Describing Body Shape Variation Between Sexes of an Endemic Eleotrid Fish *Hypseleotris agilis* (Herre, 1927), from Lake Mainit, Using Landmark-based Geometric Morphometrics

Katherine M. Unito-Ceniza, Mark Anthony J. Torres and Cesar G. Demayo

Abstract— Advanced methods in computer science, biology, imaging and statistics paved the way of advancing the science of biology from being qualitative to being quantitative. One of the advanced methods developed in studying images of biological structures is the method of geometric morphometrices which is involves image analysis using the tools of computer science, statistics, geometry and bioinformatics. In this study, geometric morphometric analysis aimed to identify morphological variations between sexes of *Hypseleotris agilis* (Herre, 1927) from Lake Mainit, Surigao del Norte using landmark-based Geometric Morphometrics was employed. A total of 129 images of the fish (77 females, 62 males) were examined. The size range of female is 7.5-13.5 cm and 7.1-12.0 cm for males. Nineteen landmark points were assigned on the specimen. The resulting partial warp scores were used in Discriminant Function Analysis (DFA) for maximal discrimination. DFA suggests sexual dimorphism of the species. Thin plate spline plot visualizes the deformed grids (Female; d^2= 0.03704; Male; d^2= 0.04808) illustrating changes of shape in *H. agilis* morphology. The mouth of the male is bigger wherein its premaxilla levels to the anterior edge of the eye but not extending past through it. The interneural gap of dorsal fins in females is shorter than in males and the form of the caudal peduncle of the males is broader relative to female. The broader caudal peduncle of the males could suggest the efficient fanning and guarding of eggs laid by the females to protect from predators.

Keywords---Hypseleotris agilis, geometric morphometrics, partial warp scores, discriminant function analysis, thin plate spline

I. Introduction

LAKE Mainit located in Mindanao, Philippines, holds the once endemic eleotrid, *Hypseleotris agilis* which is locally known as *bugwan*. It has been endemic to the lake since its accidental introduction along with the stocking of the Nile tilapia to Lake Lanao [1]. Among *Hypseleotris* Gill 1863 (carp gudgeons) species, which have been characterized by having strongly compressed head and body, a small mouth, an elongate body cavity with several anal pterygiophores preceding the first vertebral hemal spine, and a ovoid blotch at the dorsal base of the pectoral fin, *H. agilis* has the most distinctive feature, a pattern of eight spots mediolaterally and three stripes radiating posteriad from the eye [2,3].

Hypseleotris species in Lake Mainit is of interests because their biology and taxonomy are poorly known because of their small in sizes [3,4]. The *H. agilis* in Lake Mainit, although not that commercially viable when compared to other bigger fishes like tilapia and carp, is shown to be important in the biodiversity of the lake and has been recommended for conservation[5].

Studying the biology of this species could provide information on how to protect and preserve it. Without such information it is difficult to suggest appropriate management practices. Thus, this study was conducted to gather information on the variations in the morphology between sexes of *H. agilis* found in Lake Mainit with the use of landmark-based geometric morphometrics.

GM is a statistical tool used to quantify and analyze the overall shape based on the landmark configurations which allows hypothesis testing [6-11]. The use of morphometrics has been also successful in relating the body shape differentiation to ecology or phylogeny and proved the effectiveness in showing differences in morphological aspects of fishes that discriminate both within and between species populations [12-17].

II. Methodology

Collection of Samples

Hypseleotris agilis samples were collected from Lake Mainit (Figure 1). The Lake is a shared resource of Agusan del Norte and Surigao del Norte in Mindanao Islands, Philippines. It is considered as the fourth largest (17,060 ha) lake and distinguished as the deepest lake having the maximum depth of 219.35 meters [18]. There are twenty-eight river tributaries that contribute to the water volume of Lake Mainit and drained by a single outlet, the 29-km Kalinawan River that flows into Butuan Bay. The Lake has a total shoreline measurement of 62.1 km long and a watershed area of 87,072 hectares.

