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Application of geometric morphometrics in the body shapes of flying fish (*Parexocoetus brachypterus*) in Maitum, Sarangani Province

[1]Edzelle O. Digo, [1]Kirstin Lyza M. Abad, [1]Ivy Joy B. Guino-o, [1]Lynette Kryss C. Samillano, [2]Ramon M. Eduque Jr., [3]Mark Anthony J. Torres, [2]Elani A. Requieron

[1]Science Department, College of Education, Mindanao State University – General Santos City9500, General Santos City, Philippines;
[2]Science Department, College of Natural Sciences and Mathematics, Mindanao State University – General Santos City9500, General Santos City, Philippines;
[3]Department of Biological Sciences, College of Science and Mathematics, Mindanao State University – Iligan Institute of Technology 9200, Iligan City, Philippines.
Corresponding author: E. O. Digo, edigo36@gmail.com

Abstract. Environmental factors contribute to the phenotype of organisms like flying fish (*Parexocoetus brachypterus*). Extreme environmental conditions along with anthropogenic factors such as pollution can put on pressures to organisms. These organisms may be observed in their morphological aspect. Furthermore, this study was conducted to describe the body shapes within the flying fish (*Parexocoetus brachypterus*) species as well as to determine significant differences in their morphological aspect as affected by environmental pressures. Sixteen landmarks from images of 60 individuals (30 per sex) were subjected to Relative Warp (RW) Analysis, Multivariate Analysis of Variance (MANOVA), and Discriminant Function Analysis (DFA) using Geometric Morphometrics. Results revealed no significant difference between the male and the female *P. brachypterus*. Morphological Variations within sexes are attributed to effects of environmental pressures.

Key Words: Family Exocoetidae, body formation, morphological variations, environmental aspects, landmarks.

Introduction. Morphometric is the study of any quantitative measurement and analysis of morphological traits affecting on it. One of the advances in the field of morphometrics today is the Geometric Morphometric, also known as GM. In this sense, Geometric Morphometric could be defined as the study of form in two or three dimensional spaces (Bookstein 1982) allowing in-depth investigation of morphological change (Talu & Giovanzana 2012; Solon et al 2012; Albutra et al 2012ab). In this new approach, the actual shape of the specimen is preserved allowing more precise statistical analysis of specimens' stage (Torres et al 2011; Moneva et al 2014; Camama et al 2014; Eagderi et al 2015).

Morphological variation within the species in intraspecific is primarily caused by environmental factors (Talu et al 2012). Environmental-caused variations among individuals of the same species differ and depend on the individuals' ability to buffer the problems in the environment.

For instance, *Parexocoetus brachypterus* is one of the endemic fish species found in Sarangani Bay (specifically located in Maitum, Sarangani Province, Mindanao, Philippines; Figure 1). It belongs to Class Actinopterygii, Order Belonifomes and Family Exocoetidae. Due to its advantageous characteristics and high adaptability in any environmental conditions, the researchers use the *Parexocoetus brachypterus* species as an ideal subject for the study. The researchers intend to determine intraspecific morphological variations in *Parexocoetus brachypterus* and to determine the possible body shape dimorphism among different sexes of the species.

