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Determining sexual dimorphism in Bigtooth Pomfret, *Brama orcini,* in Tuka Bay, Kiamba, Sarangani Province

[1]Joseph Kennedy O. Cantabaco, [1]Shiela F. Celedio, [1]Czarina Mae B. Gubalani, [1]Rosie J. Sialana, [2]Mark Anthony J. Torres, [1]Elani A. Requieron, [1]Tres Tinna B. Martin

[1]Science Department, College of Natural Sciences and Mathematics; Science Department, College of Education, Mindanao State University – General Santos City 9500, Philippines; [2]Department of Biological Sciences, College of Science and Mathematics, Mindanao State University – Iligan Institute of Technology 9200, Iligan City, Philippines. Corresponding author: J.K. Cantabaco, cjosephkennedy@yahoo.com

Abstract. *Brama orcini* (Cuvier, 1831), the bigtooth pompret, or locally known as "Tabas" is considered as one of the most highly migratory fishes in the world. Despite being abundant in the marine sanctuary of Tuka Bay, Kiamba, Sarangani Province, not much attention is given to this species although it is one of the food fish in the region. *B. orcini* is often subjected to misidentification because of its resemblance to congeneric species in the genus *Brama*: *Brama japonica*, *Brama dussimieri* and *Brama myersi* respectively. This study aimed to identify the morphological variations of body shapes between the male and female *B. orcini* for proper management programs in fishery. In order to identify the possible sexual dimorphism of the said species, 100 samples were collected at Tuka Bay (male=64, female=36). Samples were processed using Geometric Morphometrics or GM. Nikon D200 was used to capture digital images of the species and utilized landmark based GM to further elucidate the images. Result shows that males have slender bodies while females exhibit stout body outlines. The body shape variations within *B. orcini* species were proven by the quantitative data gathered in (MANOVA). The study shows significant differences between sexes (p= 0.04014); Pillai Trace = 0.1893, Wilk's Lambda = 0.8107 and Eigenvalues = 0.2334 and 5.69E-16. In conclusion, female *B. orcini* exhibited a high significant difference in their body shapes than in male species due to thinner stout abdominal structure while males have a more elongated caudal fins.
Key Words: Relative warp, landmark-based, Bramidae, geometric morphometric.

Introduction. Comparing the biological structures and anatomical features of various organisms has long been the focus of many investigations (Natividad et al 2015; Solon et al 2012; Talu & Giovanzana 2012; Angeles et al 2014), especially in the field of ichthyological science (Adams & Collyer 2009; Adams & Otarola-Castillo 2013). With the advancement of technology, a revolutionary tool in studying morphology is being introduced (Adams & Collyer 2009). Geometric Morphometrics otherwise known as GM uses landmark configurations in representing the shape of a specific specimen giving us a more precise statistical analysis of the specimens' morphology because the actual shape is being preserved. It is utilized to determine phenotypical changes in relation with species interactions (Adams et al 2004; Langerhans et al 2004; Adams & Otarola-Castillo 2013; Moneva et al 2014). Thus, it is considered as one of the most reliable techniques in describing and interpreting patterns of morphological variations among groups of organisms in this century. Progressive innovations in image analysis, computer technology, biology as well as statistics such as the use of geometric, the study of shape have contributed to the improvement of biometric analysis like in fish populations like in *B. orcini* (Bookstein 1997; Adams & Collyer 2009; Santos & Quilang 2011).

B. orcini popularly known as Bigtooth or Bigbelly Pomfret worldwide is considered as one of the top 10 most highly migratory fishes in the world as what is being stated in the Article 64 Annex 1 on the Convention on the Law of the Sea (Burke 1982; see also Maguire et al 2006). Generally, according to Lee et al (2014), its body is strongly compressed. It has a very high body depth, a large head, slightly arched forehead, a short snout, and strongly oblique mouth. It can be found near land masses. According to Last & Moteki (2001) as cited by Bos & Gumanao (2013), *B. orcini* is an epipelagic species and can be found at depths ranging from 1 m to 100 m. Mundy (2005) reported specimens as deep as 1229 m. It is known from tropical and sub-tropical seas in the Indo-Pacific with records from Japan, Papua New Guinea, Hawaii, Maldives and Australia (Froese & Pauly 2012). Studying the morphological variations of this species is very essential for they are economically important fishes especially for communities who are relying on fishing as their major livelihood. Because of the abundance of this species, its important ecological role in these oceanic regions, and its potential for sustaining a large commercial fishery, better understanding of the biology and ecology of this species is needed (Shimazaki 1989). Further, Bos & Gumanao (2013) emphasized that *B. orcini* has an indo-pacific-wide distribution with a subsistent fish catch in Southern Mindanao and can be considered as a food fish in the region. Species among the genus *Brama* have slight phenotypical variations that are hard to determine without a thorough examination of its morphological features (Pearcy et al 1993; Thompson 2013). The congeneric species, *B. orcini, B. dussumieri,* and *B. japonica*, are often subjected to misidentification. However, as mentioned by Lee et al (2014), *B. orcini* is distinguished by having 16 gill rakers, 54 lateral line scales, and 36 vertebrae.