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A total of 139 samples of were collected from the lake by local fishermen using beach seine, 77 of which are females and 62 are males. The freshly caught samples were sexed and put separately on different containers.

Figure 1. Map showing Lake Mainit in the Northeastern part of Mindanao.

Processing of Samples

Each sample was flanked on a Styrofoam and its fins pinned to a board to show its points of origin. It was then measured and photographed capturing the left side of its body using a 12.1 megapixel Sony digital camera. The saved photographs were transferred to computer for digitization of the body shape and further analysis.

Land-marking of the Samples

The images of the fishes were digitized using TPSDig version 2.12 software [19] with 19 landmarks shown as shown in Figure 2.

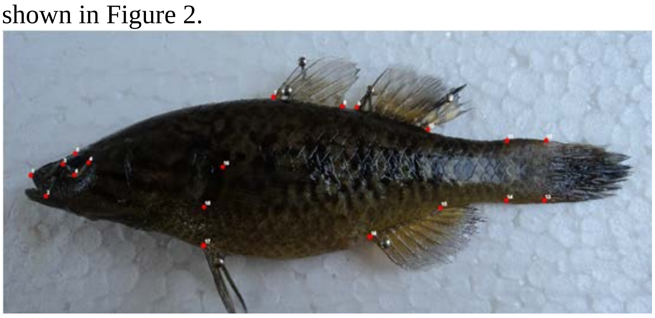

Figure 2. Location of landmarks used in the morphological analysis of *Hypseleotris agilis.* Line diagram of the left flank of the sample, showing the locations of 19 landmarks that were used for morphological analysis. These landmarks corresponds to: (1) the tip of the premaxilla, (2) the axis of the jaws, (3-6) the anteriormost, posteriormost, uppermost and lowermost point of the orbital circumference, (7-8) anterior-and-posterior-most edges of the first dorsal fin, (9-10) anterior-and-posterior-most edges of the second dorsal fin, (11) the beginning of the caudal fin, where the membrane contacts the dorsal surface, (12-13) the upper and lower portion of the hypural fan, (14) the beginning of the caudal fin , where the membrane contacts the ventral surface, (15-16) the posterior and anterior-most edges of the anal fin, at the points where it emerges from the ventral surface, (17) the anteriormost edge of the pelvic fin, (18-19) the lower and upper poits of the pectoral fin base.

Morphological Analysis

The 19 landmarks were subjected to landmark-based geometric morphometrics and thin-plate spline analysis. Using the TPS software developed by Rohlf, 2001, the x and y coordinates (using TPSdig program) were collected and the resulting coordinates was superimposed by means of Generalized Procustes Analysis (GPA) using the TpsRelW software that preserves all information about shape while removing only the information not related to shape (e.g., position and orientation) [11,20,21]. In this procedure, the landmarks of each specimen were translated, scaled to unit centroid size, and rotated to minimize Procrustes distance between all landmarks computed as the mean landmark configuration of all specimens. The resulting Procrustes distances were used to compute sexual dimophism in body shapes using the multivariate analysis of variance (MANOVA) in the PAST software [22].

The thin plate spline technique[21, 23] was used to visualize any shape changes in particular body region. In this method, shape changes are shown as deformations by fitting an interpolation function to the aligned landmark coordinates of each specimen against the reference configuration so that all homologous landmarks coincide.

The average body shape of each sex was determined and included in the analysis. Discriminant Function Analysis (DFA) was also used to assess the total amount of variation between sexes of *H. agilis.*

III. Results and Discussion

Morphological variations within a species can be explained by sexual dimorphism. As shown in Figure 3, species shows a distinction of some morphological attributes between sexes of *H. agilis*. Fig. 4 also illustrates the morphological differences of the two sexes in a particular region of the body (female; d2= 0.03704 and male; d2= 0.04808).