Material and Method

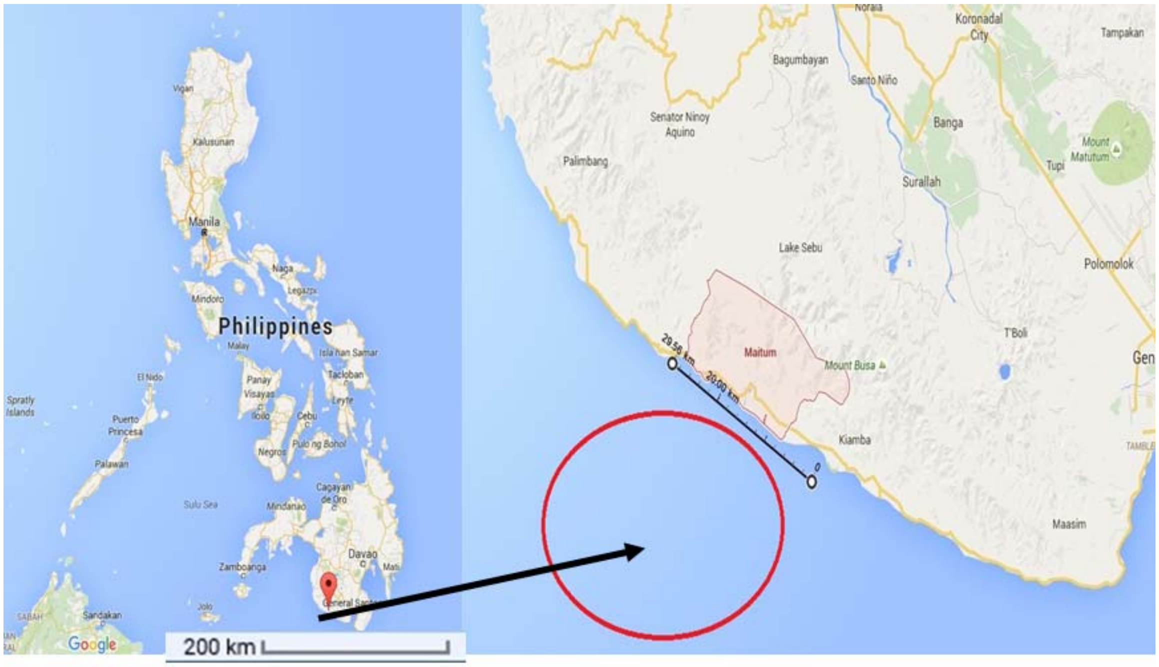

Figure 1. Map showing the study area in Maitum, Sarangani Province were the red circle indicates the area the specimen taken (Google Map 2015).

A. Fish specimens

A total of 100 individuals (60 males and 40 females) were caught from Maitum, Sarangani Province as a sampling area through traditional fish-netting technique to make sure that we can have at least 30 samples per sex. The samples were placed in a Styrofoam box with ice to preserve its freshness and were processed eventually. Males and females were identified through direct examination of the gonads. Samsung ST66 digital camera was used to take digital images of the specimen of high quality. Females had yellowish coarsely textured gonads with eggs while male fishes had whitish soft textured gonads. Sexes were later confirmed by the direct inspection of the gonads. Only the left side of the fish samples was used in the analysis (see Figure 2).

B. Landmark selection and digitization

To trace the morphology of the fishes, a total of sixteen landmarks (equivalent 16 X and 16 Y Cartesian coordinates) were used. Standard points were used in fish morphometrics which are designated due to their evolutionary and functional significances in digitizing the landmarks. The software used in digitizing fish samples was TpsDig ver. 2.1225 (Rohlf 1993).

C. Shape analysis

Since the focus of the study is shape differences, other non-shape differences are eliminated during analysis through Generalized Procrustes Analysis (GPA) using TpsRelw ver. 1.5326. All specimens in morpho-space, removing size and rotational/translational differences were aligned using GPA. The variability in body shapes was showed using the relative warp scores. To assess the morphological differences of the *P. brachypterus* species, the said scores were subjected to Multivariate Analysis of Variance (MANOVA) and Discriminant Function Analysis (DFA) using the Paleontological Statistics or PAST software ver. 1.27.

Figure 2. A male flying fish, *Parexocoetus brachypterus*, showing the 16 landmarks used in the study. Landmark's description: (1) rostral tip of premaxilla, (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 of caudal border of hypural plate, (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 maxilla, (13) anterior margin through midline of orbit, (14) posterior margin through midline of orbit, (15) dorsal end of opercle, (16) dorsal base of pectoral fin.

Results and Discussion. The Table 1 shows the total variation of relative warps in male population is 85%. In RW1 yields 33.95% illustrating the head region bends upward or downward while the caudal region constricts; RW2 yields 18.93% illustrating variations in the head and caudal region; RW3 yields 14.24% illustrating deflection in the head, body, and caudal region; RW4 yields 7.26% illustrating the variations between the head, body, and caudal region; RW5 yields 5.84% illustrating the constriction in the head, body, and caudal region; and RW6 yields 4.78% illustrating the widening and deflecting in the head, body, and caudal region.

