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Variations in body shapes among various populations of Spotted Barbs (*Puntius binotatus*) in the Rivers of Bayog, Zamboanga del Sur

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Abstract

Puntius binotatus is a native fish species in Bayog, Zambaonga del Sur, Philippines. It is locally know as ”paitan or pait-pait”. Exposure to various environmental conditions, fishes are capable of making adaptations to enable them to survive. One of the manifestations of environmental conditions is their body shape morphology. It is a and indication of what kind of environment that the fishes are living in or the habit that they have. The environment is indeed known as a major force in modelling the morphology of an organism during ontogeny. This study aims to determine the body shape variation within and between the three populations (Depore River, Dipili River and Sibugay River) of Puntius binotatus, using landmark-based geometric morphometrics. A total of 102 specimens were collected and digitized, 34 for each river (Depore, Dipili and Sibugay river) with 17 males and 17 females. Twenty landmark points were used to digitized the specimens where relative warp scores were derived. PCA, DFA and Thin Plate Spline plot showed that the three populations display sexual dimorphism. Morphology between populations also exhibit significant difference, with Depore and Dipili river spotted barbs having slimmer body and stretched head aspect ratio. These morphological characteristics suggest a more active habit or a habitat with fast water flows.

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Introduction

The spotted barb (*Puntius binotatus)* is an indigenous fish species in Asia that belongs to the Family Cyprinidae, (Lim *et al.,* 2014). This fish is synonymous with *Barbus binotatus, Systomus binotatus, Barbus maculatus, Barbus oresigenes, Barbus blitonensis*, *Barbus kusanensis, Barbus polyspilos*, *Systomus goniosoma, Barbus palavanensis, Barbus quinquemaculatus, Barbus ivis*, *Barbus maculatus hagenii* and *Puntius sibukensis* (Roberts, 1989; Robins, 1991; Roberts, 1993; Pethiyagoda *et al.,* 2012; Jordan and Richardson, 1908; Doi *et al.,* 2001). Usually, it can easily be found in the mountain streams, rivers and lakes, and is omnivorous, that feeds on zooplanktons, insect larvae and some vascular plants (Rainboth, 1996). *Puntius binotatus* is commonly known as paitan or pait-pait in the Philippines, particularly in Mindanao.

Exposure to different environmental conditions, all organisms, like fishes, are dexterous of making adaptations to enable them to survive. Indeed, the environment is known as a major force in modelling the morphology of an organism during ontogeny (Costa and Cataudella 2007). Evidence showed that body shape is a reliable indicator of the swimming behaviour and habitat choice of fishes (Webb 1982). Therefore, body shape is not only a reflection of its genotype, but its environment and its habit as well (Guill *et al.,* 2003). Study showed that active swimming fish and fish that live in fast moving waterways have fusiform shaped bodies (Barlow 1961), while fish that lives in static waters frequently have more compressed and deeper bodies (Ostrand *et al.,* 2001).

In Bayog, Zamboanga del Sur, Mindanao, Philippines, where the study was conducted, *P. binotatus* is a native species and considered an important fish in the major rivers. It contributes a large number of fishes caught by fishermen in everyday basis. However, its commercial value is lesser unlike tilapia, carp, catfish, etc. because of its abundance and size that could only reach to 90-110 mm (Yagos, 2021). The area is gifted with several rivers. Sibugay river is one of it, and it is known to its depth and wide area with a static flow of water and while Depore and Dipili rivers are more likely opposite to the Sibugay River with a shallow and fast flowing water. As such different physical characteristics, the three populations of *P. binotatus* living in these areas may be exposed to opposing habitat conditions and may possess local adaptation in morphological variations that separates them from other populations of the same species.

The major objective of this study is to compare the body shape variation within and between the three populations of *P. binotatus* from three rivers in Bayog, Zamboanga del Sur, using landmark-based geometric morphometric.

Materials and methods

Study area

The study was conducted in the three major rivers in Bayog, Zamboanga del Sur, Mindanao, Philippines, the Depore River, Dipili River and Sibugay River. Sibugay River which has a deeper bottom and wide area with a static flow of water while the Depore River and Dipili River have a shallow and fast-moving water.

(Source: Googlemap)

Fig. 1. Shows the locations of the study areas in the Municipality of Bayog. In set is the map of the Philippines and map of Zamboanga del Sur.