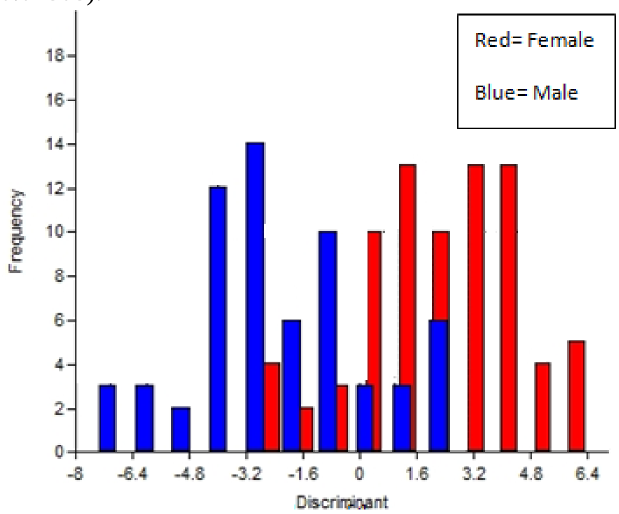

Figure 3. Graph of the Discriminant Function Analysis of the pooled partial warp scores of body shape of *H. agilis* between sexes. The graph shows a significant difference between sexes of *H. agilis* (p=0.004095).

The mouth of the male is bigger wherein its premaxilla levels to the anterior edge of the eye but not extending past through it. The interneural gap in dorsal fins of the females is shorter than in males and the form of the caudal peduncle of the males is broader. The broader caudal peduncle of the males could suggest the efficient fanning and guarding of eggs laid by the females to protect from predators [4].

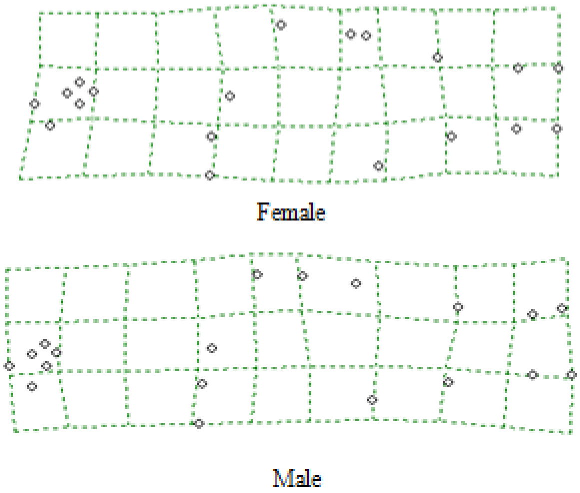

Figure 4. Thin Plate Spline plot of both sexes of *H. agilis* . It shows the bigger mouth and broader caudal peduncle of the male and the shorter distance of the interneural gap of the dorsal fins of the female. (Female; d^2= 0.03704; Male; d^2= 0.04808).

The bigger mouth of males can be related to the kind of food that they eat. In the study of Nacua et al [14], the carnivorous *G. giuris* have large head and large mouth with sharp teeth that would be advantageous in capturing their prey. Parallel results were observed in the study cichlid fish [24], cichlids with mollusk diet have larger pharyngeal jaws and muscles, compared to those cichlids of soft-food diet.

Wiley et al [25] suggests that sexual dimorphism can be caused by evolutionary and ecological forces wherein the two sexes have different habits conforming their functional adaptations. Like in *Glossogobius giuris* species, males have bigger and deeper head and bigger gape, shallower body and elongated anal fins compared to females [14] which could suggest different habits, being more active and efficient carnivores.

Gunawickrama [26] studied the intraspecific morphological variation of a Cyprinid, *Punitus singhala*, among six populations. Some morphometric characters showed a significant variations contributing sexual dimorphism, with males having long dorsal fins compared to females, significant for dispersal capabilities.

Hypseleotris agilis exhibits secondary sexual dimorphism and dichromatism in which the genital papilla of males is narrow and tapered to the end while the female have broad, lunate and bilobed papillae [5]. The coloration pattern of males appearing bright yellow in deep water and blue to dark in shallow water while females are pale, dusky to light body coloration.