On the other hand, the total variation of relative warps in female population is 84.31%. In RW1 yields 43.61% illustrating the curvature and constriction in the head, abdominal, and caudal region; RW2 yields 14.43% illustrating the variations in the head, body, and caudal region; RW3 yields 13.67% illustrating the bending and contraction in the head, body, and caudal region; RW4 yields 7.09% illustrating the variation between the head and caudal region; and RW5 yields illustrating the deflection in the head and caudal region.

Furthermore, the Figure 3 summarizes the relative warps and variations in the body shapes between the males and females of flying fish, *P. brachypterus*, through frequency from the negative and positive extreme compared to the mean shape.

Then, the body shape variations within flying fish (*Parexoceotus brachypterus*) species were proven by the quantitative data gathered in Multivariate Analysis of Variance (MANOVA). It shows significant differences between sexes that was determined by the p value (p=0.9515) which is greater than 0.05 level of significance (p>0.05). Further, other multivariate test methods also revealed significant results such as Pillai Trace = 0.02894, Wilk's Lambda = 0.9711 and Eigenvalues = 0.0298 and 3.024E-16.

Moreover, the Discriminant Function Analysis (DFA) graph in Figure 4 summarized the extent of variation between the two male and female *P. brachypterus* species. This manifests the sexual dimorphism between the sexes. Differences in the body shape of the sexes vary in the functions. Since *P. brachypterus* is a carnivorous fish, its head region is considerably big specifically the snout. Females tend to have heavier weights than males. The variations in the head, body, and caudal region observed in this study are believed to support their balance and maneuverability in the water. As what we can see in the DFA, there are overlapping bars which imply slight similarities between their shape variations. However, although there have been similarities in their body shapes, there are still relative amount of variance present within the sexes that distinguishes them. Most female samples possess bigger body if they are compared to males. Sexual dimorphism indicates the reproductive role which influences the morphological structure of the species, males need adaptation to compete with their mate while females adapt to produce offsprings.

