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Sexual dimorphism in body shapes of the spotted barb fish, *Puntius binotatus* of Lake Buluan in Mindanao, Philippines

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Abstract. The objective of this study is to describe differences in body shapes between sexes of the spotted barb, *Puntius binotatus* (Valenciennes, 1842) using landmark-based geometric morphometrics. A total of 100 fish individuals (50 per sex) collected from Lake Buluan in Mindanao were digitized using twenty landmarks. Results revealed significant variation in body shapes between the two sexes. Body shapes were more slender for males than females. Females have bigger head region, deeper body depth and shorter tail region than males. Males have also wider anal fin bases. Such dimorphic traits are believed to be outcome of selective pressures for shape differences between the sexes.

Key Words: landmark-based geometric morphometrics, relative warps, spotted barb.

Introduction. The study of body shape in fishes has been a subject of research investigations for over a century. Recent advances in morphometric analytical techniques have provided statistically powerful methods for the analysis of shape (Rohlf & Slice 1990). These techniques, inclusively named geometric morphometrics (Rohlf & Marcus 1993; Adams et al 2004), describe the spatial arrangement of anatomical features of organisms, providing statistical (Rohlf 1999) and visual (Caldecutt & Adams 1998) advantages to traditional approaches based on linear distance measures. These techniques have been used increasingly over the past decade with morphological data from fishes for studies of phylogenetics and species descriptions (Cavalcanti et al 1999; Douglas et al 2001), ontogenetic allometry (Walker 1997; Hood & Heins 2000); trophic morphology (Caldecutt & Adams 1998), ecological morphology (Corti et al 1996). Geometric morphometric methods allow fine-scale assessment of shape differences and, therefore, could be valuable for discerning patterns of intraspecific morphological variation. However, none of these studies determined sexual dimorphism in body shapes of the spotted barb, *Puntius binotatus* (Valenciennes, 1842), an economically important fish species collected from Lake Buluan in Mindanao, Philippines. *P. binotatus*, originally described as *Barbus binotatus* by Achille Valenciennes in 1842, is a native fish of Lake Buluan. It is a benthopelagic fish. The body is silvery gray, darker dorsally and paler on throat and belly. Body markings (spots or band) may be absent on large specimens, except a spot on caudal base. It mostly feeds on zooplankton, insect larvae and some vascular plants (http://www.fishbase.org/Summary/SpeciesSummary.php?id=5180). Thus, this study was conducted.

Materials and Methods. A total of one hundred *P. binotatus* (50 per sex) were sampled from Lake Buluan, Lutayan, Sultan Kudarat, Philippines (Figure 1). Only sexually mature fishes were used to reduce the amount of intrapopulation shape variation based on ontogeny. The individuals were transported in styropore box with ice and processed immediately. Sexing was based on external morphology and was later confirmed by direct examination of the gonads (Conlu 1986). Both the left and right sides of the fish individuals were scanned at uniform dpi (600) using a CanoScan model D646U flatbed scanner.

Figure 1. Location of Lake Buluan in Lutayan, Sultan Kudarat, Mindanao, Philippines.

A total of twenty landmarks (equivalent to 20 X and 20 Y Cartesian coordinates) were selected to provide a comprehensive summary of the morphology of the fishes (Figure 2). The landmarks digitized in this study are standard points used in fish morphometrics and are said to have both evolutionary and functional significance (Turan 1999; Turan et al 2004; Costa & Cataudella 2007; Buitrago-Suarez & Brooks 2007; Vasconcellos et al 2008). The landmarks were digitized on both sides of each specimen image using the TpsDig ver. 2.10 (Rohlf 2006). Digitization was done in tri-replicates for each fish sample.

The X and Y coordinates of the landmark points that were digitized from the left and right images of the fishes contain both shape and non-shape (differences in the position and orientation of the fishes in the flatbed scanner; size differences) components of variation (Adams 1999; Kasam et al 2004). Since this study focused only on shape differences, the non-shape components were necessarily removed prior to analysis via Generalized Procrustes Analysis (GPA) using TpsRelw 1.36 (Rohlf 2003). GPA aligned in all the specimens in morpho-space, eliminating size and rotational/translational differences. GPA proceeded as follows: first the landmark coordinates were translated to a common centroid at the origin of the reference coordinate system or at point (X=0, Y=0). Then, the set of landmark coordinates of each fish sample were scaled to unit centroid size, thereby removing size differences and permitting analysis of body shape. Finally, the landmark configurations of all fishes were rotated to minimize the sum of squared distances between corresponding landmarks (Bookstein 1991). This step removed residual translational and rotational differences among the individuals resulting from differences in the way the fishes were oriented in the flatbed scanner when the digital images were taken.

