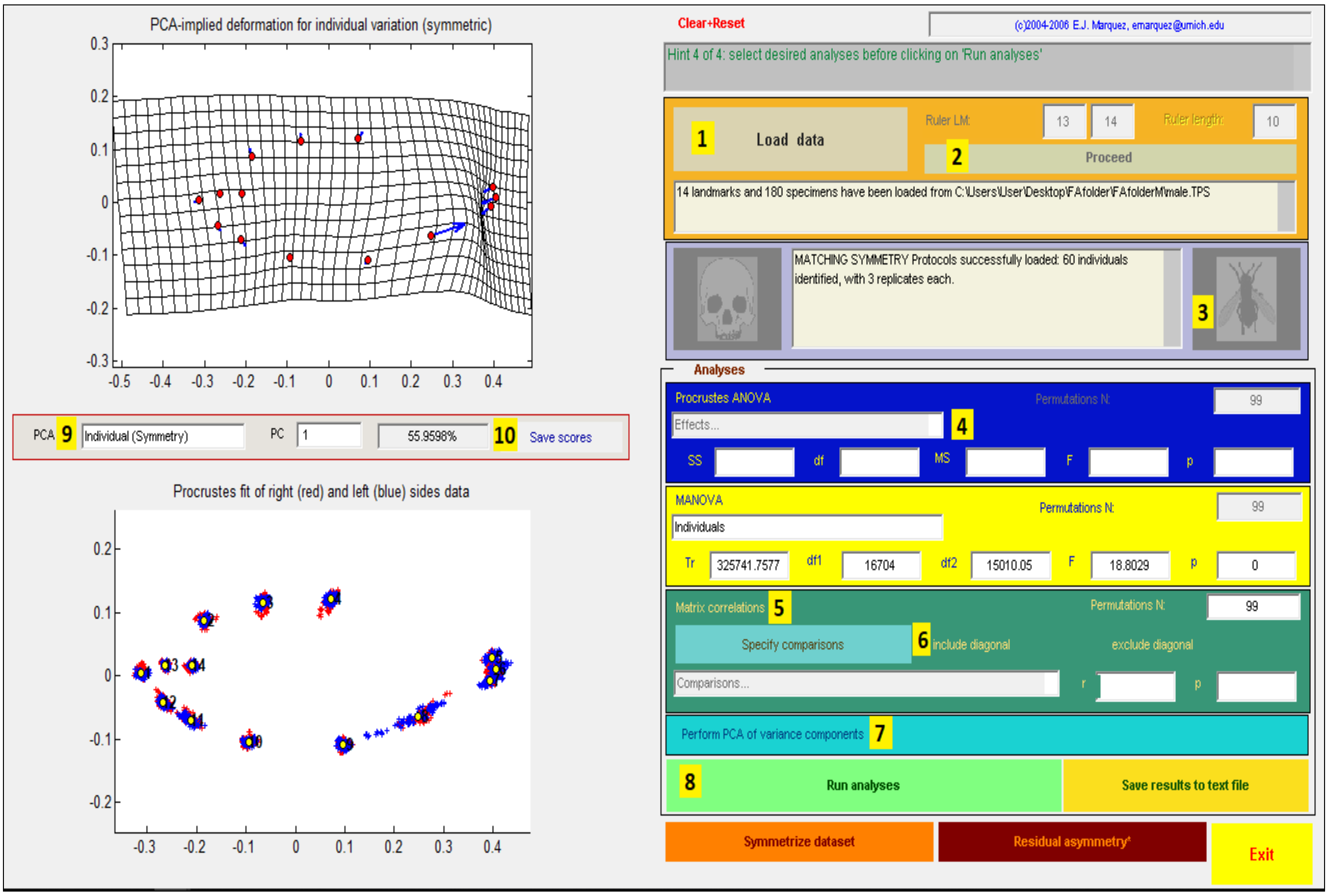

Fig 3: Overview of the schematic flow of shape analysis using **(SAGE).**

3. Results and Discussion

Table 2: Procrustes ANOVA on body shape of *S. leptolepis* in terms of sexes.