In addition, the genetic makeup of an organism as well as environmental factors generally dictates its phenotype. It is known that variations in morphological characteristics of fishes are due to species variations within and between populations, genetically and phenotypically. When no physical barriers to gene flow, morphological variations are less likely driven by significant genetic differences [17]. But if there gene flow barrier such as geographic separation more likely variations are caused by differences in water quality and other environmental conditions. Sometimes phenotypic discreteness would suggest a direct relationship between the extent of phenotypic divergence and geographic separation, which indicates that geographic separation, is a limiting factor for gene flow [13].

In this study, there is a limited difference in morphological features of *H. agilis* because the samples are of the same species caught in the same area with no geographic barriers.

H. agilis is a sexually dimorphic species. Some morphological characteristics differ between sexes. The mouth of the male is bigger wherein its premaxilla levels to the anterior edge of the eye but not extending past through it. The interneural gap in dorsal fins of the females is shorter than in males and the form of the caudal peduncle of the males is broader. They also exhibit secondary sexual dimorphism and dichromatism in which the genital papilla of males is narrow and tapered to the end while the female have broad, lunate and bilobed papillae and the coloration pattern of males appearing bright yellow in deep water and blue to dark in shallow water while females are pale, dusky to light body coloration.

Sexual dimorphism can be caused by evolutionary and ecological forces wherein the two sexes have different habits conforming their functional adaptations. In addition, the genetic makeup of an organism as well as environmental factors generally dictates its phenotype. It is known that variations in morphological characteristics of fishes are due to species variations within and between populations, genetically and phenotypically.

The study of biology of *H. agilis* is important in preservation of the species and its importance in biodiversity and evolution studies.

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References

[1] Guerrero, R.III. (2002). Invasive aquatic animals in the Philippines. Special Reports, ASEAN Biodiversity.

[2] Herre, A. W., 1927. Gobies of the Philippines and the China Sea. Bur. Sci. Manila. Monograph, 23: 1-352. Pls. 1-30.

[3] Thacker, C. and Unmack, P. 2005. Phylogeny and Biogeography of the Eleotrid Genus *Hypseleotris* (Teleostei: Gobioidei: Eleotridae), With Redescription of *H. cyprinoides*. Records of the Australian Museum 57(1): 1-13.

[4] Unmack, P. J. 2000. The Genus *Hypseleotris* of Southeastern Australia: its identification and breeding biology. Journal of the Australia New Guinea Fishes ssociation, 14:1.

[5] A.B. De Guzman, W.H. Uy, J.G. Gorospe, A.E. Openiano, R.E. Acuña, R.L. Roa, J.P. Garcia, M.M. Ologuin and J.R. Santamina. Sustainable Fisheries Management Program for Lake Mainit. Phase II: Comprehensive Resource Assessment. Final Report. MSU at Naawan Foundation for Sciene and Technology Development, Inc. Funded by the International Fund for Agricultural Development- Northern Mindanao Community Initiatives in Resource Management (IFAD-NMCIREMP), Department of Agrarian Reform 13, Lake Mainit Development Alliance (LMDA) and PCAMRDDOST. 73p., 2009.

[6] Rohlf, F.J. and L.F.,Marcus. (1993). A revolution in morphometrics. Trends in Ecology and Evolution, 8:129–132.

[7] D.C. Adams. Methods for shape analysis of landmark data from articulated structures. Evolutionary Ecology Research, 1:959-970, 1999.

[8] M.J. Cavalcanti, L.R. Monteiro and P.R. Duarte Lopes. Landmark based morphometric analysis in selected species of serranid fishes (Perciformes: Teleostei). *Zoological Studies* 38(3): 287-294, 1999.

[9] M.E. Douglas, M.R.., Douglas, J. Lynch, D.M. McElroy. Use of Geometric Morphometrics to Differentiate Gila (Cyprinidae) within the Upper Colorado River Basin. Copeia 2: 389-400, 2001.