Lower number of ichthyological studies has been conducted in the southern bays of Mindanao as this region has suffered under scenes of political unrest (Bos & Gumanao 2013). Hence, the enhancement of a research infrastructure has been hampered and information about Mindanao's natural resources is very limited. The Tuka Bay, located in southernmost part of Mindanao facing Celebes Sea, is a marine sanctuary wherein little biological data is known. According to Carpenter & Springer (2005) as cited by Bos & Gumanao (2013), chances of discovering new species or observing first record are relatively high along the coasts of Mindanao. Thus, scientific research efforts would be beneficial for the development and preservation of marine resources in Mindanao (see also Carpenter et al 2011).

In this study, an attempt was made to identify the shape variations among sexes of *B. orcini* that can be found in the Philippines as well as the role of sexual selection on morphological diversity through the use of landmark based Geometric Morphometrics coupled with Multivariate Statistical Analysis.

Material and Method

Study Area. The researchers conducted this study from September 3 – 4, 2015 at Tuka Bay, Kiamba, Sarangani Province, which can be found at Southern Mindanao, geographically situated 5° 59' 22" N and 124° 37' 27" E (Figure 1) and is bordered by South Cotabato on the north, Celebes Sea on the south, Maitum on the west and Maasim on the east. As one of the municipalities of Sarangani Province, Kiamba is a protected asylum for different marine species like colorful fishes and corals. It is also one of the known spots for snorkelling and diving.

Fish Specimens. A total of 100 individuals (64 males and 36 females) were collected in the 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. Nikon D200 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 (Figure 2).

Fig. 1. Map showing the study area, Tuka, Kiamba, Sarangani Province (modified from Google Earth).

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 TpsDigver 2.12.

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. 1.36 (Rohlf 2007). All specimens in morpho-space, removing size and rotational/translational differences were aligned using GPA. The variability in body shapes were showed using the relative warp scores. To assess the morphological differences of the *B.orcini* 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 (Hammer et al 2001).