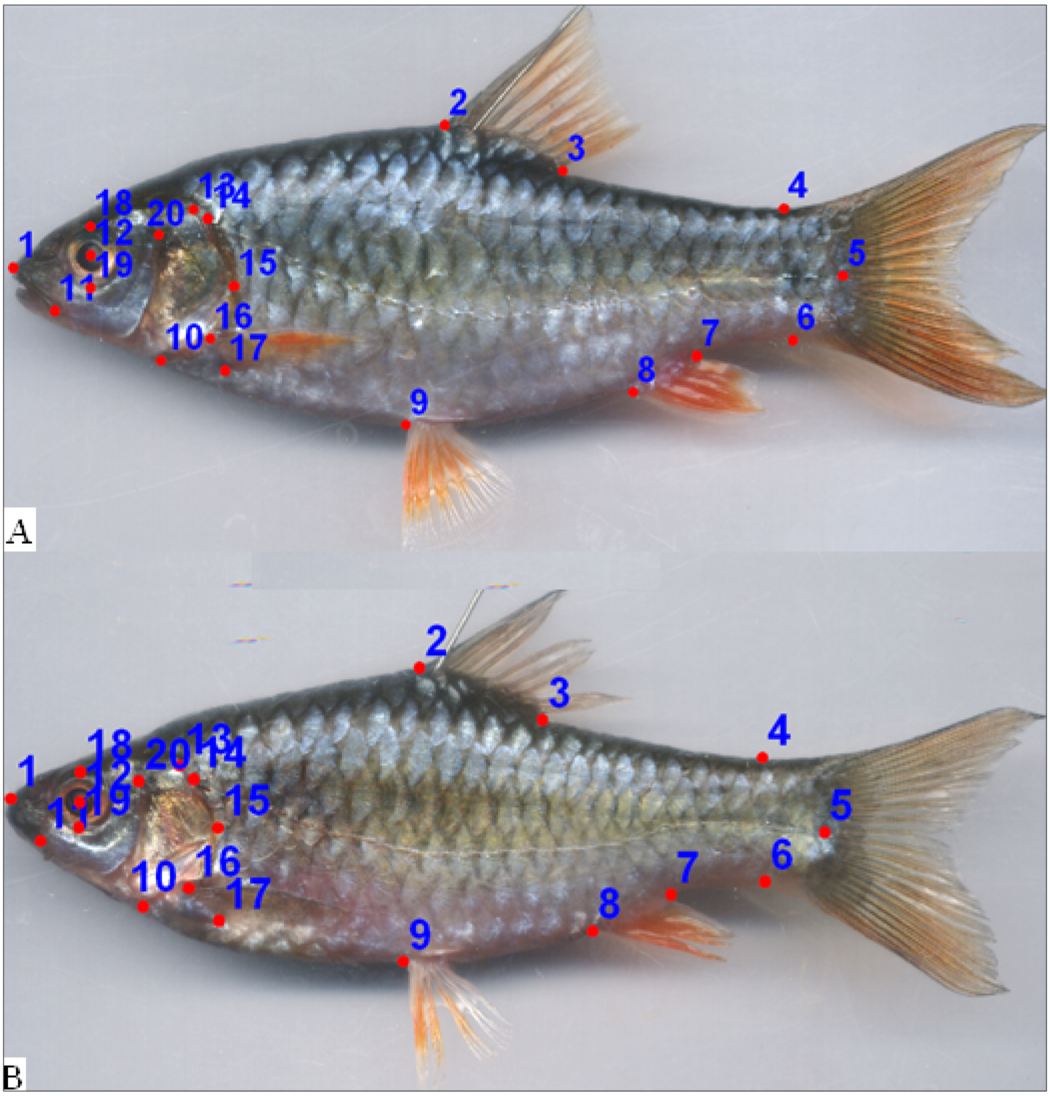

Figure 2. Landmarks' description of the spotted barb, *P. binotatus* (A. female, B. male): (1) snout tip; (2) and (3) anterior and posterior insertion of the dorsalfin; (4) and (6) points of maximum curvature of the peduncle; (5) posterior body extremity; (7) and (8) posterior and anterior insertion of the anal fin; (9) insertion of the pelvic fin; (10) insertion of the operculum at the lateralprofile; (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) superiorand inferior margin of the eye; (20) superior margin of the preoperculum.