Factors	SS	DF	MS	F	P-value
Female					
Individuals	0.1586	696	0.0002	0.9173	0.08724
Sides	0.0138	24	0.0002	2.3064	0.0004
Individual x Sides	0.1729	696	0.0002	17.8581	0.0001**
Measurement Error	0.0401	2880	0	-	-
Male					
Individuals	0.1513	696	0.0002	0.7027	0.0001**
Sides	0.0244	24	0.001	3.2853	0.0001**
Individual x Sides	0.2153	696	0.0003	42.1359	0.0001**
Measurement Error	0.0211	2880	0	-	-

** (P<0.0001) highly significant

Table 2 shows the Procrustes ANOVA on the body shape of *S. leptolepis* in terms of sexes. There were three parameters (Individuals, Sides, and Individuals by sides) analyzed to determine shape deformities among the fish population. In the female, the result shows a highly significant (P<0.0001) found in the Individual by sides. While in male, the three factors denoting (P<0.0001) highly substantial. This observation is indicating a body shape variations among the female and male samples. A study has shown that the differences among members of the same species and the dissimilarities in pattern and color between the variants are linked to individual fish cycling through a series of modifications associated to seasonal gonad development [16]. While, in the same study performed on *C. temensis*, this phenomenon has been associated with spawning annually in their natural, flood-pulse river environment [17] possibly owing to the integration of fluctuating availability of spawning sites, prey, and potential forage for their fry. The correlation concerning ecology and morphology in fishes has long been recognized, and limited studies have utilized multivariate morphometric approaches to examine ecomorphological outlines in multi-species fish populations [18]. Further, intraspecific examination of morphological polymorphisms existed to be the subject of pervasive study [19]. Polymorphism might be influenced through ecological inputs, either entirely or by genetic control and may result from gene-environment interaction [20]. In the event of gene regulation, polymorphism may evolve itself, expected to the modular nature of various gene linkages [21, 22]. Moreover, morphological distinctions amongst species can be plotted in a morpho-space where the organisms distribution and occupied space support functional information on the structural complexity of the community [23, 24].

Table 3: Principal component scores showing the values of symmetry and asymmetry scores

PCA	Individual (Symmetry)	Sides Directional Asymmetry	Interaction (Fluctuating Asymmetry)	Affected Landmarks
Female				
PC1	53.3353%	100%	54.4203%	5, 6, 7, 8, 11, 12
PC2	20.5923%		15.8745%	1, 2, 3, 4, 6, 8, 9, 10, 11, 12, 13, 14
PC3	10.0825%		14.7165%	1, 3, 4, 6, 7
PC4	6.0589%		4.6813%	1, 3, 4, 5, 6, 7, 8, 9, 11, 13
	90.069%		89.6926%	
Male				
PC1	55.9598%	100%	75.1336%	1, 3, 4, 5, 6, 8, 10, 11, 13, 14
PC2	16.7638%		6.7343%	1, 3, 4, 5, 6, 7, 10, 11, 12
PC3	8.252%		5.8203%	2, 3, 10, 11, 12, 14
PC4	7.3170%		3.9353%	1, 2, 4, 5, 6, 7, 9, 10, 11
	88.2926		91.6235%	

On the other hand, Principal Component Analysis (PCA) was presented (Table 3) to show the values of symmetry and asymmetry scores among the fish samples. It was observed that female population, generated four (4) PC scores accounting to 90.06% and Interaction (Fluctuating Asymmetry) of 89.69%. While the common affected landmarks where 6 (Midpoint or lateral line) and 7 (Ventral insertion of caudal fin). The male population, generated four (4) PC scores accounting to 88.29% lower than of the female. Also, Interaction (Fluctuating Asymmetry) has a percentage of 91.62% higher than of the female. The common affected landmarks where 10 (Dorsal base of pelvic fin) and 11 (Ventral end of lower jaw articulation). It was observed that affected landmarks among of the two sexes were dissimilar and thus it denotes variations among the landmark points In relation, benthic species, such as frogfishes or many scorpionfishes, practice synchronized movements of pectoral and pelvic fins to move over the substrate or to sustain static positions in alert, defensive or rest behaviors [25, 26], or changes of pelvic fins into suction discs that support to the adherence on the substrate happens in gobies [27]. Nonetheless, the alterations or modification of pelvic fins used throughout courtship or aggressive behaviors are also noticeable in balistoids [26]. At the same time the existence of spines in the pelvic, median and dorsal fins for protection and propulsion functionalities in scorpionfishes and gasterosteids [25].