Sample Collection

A total of one hundred two *P. binotatus* (17 per sex per population) were sampled from three major rivers in Bayog, Zamboanga del sur. Sexually matured fishes were chosen to minimize the intrapopulation variations in the body shape based on ontogeny. The samples were transported in styropore box and processed immediately. Sexes of samples were determined based on external morphology and was validated by the examination of the gonads (Dorado *et al.,* 2012).

Data Acquisition

To show its points of origin, the samples were then flanked on a plain Styrofoam and pinned its fins. It was then photographed with a measuring tape for the basis of its size, capturing the left side of its body using a 12.2 megapixel Cannon 450D DSLR camera. The images were processed for the landmarking of the body shape and further statistical analysis.

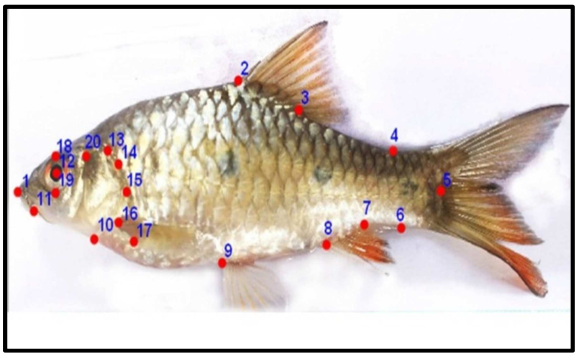

Fig. 2. Landmarks' descriptions of the spotted barb, *P. binotatus* (A. female, B. male): (1) snout tip; (2) and (3) anterior and posterior insertion of the dorsal fin; (4) and (6) points of maximum curvature of the peduncle; (5) posterior body extremity; (7) and (8) posterior and anterior insertion of the anal fin; (9) insertion of the pelvic fin; (10) insertion of the operculum at the lateral profile; (11) posterior extremity of premaxillar; (12) centre of the eye; (13) superior insertion of operculum; (14) beginning of the lateral line; (15) point of maximum extension of operculum on the lateral profile; (16) and (17) superior and inferior insertion of the pectoral fin; (18) and (19) superior and inferior margin of the eye; (20) superior margin of the pre-operculum.

Morphological Analysis

A total of twenty landmarks were selected to provide a comprehensive summary of the morphology of the fish (Fig. 2). The landmarks digitized in this study are standard locations used in fish morphometrics and are claimed to have evolutionary as well as functional importance (Turan 1999; Turan *et al.,* 2004; Costa & Cataudella 2007; Buitrago-Suarez & Brooks 2007; Vasconcellos *et al.,* 2008). The TpsDig ver. 2.10 software were used to digitize the landmarks (Rohlf 2006).

The X and Y coordinates of the landmark points that were digitized from the left image of the samples contain both shape and non-shape (e.g. differences in the position, orientation, and size) components of variation (Adams 1999; Kasam *et al.,* 2004). The focus of this study was solely on shape differences, the non-shape components had to be deleted prior to analysis using Generalized Procrustes Analysis (GPA) in TpsRelw 1.45 software (Rohlf 2003). GPA aligned all of the specimens in morpho-space, removing disparities in size and rotation/translation.

The weight matrix from the aligned specimens, encompassing uniform and non-uniform shape components, was constructed using the thin-plate spline equation and the usual formula for uniform shape components (Bookstein 1991). The resulting partial scores of the images were subjected to relative warp analysis using the TpsRelw version 1.45 (Rohlf 2003), which used to quantify the body shape of the samples. It also maps the landmarks in a two-dimensional morpho-space warp grid, with variations represented by grid deformations. Images from the three groups were separated into male and female to generate the consensus configuration of each sex for comparison between the sexes. The photos of the two sexes were combined in one analysis to assess the shape variability of each group.

Relative warp scores of the sexes from the three populations were subjected to Discriminant Function Analysis (DFA) and Canonical Variance Analysis to determine if shape varied significantly among and between populations. The partial warp scores were treated as independent variables in DFA, and a multivariate function was created to differentiate males and females to the greatest extent possible.

The software tpsSpline (version 1.20) was used to have a visualization in the variation between compared shapes. The Procrustes distance (d2) is the standard measure for the size of shape deviations used in geometric morphometrics, and it is utilized in this comparison (Bookstein, 1996). Because Procrustes distance is an absolute measure of the degree of shape difference between different configurations, it is unaffected by factors such as variation within samples.