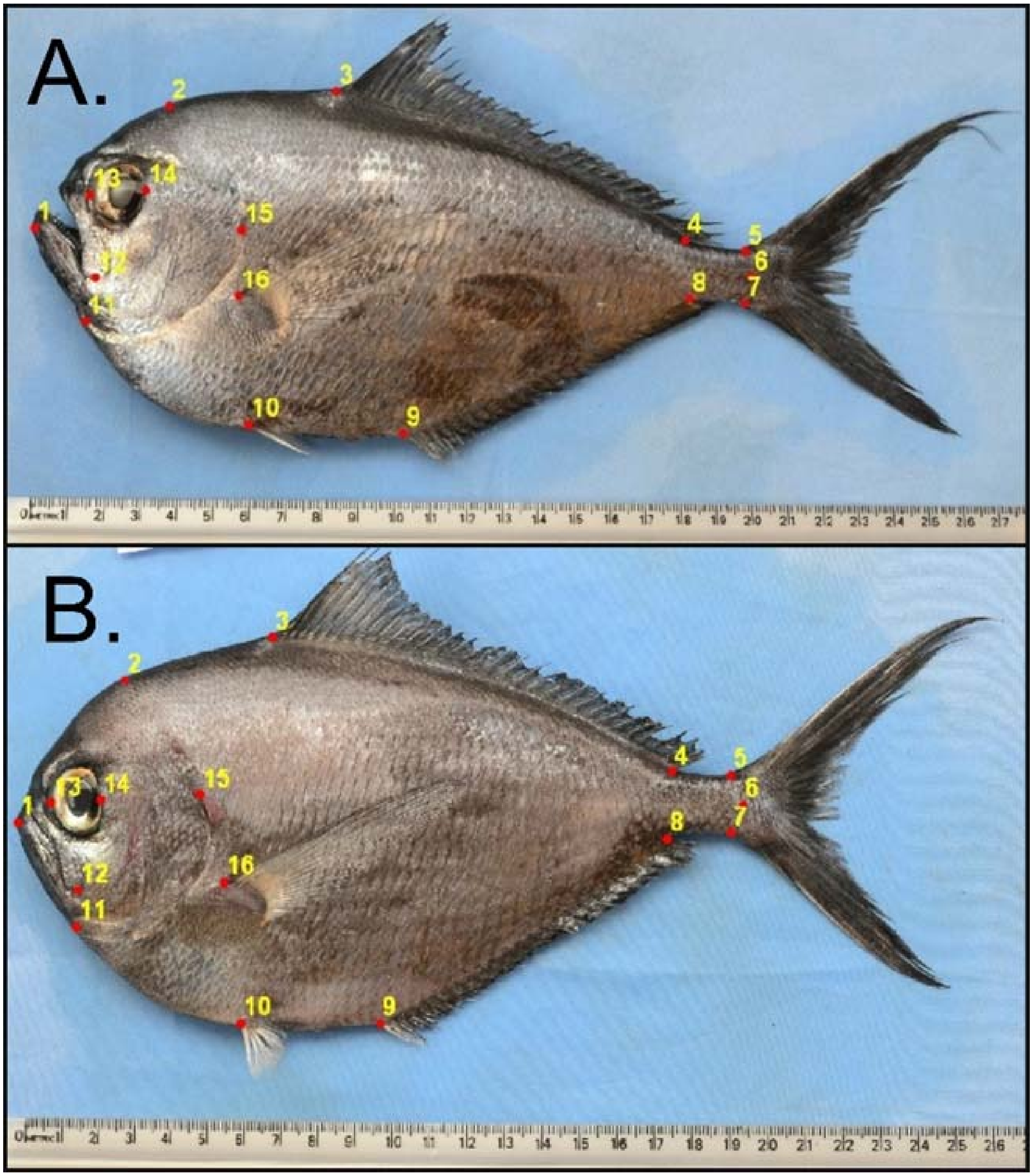

Fig. 2. A male *B. orcini* (A) and female (B), showing the 16 landmarks used in the study. Landmark's description: (1) rostral tip of premaxilla, (2) posterior end of nuchal spine, (3) anterior insertion of dorsal fin, (4) posterior insertion of dorsal fin, (5) dorsal insertion of caudal fin, (6) midpoint 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 summary of the patterns of shape variation is shown through the Relative Warp Analysis. The first six relative warps in male population showed 75.69% of the total variation. From this, RW1 yields 21.51% describing constriction in the head region specifically in the lower jaw articulation, posterior end of maxilla, and dorsal end of operculum, RW2 yields 16.02% describing variations in the head, body and caudal region, RW3 yields 13.26% describing variations between head region and body length, RW4 yields 10.10% describing constriction and curvature in the caudal region, RW5 yields 8.90% describing slight constriction in the head part, and RW6 yields 5.90% describing the variations in the head and caudal region.

The first five relative warps in female population showed 79.71% of the total variation. From this, RW1 yields 28.94% describing constriction in the head region particularly between the dorsal end of opercle and the dorsal base of pectoral fin, RW2 yields 21.67% describing extent variations in head, body and caudal regions, RW3 yields 13.84% describing the opposite variation in head, body and caudal regions between the positive and negative extreme, RW4 yields 8.32% describing curvature in the head region and RW5 yields 6.94% describing variations in the head and body region (Figure 3).