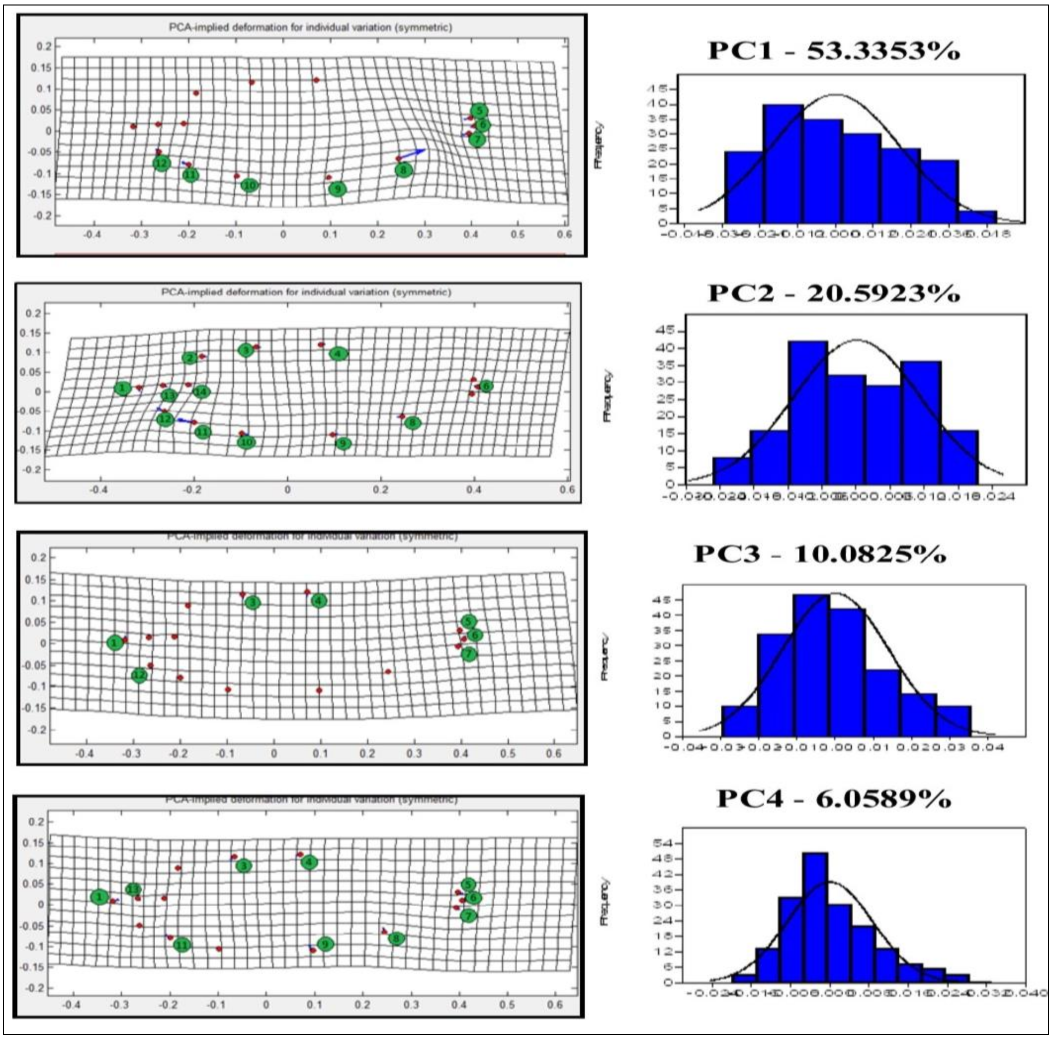

Fig 4: Principal components implied deformation grid and a histogram of individual symmetric) in *S. leptolepis* female sample.

Variation of the morphology of the organism was also correlated to its adaptive ability to sustain the environment inhabits. Ecologically it is exciting because diagrams can describe critical ecological features of communities, such as structural complexity or interactions amongst species [28, 29], where organisms that are close together interrelate in the same habitat and take advantage of the reserves. Thus, the significance of this application entails different approach and therefore measure effective mechanism for analyzing the species position within morpho-space (Fig. 4 & 5) [30, 31].