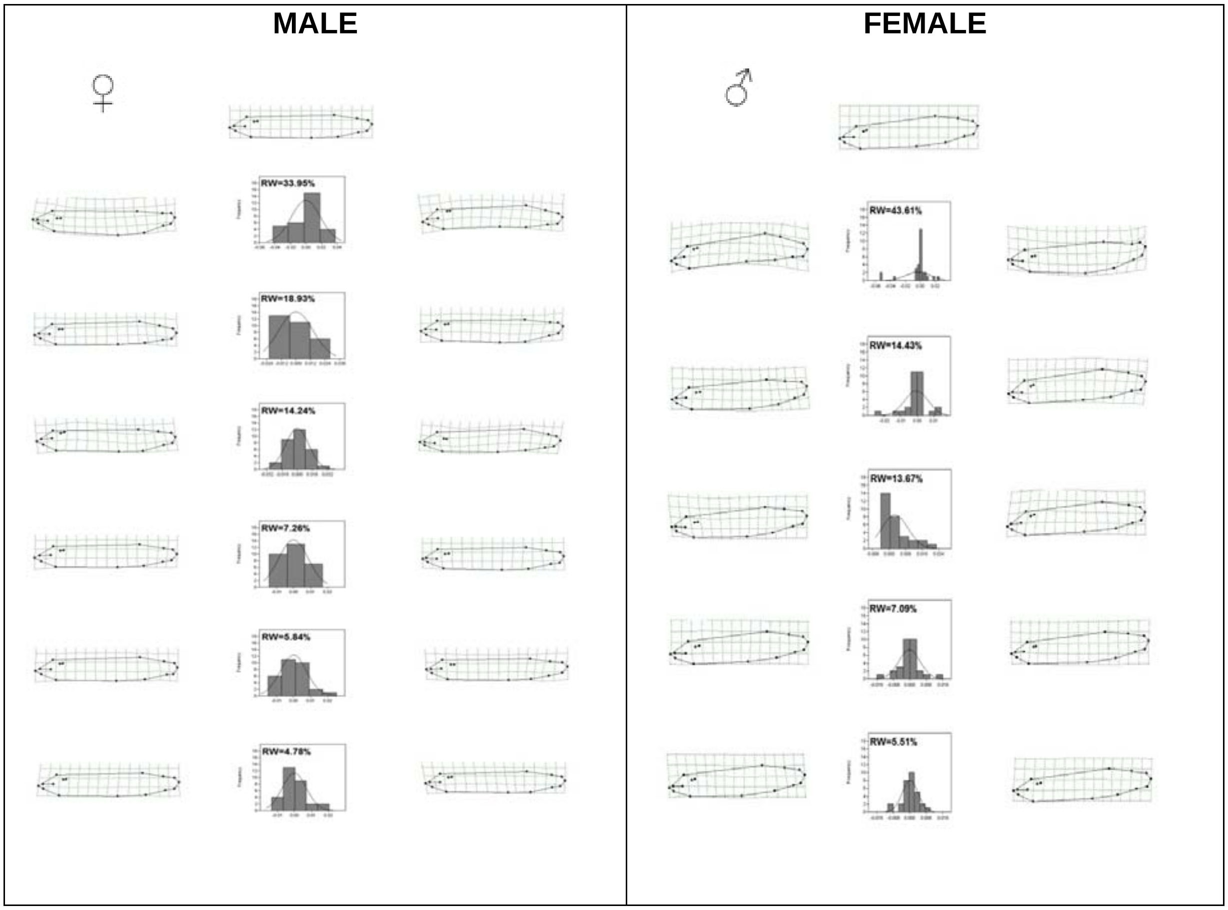

Figure 3. Summary of the geometric morphometrics in male and female *Parexocoetus brachypterus* species showing the relative warps and the variation in body shapes from negative extreme (left) towards positive extreme (right) as compared to the mean shape (top).

The flying fish (Family Exocoetidae) is a group of small fish comprising 52 species and is distributed globally in the tropical and subtropical areas of three oceans (Nelson 2006; Chang et al 2012; Xu et al 2012). Exocoetids are low-tropical level species (Wu et al 2010) and food source for dolphin fish, tuna, and swordfish (Wu et al 2006). They are known as flying fish due to their ability to glide in the air over the water using large and elongated pectoral and asymmetric caudal fins (Davenport 1994). This uncommon flying behavior serves as an escape mechanism from predators and is uniquely present in extant fish species of the (Family Exocoetidae) group as a homoplasy of the extinct family Thoracopteridae (Kutschera 2005; Xu et al 2012).

The flying fish is economically important in the Caribbean and the western Pacific Ocean (Rennie 2002; Potts et al 2003). It is the national fish and one of the national cuisines of Barbados (Cumberbatch & Hinds 2013). In Taiwan, it supports the stability of the Kuroshio ecosystem as an intermediary in the bioenergetics transfer, the sustainability of artisanal fisheries as a locally consumed dish and major bait for fishing, and the continuity of aboriginal Tao culture as a respectable emblem (Chang et al 2012).

Table 1

Variations in the body shapes between the male and female of the flying fish (*Parexocoetus brachypterus*)