Results and discussion

Sexual Dimorphism Within the Three Populations

Variation in body shape of a population can be explained by sexual dimorphism. Fig. 3 shows the principal component analaysis and Discriminant function analysis plots of the relative warp scores of *Puntius binotatus* populations from Depore River, Dipili River and Sibugay River. As shown in fig. 3, all populations exhibit sexual dimorphism as conformed by PCA and DFA with p-values lesser than 0.05 (Table 1). Separation of sexes in each of the populations in the PCA and DFA plot shows significant differences. The PCA plots within population explain the variation existing between the two sexes and could explain for almost 100% of the variance. The Discriminant Function Analysis further emphasizes the variation within population as shown by the overlapping of some of the morphological attributes. Fig. 4 further showed a clearer distinction of shape between sexes of Sibugay River population of *P. Binotaus* with d^2 = 0.03025 compared to Dipili River and Depore River with d^2 = 0.01947 and d^2 = 0.01512 respectively.

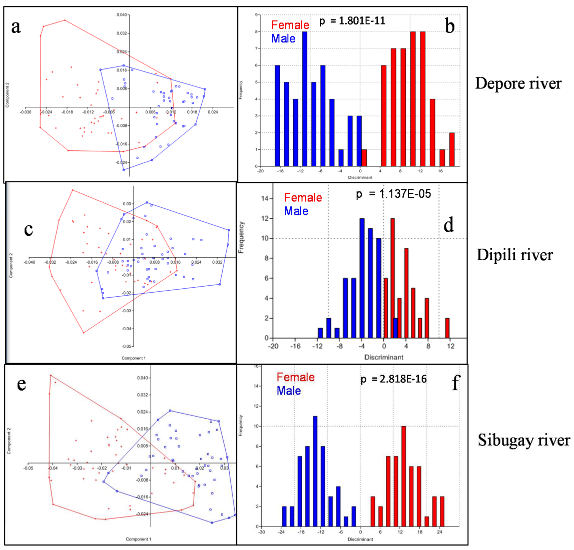

Fig. 3. Principal Component Analysis (PCA) (a, c, e) and Discriminant Function Analysis (DFA) (b, d, f) plots of the relative warp scores of *Puntius binotatus* populations from (a,b) Depore River, (c,d) Dipili River, (e,f) Sibugay River.

Table 1. Summary of the DFA results within the three populations of *Puntius binotatus*.

	P-Value	Correctly Classified (%)
Depore	1.801E-11	97.78%
Dipili	1.137E-05	100%
Sibugay	2.818E-16	100%

Mean shapes using relative warp analysis show sexual dimorphism in the body shapes of *P. binotatus*. Fig. 4 showed the body shape difference between sexes for the three populations, with the females having bigger head and a deeper body depth. The relative warp scores (RW) further reveal that females display greater curvature of the body than the males. This could be the consequence of their reproductive role resulted to the females' bulkier bellies. Study of Monet *et al.* (2006) observed similar body curvature in the body shapes of trout *Salmo trutta* but connected to size and independent of genetic and sexual dimorphism. .

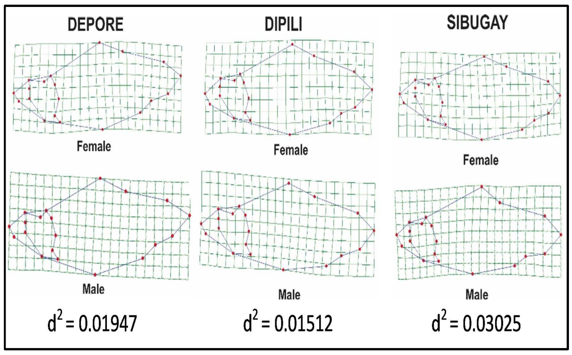

Fig. 4. Thin Plate Spline plot of the three populations with their Procrustes distance values.

Insertion of the pelvic fin in the abdominal part of male is shallower that would create a streamlined-like body shape for males. Male also have wider and deeper posterior insertion of the anal fin that could increase success in male-male competition or likely to be chosen by female observed in the genus *Petrotilapia* (Kassam *et al.,* 2004). Larger fin base shall aid the stability and control during swimming. It may also help males to position themselves properly relative to females during spawning in order to maximize fertilization success (Casselman & Schulte-Hostedde 2004). In the other hand, females have shallower posterior insertion of the anal fins that would aid for easy access of male during mating.