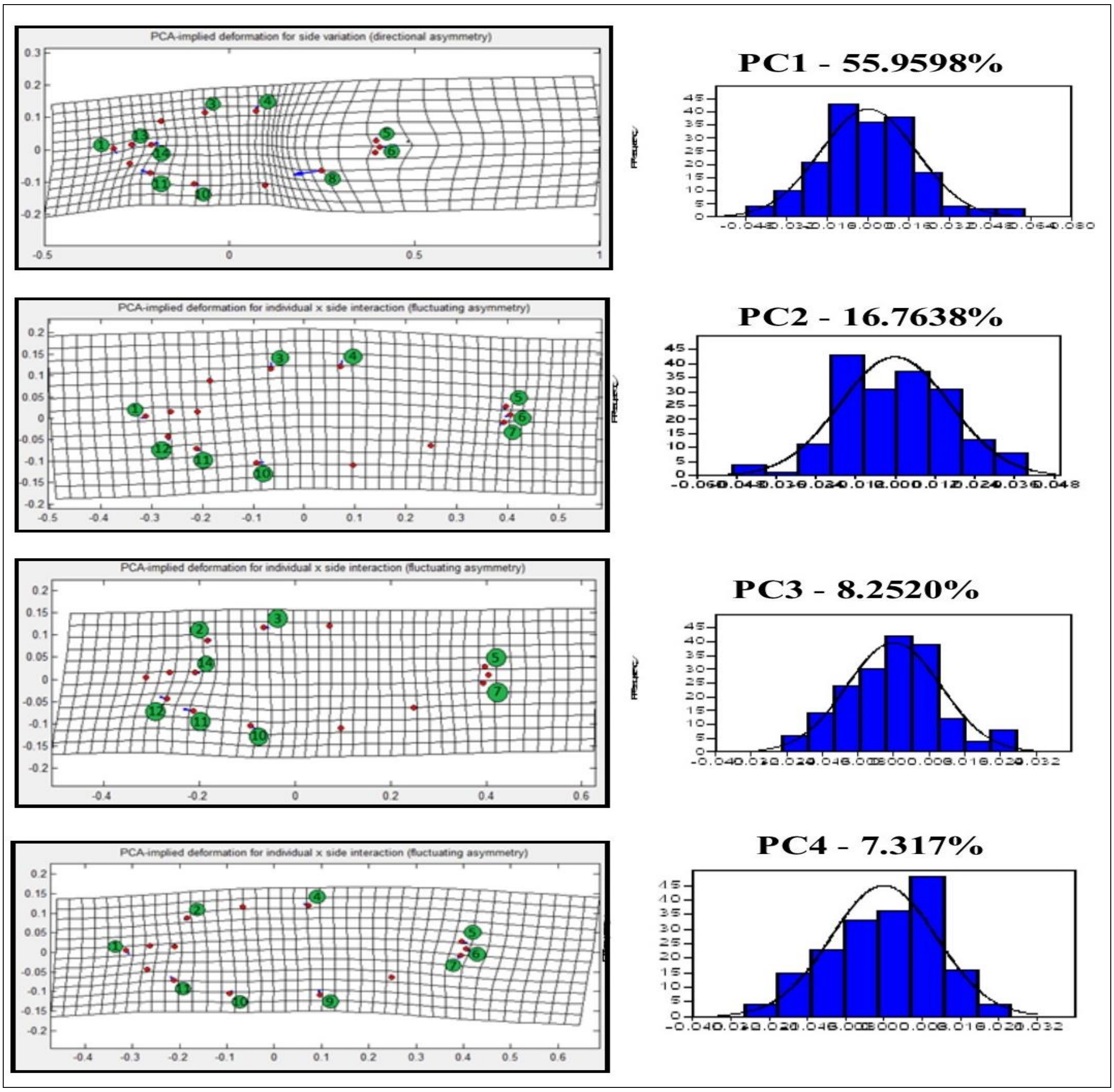

Fig 5: Principal components implied deformation grid and a histogram of individual symmetric) in *S. leptolepis* male sample.