Using the thin-plate spline equation and the standard formula for uniform shape components, a weight matrix (containing uniform and non-uniform shape components) from the aligned specimens were generated (Bookstein 1991). Variability in body shapes was then examined via relative warp (RW) analysis of the set of uniform and non-uniform components of shape using TpsRelw 1.36 (Rohlf 2003). The RW scores were then subjected to Multivariate Analysis of Variance (MANOVA) and Canonical Variate Analysis (CVA) to test for sex differences in body shapes in *P. binotatus* using the PAST software (Paleontological Statistics, version 1.27; Hammer et al 2001).

Results and Discussion. Significant differences in body shapes of the two sexes of *P. binotatus* (right image) (Figure 3a) can be seen based on the distribution of the individuals along the first two canonical variate axes (Wilks' lambda = 0.3576; Pillai trace = 0.6424; P-Value = 9.91E -41). As shown in Figure 3a, the first axis explains much of the variation between the sexes and accounts for nearly 100% of the variance (Eigenvalues for CV1 and CV2 are 1.796 and 2.206E-15, respectively).

Significant differences in body shapes of the two sexes of *P. binotatus* can be seen also at the left image (Figure 3b) based on the distribution of the individuals along the first two canonical variate axes (Wilks' lambda = 0.3576; Pillai trace = 0.2464; P-Value = 9.91E -41). As shown in Figure 3b, the first axis explains much of the variation between the sexes and accounts for nearly 100% of the variance (Eigenvalues for CV1 and CV2 are 1.796 and 2.206E-15, respectively).

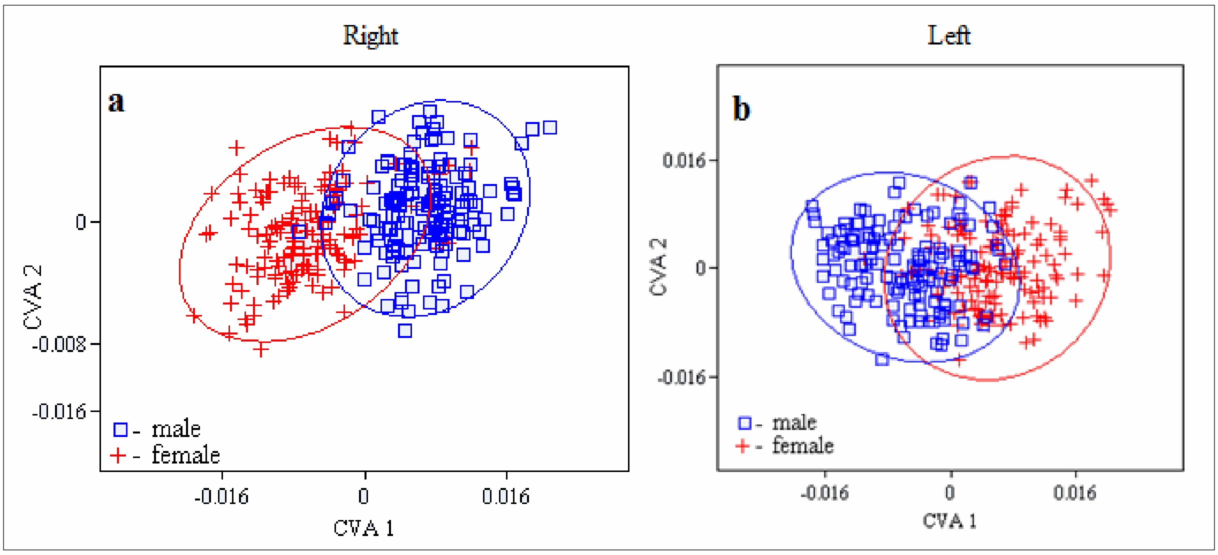

Figure 3. Distribution of the two sexes of *P. binotatus* along the first two canonical variate axes.