This observed sexual dimorphism in *P. binotatus* is in concordance with the study of Dorado *et al.* (2012) about the sexual dimorphism in the body shape of spotted barb of lake Buluan, Mindanao, Philippines with the females showing larger heads and deeper bodies. Sexual selection somehow explains sexual dimorphism, as mostly these traits would mean superior character which would be favored by the opposite sex. These traits become very evident especially in the spawning season, as in Salmon for instance (Wilson, 1997). However, one study suggests that sexual dimorphism might be an adaptation for different habits of the opposing sexes (Spoljaric and Reimchen, 2008).

Difference in the reproductive role is one determinant of sexual dimorphism that would influence patterns of selection and eventually lead to the variation in morphology. Males should acquire adaptations that favor them in acquiring mates and excel in male-male competition. Females should be under selection to acquire process and store energy to facilitate the production of offspring (Casselman & Schulte-Hostedde, 2004).

Body Shape Differences Between the Three Populations

Table 5 showed the results of the Canonical Variate Analysis (CVA) plots (fig. 5a and 5c) and DFA plots (fig. 5b and 5d) of the relative warp scores of females (5a, 5b) and males (5c, 5d) between the three populations. Body shapes between males and females from the three populations showed significant differences through the separation of each of the population in CVA plots. These plots explain the variation existing within sexes and explained for more than 90% of the variance. Furthermore, Discriminant Function analysis emphasizes the difference between populations as shown by the slight overlapping of some morphological attributes as shown in fig. 5.

Additionally, p-values from MANOVA and DFA, as summarized in table 2 and 3, further highlights that there are differences in the morphology between populations as the p-value is less than 0.05.

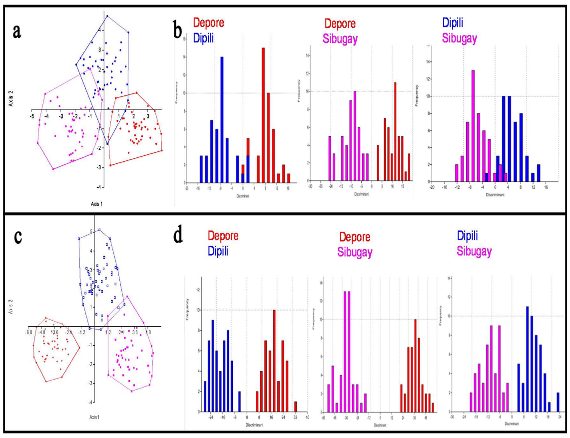

Fig. 5. Canonical Variate Analysis (CVA) (a, c) and Discriminant Function Analysis (DFA) (b, d) plots of the relative warp scores of *Puntius binotatus* females (a, b) and males (c, d).

Table 2. Summary of the MANOVA results for *Puntius binotatus* females and males between the three populations.

	Females	Males
Wilks' lambda	0.08569	0.02771
Pillai trace	1.4	1.641
P-Values	7.837E-27; 2.607E-26	8.504E-53; 3.754E-50
Eigenvalue 1	3.27	7.919
Eigenvalue2	1.733	3.045

Table 3. Summary of the DFA results for *Puntius binotatus* females and males between the three populations.

	Females		Males	
	P-Value	Correctly Classified (%)	P-Value	Correctly Classified (%)
Depore Vs. Dipili	1.714E-09	94.44 %	3.327E-19	100%
Depore Vs. Sibugay	2.353E-13	100%	1.498E-25	100%
Dipili Vs. Sibugay	1.433E-08	95.7%	1.485E-15	100%

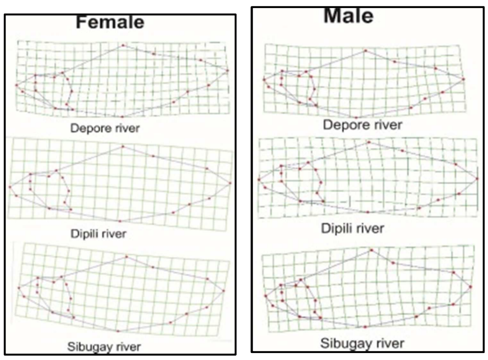

Fig. 6. Thin Plate Spline plot of the males and females based on their Procrustes distances.