4. Conclusion

The results of this study propose that referring to the objectives, different approaches of landmark selection are capable of showing the location of organisms within morpho-space [32]. The application stands as an essential mechanism to differentiate the species and illustrate them environmentally and biologically, as the results exhibit. Hence, an assessment of fish assemblages where groups of distinct morphologies may exhibit, the method considering the anatomical features in the landmark coordination supports a suitable and exact depiction of the three-dimensional position of species, serving to develop the identification of the structural complexity and environmental developments of fish communities. Likewise, graphical techniques are in wide-ranging and further easy to understand. The integration of diverse analytical approaches, comprising graphs and numbers, is the most efficient and comprehensive selection for evaluating the internal position among morphology and spaces where organisms inhabited.

5. References

1. Jensen RJ. Detecting shape variation in oak leaf morphology: a comparison of rotational-fit methods. American Journal of Botany. 1990; 77:1279-1293.
2. Bookstein FL. Combining the tools of geometric morphometrics. In: Marcus LF, Corti M, Loy A, Naylor GJP and Slice DE. (Edition). Advances in morphometrics. New York, Plenum, 1929; 131-51. The corn borer, *Pyrausta nubilalis* Hubner (Pyralidae, Pyraustidae, Lepidoptea). Philipp. Agriculture. 1996; 17(8):397-450.
3. Rohlf FJ. On applications of geometric morphometrics to studies of ontogeny and phylogeny. Systematics Biology. 1998; 47(1):147-67.
4. Vieira M, Joseph Mayo S, Moreira de Andrade I. Geometric morphometrics of leaves of *Anacardium microcarpum Ducke* and *A. occidentale* L. (Anacardiaceae) from the coastal region of Piauı´, Brazil.