	Variation	Male	Variation	Female
RW1	33.95%	There are variations in the head and caudal region of the body. In the negative extreme, there is bending upward in the head region specifically in the lower jaw. It can also observe that there is contraction in the caudal part of the body region especially in the dorsal and ventral insertion of caudal fin and posterior insertion of anal fin. On the other hand, in the positive extreme, the head and caudal region of the body express bending downward. In addition, the posterior end of nuchal spine and the anterior insertion of dorsal fin express broadening of those parts.	43.61%	There are variations in the head, abdominal, and caudal region. In negative extreme, the head region moves downward specifically in the mouth. In addition, the dorsal base and anterior insertion of pelvic fin are slightly deflected while the caudal region expresses bending downward primarily in the anterior and posterior insertion of dorsal fin and dorsal insertion of caudal fin. On the other hand, positive extreme has curvature and deflection in the head, abdominal, and caudal region.
RW2	18.93%	There are variations in the head and caudal region of the body. In the negative extreme, the caudal region bends downward specifically in the dorsal and ventral insertion of caudal fin and posterior insertion of anal fin. On the other hand, in positive extreme, there is a constriction of the head region especially in the mouth while the latter bends upward primarily in ventral insertion of caudal fin and posterior insertion of anal fin.	14.43%	There are variations in the head, body, and caudal region. In negative extreme, there is a constriction of the head and caudal region. While in positive extreme, the head region has deflected. In addition, the posterior end of nuchal spine and anterior insertion of dorsal fin has expanded while the latter has deflected and moves a little downward.
RW3	14.24%	There are variations in the head, body, and caudal region. In negative extreme, there is a constriction head and caudal region while the body region is slightly deflected. On the other hand, in positive extreme, the head region is slightly bending upward while the caudal region is bending downward. In addition, the body also expresses deflection.	13.67%	There are variations in the head, body, and caudal region. In negative extreme, the head region moves upward primarily in the mouth. In addition, there is broadening in the posterior end of nuchal spine and anterior insertion of dorsal fin while the caudal region is constricted. On the other hand, in positive extreme, the head region specifically in the mouth bends downward while the caudal region has been constricted.
RW4	7.26%	There are variations in the head and caudal region of the body. In negative extreme, the head region moves downward while the caudal region contracts. In contrast, positive extreme has a curve specifically in the jaw while the latter constricts.	7.09%	There are variations in the head and caudal region. In negative extreme, the head region specifically in the jaw moves upward while the caudal region is deflected. In contrast, positive extreme's head region bends downward while there is a constriction primarily in the dorsal and ventral insertion of caudal fin and posterior insertion of anal fin.
RW5	5.84%	There are variations in the head, body, and caudal region. In negative extreme, the head region moves slightly downward specifically in the mouth while the body and caudal region are deflected. While in positive extreme, the head, body, and caudal region are shrinking.	5.51%	There are variations in the head and caudal region. In negative extreme, the head region bends downward especially in the mouth while there is a contraction in the caudal region specifically in the posterior insertion of dorsal fin and dorsal insertion of caudal fin. While in positive extreme, the head region is deflected while the posterior end of nuchal spine and anterior insertion of dorsal fin have been widen.
RW6	4.78%	There are variation in the head, body, and caudal region. In negative extreme, there is broadening in the posterior end of nuchal spine and anterior insertion of dorsal fin while the body and caudal region are slightly constricted. On the other hand, in positive extreme, it can also observe that there is widening in the posterior end of nuchal spine and anterior insertion of dorsal fin while the body and caudal region are deflected.		
	85%		84.31%	

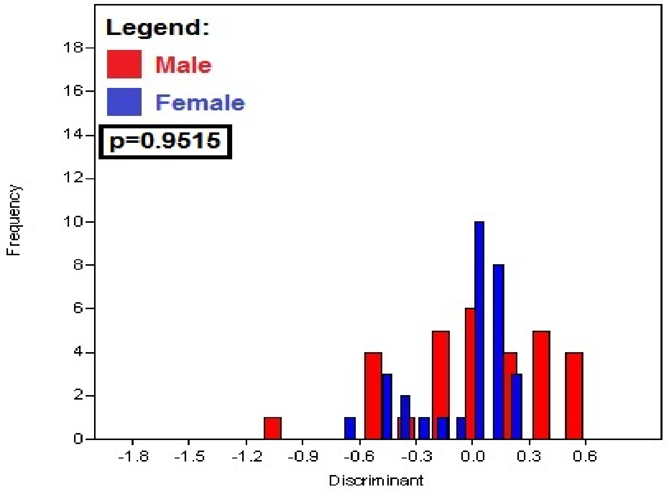

Figure 4. Discriminant Function Analysis (DFA).

At least 29 species of flying fish in seven genera are reportedly found in the waters off Taiwan and adjacent areas (Chang et al 2012). Among these, *Hirundichthys oxycephalus* (bony flying fish) is one of the dominant flying fishes in the Kuroshio Current (Chang et al 2012). The fish can be found from south of Taiwan to northwestern Japan (Ichimaru 2007; Chang et al 2012).