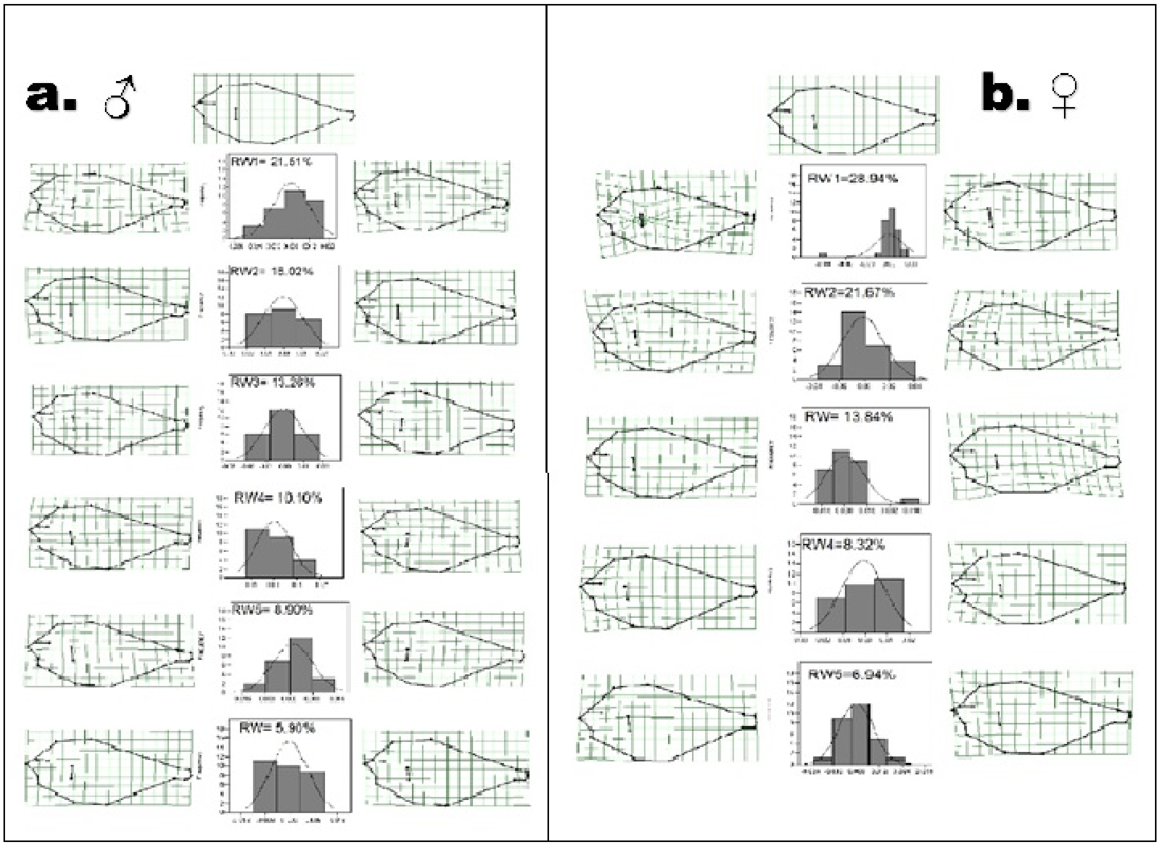

Fig. 3. Summary of the Geometric Morphometrics in male and female *Brama orcini* species showing the relative warps and the variation in body shapes from negative extreme (left) towards positive extreme (right) as compared to the mean shape (top).

The patterns of intraspecific variations in the body shapes were summarized through the Relative Warp Scores and its corresponding frequency histograms within the two sexes. Based on the Relative Warp analysis results, the body depth and belly region of female *B. orcini* is wider and bigger than males which have slim and slender bodies. Males have heads which are more confined in their ventral part, meanwhile, females are also more confined in their dorsal body part. Furthermore, this study revealed that females compared to males have greater curvature in their posterior dorsal and caudal region (see Table 1).

Table 1
Description of the designated relative warps by its overall shape variation and percentage variance of male and female species of *B. orcini*