Table 4. Summary of the results of Thin Plate Spline between population based on Procrustes distance.

	Females	Males
	d^2	d^2
Depore Vs. Dipili	0.03072	0.03635
Depore Vs. Sibugay	0.02936	0.04274
Dipili Vs. Sibugay	0.02128	0.03297

Thin Plate Spline plot and their consensus configuration further showed the differences within sexes of the three populations (Fig. 6) by taking its Procrustes distance. Table 4 summarizes the results of Thin Plate Spline based on Procrustes distance. It further showed that in the female populations, Depore versus Dipili has the highest procrustes value (d^2 = 0.03072) followed by Depore versus Sibugay (d^2 = 0.02936) and Dipili versus Sibugay (d^2 =0.02128).

Depore female population shows a semi-streamlined body, more compressed head from the insertion of the operculum at the lateral profile to the superior and inferior insertion of the pectoral fin, stretched maximum curvature of the peduncle and posterior body extremity and deeper posterior insertion of the anal fin compared to the two populations, while the Sibugay River population exhibit deeper body and compressed maximum curvature of the peduncle and posterior body extremity. In the male population, Dipili River population exhibits a streamlined body, shallow posterior insertion of the dorsal fin, expanded head from snout tip, posterior extremity of premaxillar to superiorand inferior margin of the eye, a little shallow posterior insertion of the anal fin and compressed maximum curvature of the peduncle and posterior body extremity. Sibugay River population also exhibit compressed maximum curvature of the peduncle and posterior body extremity, but its head is compressed and has a deeper posterior insertion of the dorsal fin and posterior insertion of the anal fin. Its head is more compressed from snout tip, posterior extremity of premaxillar to beginning of the lateral line and point of maximum extension of operculum on the lateral profile. In the other hand, Depore river population has also its different attributes. Its head is expanded from snout tip, posterior extremity of premaxillar to beginning of the lateral line and point of maximum extension of operculum on the lateral profile but compressed from superior margin of the eye and superior margin of the pre-operculum towards insertion of the operculum at the lateral profile and inferior insertion of the pectoral fin.

In nature, phenotypic variation among organisms may be considered as important only if these morphological differences increased the fitness among the other different groups. In an aquatic medium it means subscribing to the demands of hydrodynamic forces to conserve energy while maintaining its preferred behaviour (Nacua *et al.,* 2012).

Morphology of fishes reflect its environment or habit as it will have to conform to this. It can be predicted if it is living in an environment of strong water flows or with a habit of swimming continuously. The slimmer body shape of Depore River and Dipili River spotted barbs suggest that it has a more active habit of swimming continuously or an environment of strong water flow. Fishes living in this kind of habitat develop a streamlined body shape with lengthened body and stretched tail area (Spoljaric & Reimchen 2008, Burns *et al.,* 2009). Fish populations that were geographically isolated are likely to have morphological variations as fishes are capable to adapt by altering essential morphometrics (Hossain *et al.*, 2010). Study of Elmer *et al.* (2010) showed the non-overlapping morphospace of cichlids species. Midas cichlids inhabiting different lakes possess their own morphological characteristics that are significantly varied from the others and eminent as different species. These results showed the adaptations to local environment and influence of environmental factors.

Conclusion

The variations that were being observed between the three populations were the depth of the body, shape of head and length and positioning of the fins. Within population, this variability is attributed by sexual dimorphism and can be explained by differences of habits or reproductive role with the slimmer body of the males being adapted to a more active swimming habit that might be caused by male-to-male completion for mating. Generally, differences in morphology between the three populations is evident with Depore River and Dipili River spotted barbs exhibit slimmer bodies and stretched head ratio portion compared to Sibugay River population. These might explain their habits or the habitat they are living in.

Differences in morphology observed within population and between populations can be attributed to the habits and habitat of the fish species (Dynes et. al 1999). Body shape and morphological variations can be greatly affected by environmental conditions and adaptations. Additionally, this variation might also being influenced by environmental and physical pressure such as overfishing and pollution. Studying the biological attributes of this species is essential in biodiversity studies and proper management of fishery.

Recommendation

Environmental factors may contribute a large portion in modeling the morphology of an organism such as fishes, thus it is essential to have a further study that would determine the other factors that contribute to the variation of the morphological characteristics of *P. binotatus* in Bayog, Zamboanga del Sur.

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