MANOVA reveals that there is a significant sexual dimorphism in the overall body shapes of *P. binotatus*. This is based on the results of the two statistics calculated from MANOVA which are the Wilks' lambda and Pillai trace. Both statistics give significant values. This implies that using the tools of geometric morphometrics would reveal significant morphological variation using twenty landmarks. These results are further summarized by the canonical variate analysis (CVA). In all the CVA scatter plots presented, both male and female points overlap around zero of the first and second axes. The CVA produces a scatter plot of specimens along the first two canonical axes, producing maximal separation and second to maximal separation between the two sexes of each fish species evaluated (Hammer et al 2001). Thus, the results from MANOVA and visualized by the CVA scatter plots revealed significant differences between sexes of *P. binotatus* based on their body shapes.

A number of dimorphic traits are revealed by the histograms of the relative warp scores that differentiates female (Figure 4) from male *P. binotatus* (Figure 5). The variations observed are described in Tables 1 and 2. These include bigger head region, deeper body depth and length of the tail region. Females have bigger head region, deeper body depth and shorter tail region than males. Bigger head region would maximize buccal volume and suction velocity as an adaptation for feeding on macrobenthos (Caldecutt & Adams 1998) whereas deep bodies increase maneuverability when foraging (Webb 1982, 1984). A greater degree of convex curvature of the dorsal part of the tail region is also evident in females than in males. This could be attributed to the bulkier abdominal region of the females. Males have wider anal fin base than the females which could promote success in male-male competition or female choice as observed in the genus *Petrotilapia* by Kassam et al (2004). Larger fin base should aid in stability and control during swimming and may help males position themselves optimally relative to females during spawning in order to maximize fertilization success (Videler 1993 as cited by Casselman & Schulte-Hostedde 2004).