Botanical Society of Sao Paulo, 2014.
5. Jensen RJ. The conundrum of morphometrics. Taxonomy. 2003; 52:663-671.
6. Adams DC, Slice DE, Rohlf FJ. Geometric morphometrics: Ten years of progress following the ‘revolution’. Italy Journal Zoology. 2004; 71:5-16.
7. Frieß M, Baylac M. Exploring Artificial Cranial Deformation Using Elliptic Fourier analysis of Procrustes Aligned Outlines. American Journal Physiology Anthropology. 2003; 122:11-22.
8. Sanfilippo P, Cardini A, Mackey D, Hewitt A, Crowston J. Optic disc morphology - rethinking shape. Progress Retina Eye Research. 2009; 28:227-248.
9. Buchanan B, Collard M. A geometric morphometrics-based assessment of blade shape differences among Paleoindian projectile point types from western North America. Journal Archaeological Science. 2010; 37:350-359.
10. Van Valen, L. A study of fluctuating asymmetry. Evolution. 1962; 16:125-142.
11. Palmer R. Waltzing with asymmetry. Bioscience. 1996; 46:518-532.
12. Moller AP, Swaddle JP. Asymmetry, developmental stability, and evolution. Oxford University Press, Oxford, U.K, 2000.
13. Leamy L. Dominance, epistasis, and fluctuating asymmetry. 2003, 142-56.
14. Sinclair S, Hoffmann AA. Monitoring salt stress in grapevines: are measures of plant trait variability useful? Journal applied Ecology. 2003; 40:928-937.
15. Lappalainen JH, Mattel J, Lempa K, Wilsey B, Ossipov V. Effects of resource availability on carbon allocation and developmental instability in cloned birch seedlings. International Journal Plant Science. 2000; 161:119-125.
16. Reiss P, Able KW, Nunes MS, Hrbek T. Color pattern variation in *Cichla temensis* (Perciformes: Cichlidae): Resolution based on morphological, molecular, and reproductive data. Neotropical Ichthyology. 2012; 10:59-70.
17. Jepsen DB, Winemiller KO, Taphorn DC, Rodriguez Olarte D. Age structure and growth of peacock cichlids from rivers and reservoirs of Venezuela. Journal of Fish Biology. 1999; 55:433-450.
18. Douglas ME, Matthews WJ. Does morphology predict ecology? Hypothesis testing within a freshwater stream fish assemblage. 1992; 65:213-224.
19. Trapani J. Geometric morphometric analysis of body-form variability in *Cichlasoma minckleyi*, the Cuatro Cienegas cichlid. Environmental Biology of Fishes. 2003; 68:357-369.
20. Clark WC. The environment and the genotype in polymorphism. Zoology Journal Linneaus Society. 1976; 58:255-262.
21. Weiss KM, Fullerton SM. Phenogenetic drift and the evolution of genotype–phenotype relationships. Theoretical Population Biology. 2000; 57:187-195.
22. Abouheif E, Wray GA. Evolution of the gene network underlying wing polyphenism in ants. Science. 2002; 297:249-252.
23. McClain CR, Johnson NA, Rex MA. Morphological disparity as a biodiversity metric in lower bathyal and abyssal gastropod assemblages. Evolution. 2004; 58:338-348.
24. Clabaut C, Bunje PME, Salzburger W. Geometric morphometric analyses provide evidence for the adaptive character of the Tanganyikan cichlid fish radiations. Evolution. 2007; 61:560-578.
25. Gosline WA. Function and structure in the paired fins of scorpaenifom fishes. Environment Biology Fish. 1994; 40:219-226.
26. Yamanoue Y, Setiamarga DHE, Matsuura K. Pelvic fins in teleosts: structure, function and evolution. Journal Fish Biology. 2010; 77:1173-1208.
27. Schoenfuss HL, Blob RW. Kinematics of waterfall climbing in Hawaiian freshwater fishes (Gobiidae): vertical propulsion at the aquatic-terrestrial interface. Journal Zoology. 2003; 261:191-205.
28. Strogatz SH. Exploring complex networks. Nature. 2001; 410:268-276.
29. Dale MRT, Fortin MJ. From graphs to spatial graphs. Annual Review Ecology Evolutionary System. 2010; 41:21-38.
30. Perry GLW, Miller BP, Enright NJ. A comparison of methods for the statistical analysis of spatial point patterns in plant ecology. Plant Ecology. 2006; 187:59-82.
31. Van Bocxlaer B, Schultheiß R. Comparison of morphometric techniques for shapes with few homologous landmarks based on machine-learning approaches to biological discrimination. Paleobiology. 2010; 36:497-515.
32. Adams DC, Rohlf FJ, Slice DE. A field comes of age: geometric morphometrics in the 21st century. Italy Journal Mammalogy. 2013; 24:7-14.
33. Walker JA. Principal components of body shape variation within an endemic radiation of threespine stickleback. In Advances in morphometrics. Edited by Marcus LF, Corti M, Loy A, Naylor GJP and Slice DE. NATO ASI Series A, Life Sciences. 1996; 284:321-334.
34. O’Higgins P. Advances in approaches to the study of morphological variation in the hominid fossil record: biology, landmarks and geometry. Journal Anatomy. 2000; 197:103-120.
35. Cabuga CCC Jr, Milloria MB, Lanes JR, Varona CJL, Pondang JMD, Raules JJ *et al*. Intraspecific evaluation in the morphology of *Glossogobius guiris* using geometric morphometric analysis from Lake Mainit, Agusan del Norte, Philippines. International Journal of Biosciences. 2019; 14(1):379-387.
36. Cabuga CCC Jr, Seronay RA, Busia MA, Billuga NP, Ayaton MA, Angco MK *et al*. Geometric morphometric and heavy metals analysis of flathead grey mullet (*Mugil cephalus*) from Agusan River, Butuan City, Philippines. Journal of Biodiversity and Environmental Science. 2017; 11(1):134-151.
37. Cabuga CCC Jr, Masendo CBE, Hernando BJH, Joseph, CCD, Velasco JPB, Angco MKA *et al.* Relative warp analysis in determining morphological variation and sexual dimorphism between sexes of flathead goby (*Glossogobius giuris*). Computational Ecology and Software. 2016; 6(3):109-119.
38. Joseph CCD, Jumawan JH, Hernando BJ, Boyles LZ, Jumawan JC, Velasco JPV *et al*. Fluctuating asymmetry in evaluating the developmental instability of Glossogobius giuris (*Hamilton*, 1822) from Lake Mainit, Surigao del Norte, Philippines. Computational Ecology and Software. 2016; 6(2):55-65.
39. Jumawan JH, Cabuga CCC Jr, Jumawan JC, Cortez

EMB, Salvaleon SMN, Gamutan KJS *et al.* Probing the exposure to environmental stress using fluctuating asymmetry of metric traits in *Johnius vogleri*, (Bleeker, 1853) from lower Agusan River basin, Butuan City, Agusan del Norte, Philippines. AACL Bioflux. 2016; 9(1):122-132.