[10] Richtsmeier, J.T., Deleon, V.B., and Lele, S. (2002). The Promise of Geometric Morphometric Yearbook of Physical Anthropology, 45:63-91.

[11] Mitteroecker, P and Gunz, P. (2009). Advances in Geometric Morphometrics. Evolutionary Biology, 36:235-247.

[12] Haas, T., Blum, M., and Heins, D. (2010). Morphological responses of a stream fish to water impoundment. Biology Letters. doi: 10.1098/rsbl.2010.0401.

[13] Hossain, M., Nahiduzzaman, Md., Saha, D., Khanam, H., Alam, Md. (2010). Landmark-Based Morphometric and Meristic Variations of the Endangered Carp, *Labeo Calbasu,* from Stocks of Two Isolated Rivers, the Jamuna and Halda, and a Hatchery. Zoological Studies 49(4): 556-563.

[14] Nacua, S., Dorado, E., Torres, M.A., and Demayo, C.G.(2010). Body Shape Variation Between Two Populations of the White Goby, *Glossogobius giuris* (Hamilton and Buchanan). Research Journal of Fisheries and Hydrobiology, 5(1): 44-51.

[15] Elmer, K., Kusche, H., Lehtonen, T and Meyer, A. (2010). Local variation and parallel evolution: morphological and genetic diversity across a species complex of neotropical crater lake cichlid fishes. *Philosophical Transactions, R. Soc. B.,* 365, 1763-1782.

[16] Matondo, D., Torres, M.A., Tabugo, S.R., and Demayo, C. (2010). Describing variations in scales between sexes of the yellowstriped goatfish,*Upeneus vittatus* (Forsskål, 1775) (Perciformes: Mullidae). Egypt. Acad. J. biolog. Sci., 2 (1): 37-50

[17] Webster, M., Atton, N., Hart, P. and Ward, A. (2011). Habitat-specific Morphological variation among Threespine Sticklebacks (*Gasterosteus aculeatus*) within a Drainage Basin. PLoS ONE 6(6): e21060. Doi: 10.1371/journal.pone.0021060.

[18] Tumanda, M., Roa, E., Gorospe, J., Daitia, M., Dejarme, S., and Gaid, R. (2004). Limnological and water quality assessment of lake Mainit. Lake Mainit Project Terminal Report.PCAMRD.

[19] Rohlf, F. J. (2001). TPSDIG2: A program for landmark development and analysis. See http://life.bio.sunysb.edu/morph/.

[20] Rohlf FJ, Slice D (1990) Extensions of the Procustes method for the optimal superimpositions of landmarks.Syst Zool 39(1):40–59

[21] I.L. Dryden and K.V. Mardia. Statistical Shape Analysis. New York: John Wiley and Sons, 1998.

[22] Hammer, Ø., D.A.T. Harper and Ryan, P. D. (2001). PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica. 4(1). 9pp.http://palaeo-electronica.org/2001_1/past/issue1_01.htm.

[23] F. Bookstein. Morphometric tools for landmark data: geometryand biology. Cambridge: Cambridge University Press, 1991.

[24] S.A. Binning and L.J. Chapman. Is intraspecific variation in diet and Morphology related to environmental gradients? Exploring Liem's paradox in a cichlid fish. Integr. Zoology. Sep;5(3) 241-255, 2010.

[25] Wiley, T., Simpfendorfer, C., Faria, V. and Mcdavitt, M. (2008). Range, sexual dimorphism and bilateral asymmetry of rostral tooth counts in the smalltooth sawfish *Pristis pectinata* Latham (Chondrichthyes: Pristidae) of the southeastern United States. Zootaxa, 1810: 51- 59.

[26] Gunawickrama, K.B. S. (2008). Intraspecific variation in morphology and sexual dimorphism in Puntius singhala (Teleostei: Cyprinidae). Cey.J.Sci.(Bio.Sci.) 37(2): 167-175.