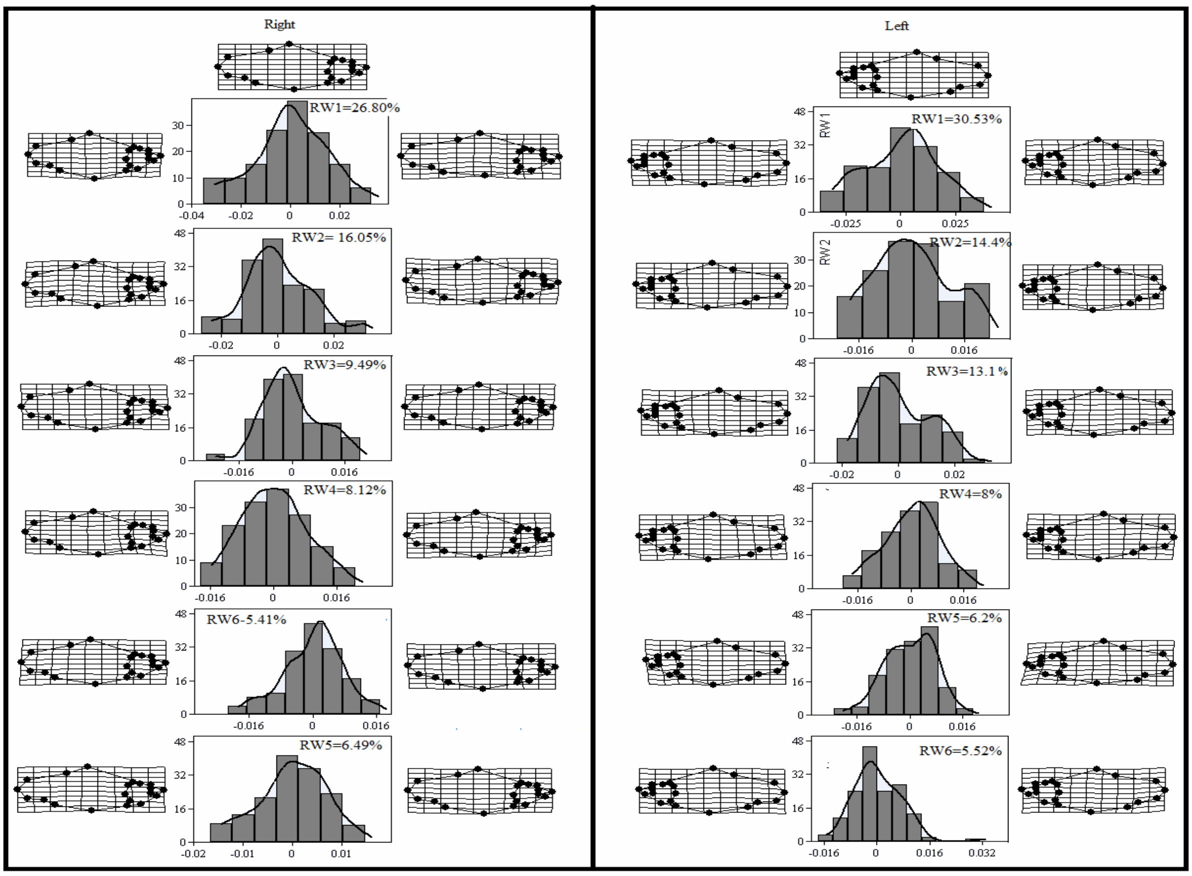

Figure 4. Summary of the geometric morphometric analysis showing the consensus morphology (uppermost panel) and the variation in body shape among the female population (right and left image) of *P. binotatus*, produced by the first six relative warps (RW).

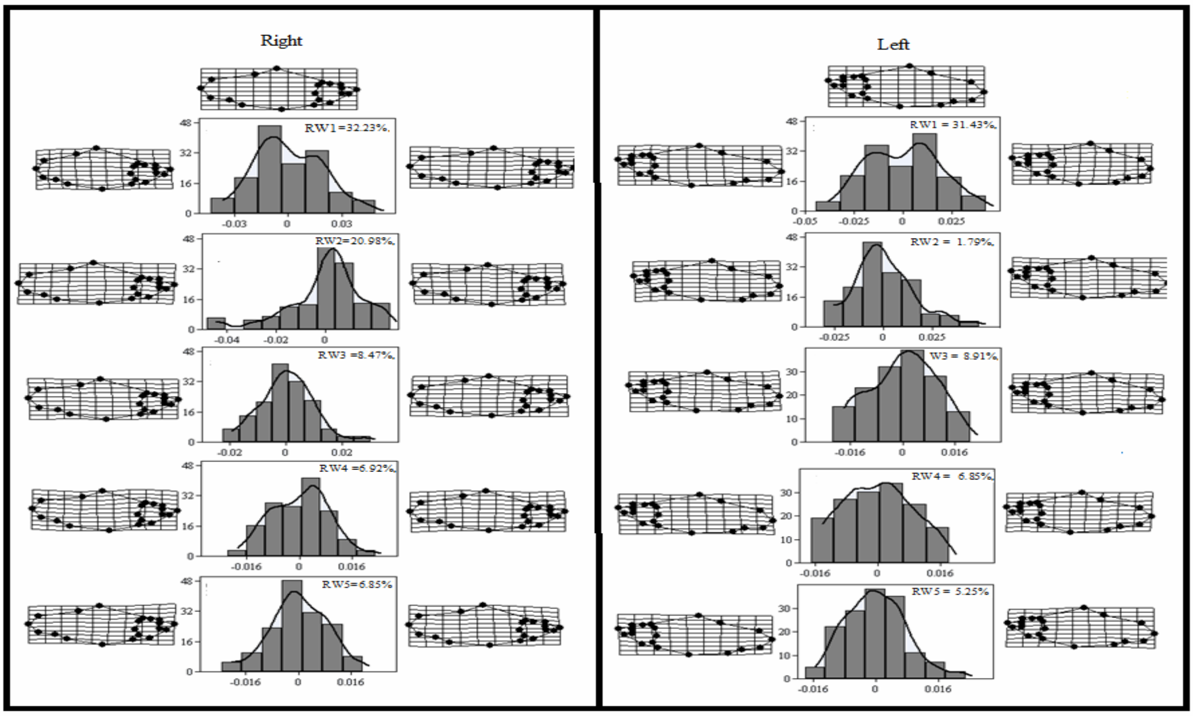

Figure 5. Summary of the geometric morphometric analysis showing the consensus morphology (uppermost panel) and the variation in body shape among the male population (right and left image) of *P. binotatus*, produced by the first five relative warps (RW).