RW	% Variance	Male	% Variance	Female
RW1	21.51	Variations in the head region and ventral of the body. In the negative extreme, there is a constriction in the head region specifically in the lower jaw articulation, posterior end of maxilla, and dorsal end of operculum. In the observation, there is compression in the ventral part of the body region while in the positive extreme the head region becomes broader particularly in the mouth. The latter also expresses bending downward in the caudal region.	28.94	There were variations in the head, body and caudal region. Negative extreme has constriction in the head region particularly between the dorsal end of opercle and the dorsal base of pectoral fin thus makes its head length shorter and head depth narrower. In contrast, positive extreme has broader body depth, longer length between the dorsal end of opercle and dorsal base of pectoral fin which results to its bigger head region. Negative extreme constrict at the head region as it approaches the positive extreme while the latter widens particularly in the head and body regions.
RW2	16.02	There were variations in the head, body and caudal region. The negative extreme manifests latitudinous mouth in the head region. On the other hand, positive extreme displays broader head, longer body depth and elongated caudal part.	21.67	There were variations in the head, body and caudal region. Negative extreme has longer head and body length while the positive extreme has shorter length between the posterior end of nuchal spine and anterior insertion of dorsal fin. In addition, the negative extreme bends downward towards the left primarily in the head region as it approaches the negative extreme while the latter bends upward towards the right particularly in the body region.
RW3	13.26	There were variations in the head region as well as in the body length. The negative extreme shows that the head region is enlarged while the body and caudal part is shortened. On the other side, the positive extreme exhibits a longer body length.	13.84	There were variations in the head, posterior dorsal and caudal region. Positive extreme has longer dorsal fin, shorter anal fin, longer length between anterior insertion of anal fin and dorsal base of pelvic fin and lastly, has longer length between ventral end of lower jaw articulation and posterior end of maxilla which is the complete opposite of negative extreme. Negative extreme bends downward particularly at the belly region while positive extreme curves upward primarily at the body region.
RW4	10.10	There were variations in the head region and posterior dorsal end and ventral insertion caudal fin. In the negative extreme, it has a greater head constriction and curvature in the ventral insertion caudal fin compare to the positive extreme. In contrast, positive extreme shows that it has a broader body depth.	8.32	There was a variation in the head region. Both positive and negative extreme has a curvature in the head region. Moreover, the snout at the positive extreme bends outward while in the negative extreme bends inward particularly in the posterior end of maxilla and posterior margin through midline of orbit.
RW5	8.90	There were variations in the head region and the body region. There is a difference in the head region because the negative extreme manifests constriction in the mouth and posterior end of nuchal spine. While the positive extreme shows that the head is larger compare to the negative extreme and it has a broader body depth.	6.94	There were variation in the body and head region. Negative extreme has a longer length in posterior fin, longer distance between the posterior margin through midline of orbit and the dorsal base of pelvic fin and it is completely opposite of positive extreme.
RW6	5.90	There were variations in the head region and caudal fin. The negative extreme has a more constricted head than the positive extreme yet it has a latitudinous mouth and a greater curvature in the caudal fin. In contrast, the positive extreme has a broader body depth.		
Total	75.69		79.71	

The body shape variations within *B. orcini* species were proven by the quantitative data gathered in Multivariate Analysis of Variance (MANOVA). The study shows significant differences between sexes that was determined by the p value (p= 0.04014) which is lesser than 0.05 level of significance (p<0.05). Further, other multivariate test methods also revealed significant results such as Pillai Trace = 0.1893, Wilk's Lambda = 0.8107 and Eigenvalues = 0.2334 and 5.69E-16.

Moreover, the Discriminant Function Analysis (DFA) graph in Figure 4 summarized the extent of variation between the two male and female *B. orcini* species. This manifests the sexual dimorphism between the sexes. Differences in the body shape of the sexes varies in the functions. Since *B. orcini* 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 manoeuvrability 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 compare to males. Sexual dimorphism indicates the reproductive role which influences the morphological structure of the species. According to Casselman & Schulte-Hostedde (2004), males need adaptation to compete with their mate while females adapt to produce offsprings.

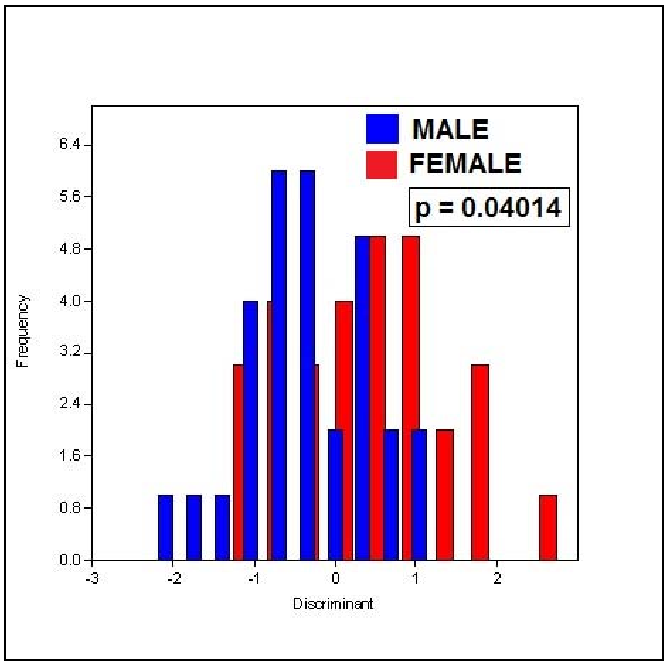

Fig. 4. Discriminant Function Analysis (DFA) Graph.