The fish was utilized as the main source of eggs for flying fish egg fisheries in northern Taiwan and the bait of long line and troll fisheries in eastern Taiwan (Chang et al 2012). Juveniles of the fish also constituted an important target of boat seine and set net fisheries in northwestern Japan (Ichimaru 2007). A decline of more than 60% in catches of the fish and its eggs occurred in Taiwan during the 2006 to 2007 period (Fisheries Agency 2010). This decline has caused serious concerns regarding the fish stock status and prompted the Taiwan government to implement a set of regulations on flying fish egg fishery since 2008, including an annual catch limit. The catch limit regulation is precautionary, however, and has no scientific support regarding the optimal fish stock abundance or, more basically, on the boundary of the fish population that is crucial for effective fishery management (Garcia 2005).

The most important physical adaptation of the flying fish is its large pectoral fins. It can swim really fast and then jump out of the water. The flying fish extends its large fins and they act like wings to escape from predators. The flying fish has more adaptations that help it fly. First, its gills can breathe in air. It allows it to survive when it's flying. Also, they have a torpedo shaped body and a really strong tail so it can reach enough velocity to fly (Yumul 2012).

Flying fish are animals that can be found in the subtropical (medium temperatures waters) and tropical waters of the ocean. The subtropical waters are found in the Atlantic, Pacific, and Indian Oceans. You can also find it in the Caribbean Sea as well (Yumul 2012). Occurs in coastal waters, rare in the open ocean, capable of leaping out of the water and gliding for considerable distances above the surface. A number of 3-4 individuals aggregate during breeding (Nakabo 2002).

The flying fish are body elongate, broadly cylindrical. Head short, snout blunt, mouth small. Gill rakers well developed. Dorsal fin with 11-13 soft rays, longest dorsal-fin rays reaching beyond origin of upper caudal-fin lobe; Origin of anal fin slightly before or under origin of dorsal fin, and with 13-14 soft rays; pectoral fins not reaching posterior end of anal fin; pelvic fin short, not extending beyond of anal fin when depressed; caudal fin deeply forked, its lower lobe longer than the upper. Scales large, cycloid, easily shed; predorsal scales 21-25; lateral line with branch at thorax, with 43-50 scales. Body deep blue above, pale below; dorsal fin black except near base; pectoral and caudal fins uniformly transparent (Nakabo 2002).

Conclusion. In this study, the result based on the Discriminant Function Analysis (DFA) that there is no significant difference between the body shapes of the male and female *P. brachypterus* (as shown in the Figure 4) can be used to discriminate the species.

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Authors:
Edzelle O. Digo, Brgy. Lunen, Tupi, South Cotabato, 9505, Mindanao, Philippines, College of Education, Mindanao State University General Santos City, e-mail: edigo36@gmail.com
Kirstin Lyza M. Abad, Block 3, Zone 3, Brgy. Fatima, General Santos City, 9500, Mindanao, Philippines, College of Education, Mindanao State University-General Santos City, e-mail: abadkirstinlyza@gmail.com
Ivy Joy B. Guino-o, Purok 6, Brgy. Poblacion, Tupi, South Cotabato, 9505, Mindanao, Philippines, College of Education, Mindanao State University-General Santos City, e-mail: ivyjoyguinoo@gmail.com
Lynette Kryss C. Samillano, Zone 10, Block 1, Brgy. Fatima, General Santos City, 9500, Mindanao, Philippiines, College of Education, Mindanao State University-General Santos City, e-mail: lynettekrysscorpuzsamillano@yahoo.com
Ramon M. Eduque Jr., Science Department, College of Social Science and Mathematics, Mindanao State University-General Santos, Fatima, General Santos City, 9500, Mindanao, Philippines, e-mail: ramon.eduque@msugensan.edu.ph
Mark Anthony J. Torres, Department of Biological Sciences, College of Science and Mathematics, Mindanao State University-Iligan Institute of Technology, 9200 Iligan City, Mindanao, Philippines, e-mail: markanthonytorres@yahoo.com
Elani A. Requieron, Science Department, College of Social Science and Mathematics, Mindanao State University-General Santos, Fatima, General Santos City, 9500, Mindanao, Philippines, e-mail: elani_requiron2003@yahoo.com

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