Table 1

Variations observed in the body shapes of female *P. binotatus*

RW (%)	*Right*	*RW (%)*	*Left*
RW 1 = 26.80	Differences in the body depth and trunk and tail regions. Individuals with positive scores have shorter head and trunk regions and have longer and narrower tail region. However, individuals with negative scores have deeper body depth and shorter tail region.	RW 1 = 30.53	Variations observed are associated with the head, trunk and tail regions. Individuals with positive scores have more compression of the head, trunk and tail regions than those with negative scores. However, individuals with negative scores have longer tail region contributing to their greater standard length.
RW 2 = 16.05	Observable body shape differences relating to the curvature of the tail region. Individuals with positive scores have their tail region curves dorsally while those with negative scores have their tail region curves ventrally.	RW 2 = 14.39	Similar observations with RW 1
RW 3 = 9.49	Individuals with positive scores have constriction of the mouth region and have longer trunk region in contrast to the individuals with negative scores having compressed trunk region.	RW 3 = 13.1	Variability in the mouth region and body depth. Individuals with positive scores have constriction of the mouth region towards the ventral side while individuals with negative scores have constriction of the mouth region towards the dorsal side. In addition, individuals with positive scores have deeper body depth than those with negative scores.
RW 4 = 8.12	Variations observed on the head, trunk and tail regions. Majority of the individuals have positive scores and are characterized with a slight compression of the tail region. However, individuals with negative scores have constriction of the ventral part of the head region and compression of the trunk region.	RW 4 = 8	Individuals with positive scores have tails slightly pointing ventrally. Individuals with negative scores have constriction of the head and trunk regions.
RW 5 = 6.49	Individuals with positive scores have longer tail region than those with negative scores.	RW 5 = 6.2	Constriction of the mouth region and compression of the head region. Individuals with positive scores have constriction of the mouth region drawing them towards the ventral side. However, constriction of the head region is observed in individuals with negative scores.
RW 6 = 5.41	Similar variations observed with RW 5	RW 6 = 5.52	Individuals with positive scores have tails pointing dorsally while individuals with negative scores have tails pointing ventrally.

Table 2

Variations observed in the body shapes of male *P. binotatus*

RW (%)	*Right*	*RW (%)*	*Left*
RW 1 = 30.23	Variation in the tail region. Individuals with positive scores have longer tail region than individuals with negative scores resulting to longer standard length. On the other hand, individuals with negative scores have compressed trunk and tail regions.	RW 1 = 31.43	Variation in the tail region. Individuals with positive scores have shorter tail region than individuals with negative scores.
RW 2 = 20.98	Differences in the head region are observed. Individuals with negative scores have compressed head region and expanded tail region in contrast to the compressed tail region of the individuals with positive scores.	RW 2 = 21.79	Differences in the head region. Individuals with negative scores have shorter head region than individuals with positive scores.
RW 3 = 8.47	Variations associated with the head length and trunk region. Individuals with positive scores have longer head length and more compressed trunk region than those with negative scores.	RW 3 = 8.91	Variation among the individuals observed in the third relative warp (RW3) is associated with the head region. Individuals with positive score have shallower head region than those with negative scores.
RW 4 = 6.92	Differences in the tail region. Individuals with positive scores have tails pointing downward while individuals with negative scores have tails pointing upward.	RW 4 = 6.85	Differences in the tail region. Individuals with positive scores have tails pointing upward while individuals with negative scores have tails pointing downward.
RW 5 = 8.47	The fifth relative warp (RW5) accounts for variations associated with the tail region. Individuals with positive scores have expanded posterior part of the tail region than those with negative scores.	RW 5 = 5.25	The fifth relative warp (RW5) accounts for variations associated with the head region. Individuals with positive scores have more compressed head than the individuals with negative scores.

Conclusion. The results of the study underlies the utility of the method of Geometric Morphometrics in discriminating sexes in *P. binotatus* and in understanding variability in the shapes of the body of this fish. Future studies should be directed towards unraveling the functional basis of the observed differentiation in the shapes of the body as well as the observed disparity in the shapes of the body between sexes.

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