In addition, variation between sexes is characterized primarily by their differences in their abdominal region. Male Bigtooth pomfrets exhibited slender bodies while females have stout body outlines (Gomes 1990; Haedrich 1977; Masuda et al 1984). Several studies about morphological variation among species within the family *Bramidae* have been carried out, but only few has given much attention about the sexual dimorphism of the species. Bos & Gumanao (2013) pointed out that observations of *B. orcini* at the Pena Plata market in Samal Island suggested that this species had no seasonal pattern of occurrence in Southern Mindanao. The specimens collected in Davao Gulf, a large bay encompassing the Samal Island, and the reported new record of *B. orcini* in Jeju Strait in South Korea as disclosed by Lee et al (2014), as well as the collected species in Tuka Bay, corresponded well with the description in the numbers of lateral line scales (54), and gill rakers (16) and vertebrae (36) as emphasized by Mead (1972ab), who studied the non-type and holotype specimens of *B. orcini* (Lee et al 2014).

Another observation in this study was the evident difference between the caudal region of the male and female *B.orcini*. Male *B. orcini* displayed a more elongated caudal region compared to females. The caudal fin in most bony fishes is not just being exploited for propelling movements of fishes, but also to generate forces and moments that can control the overall orientation of the fish (Flammang & Lauder 2009; Esposito et al 2011). The change in the length, stiffness, and number of the fin rays in the caudal region would have a significant change in the propulsion as well as in the locomotion in the lateral and lift movement of a certain fish (Alben et al 2007). This manifestation was observed in a bony fish called bluegill sunfish (*Lepomis macrochirus*) wherein it was able to regulate the speed of its movements (Alben et al 2007; Flammang & Lauder 2009; Esposito et al 2011).

An observation with the *Hypseleotris agilis* in Lake Lanao, Philippines indicated that fin elongations in males in the study would develope an additional length of the fins that would influence various characteristics of the fish especially its ability to move in their water habitat (Nacua et al 2012). In a study about sexual dimorphism of *Oreochromis mossambicus* in the same area with the latter, the study revealed that changes in fins were probably caused by sexual selection so fishes could be able to threaten rivals (Nacua et al 2011).

In line with this, a significant difference on the shape variations of male and female *B. orcini* was observed in this study. It dispensed a new knowledge about the original distribution and range of expansion of this species. Aside from that, the results manifested that morphological variations among species are greatly influenced by their reproductive role.

Conclusions. This study revolves around the significant morphological structure of sexes of *B. orcini* through the use of landmark-based Geometric Morphometrics. From the given results, males exhibit leaner bodies, while females show bold body outlines and greater degree of curvatures. Sexual dimorphism in the body shape of the *B. orcini* has significance about sexual roles during reproduction to have better mating opportunities. This proved the applicability of geometric morphometrics (GM) in identifying variations as well as similarities of shapes between and within species populations.

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Authors:
Joseph Kennedy O. Cantabaco, Mindanao State University-GSC, College of Education, Department of Secondary Education, Philippines, Barangay Fatima, General Santos City 9500, e-mail: cjosephkennedy@yahoo.com
Shiela F. Celedio, Mindanao State University-GSC, College of Education, Department of Secondary Education, Philippines, Barangay Fatima, General Santos City 9500, e-mail: shiela.celedioshiela@gmail.com
Czarina Mae B. Gubalani, Mindanao State University-GSC, College of Education, Department of Secondary Education, Philippines, Barangay Fatima, General Santos City 9500, e-mail: soshiraiseto9@gmail.com
Rosie J. Sialana, Mindanao State University-GSC, College of Education, Department of Secondary Education, Philippines, General Santos City 9500, e-mail: rosie.sialana@facebook.com
Mark Anthony Jariol Torres, Mindanao State University – Iligan Institute of Technology, College of Science and Mathematics, Department of Biological Sciences, Philippines, Iligan City 9200, Andres Bonifacio St., e-mail: torres.markanthony@gmail.com
Elani Antonio Requieron, Mindanao State University, College of Natural Sciences and Mathematics, Science Department, Philippines, Barangay Fatima, General Santos City 9500, e-mail: elani_requieron2003@yahoo.com
Tres Tinna B. Martin, Mindanao State University, College of Education, Department of Secondary Education, Philippines, Barangay Fatima, General Santos City 9500, e-mail: tres1203@yahoo.co

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