

# EVIDENCE OF SHAPE SEXUAL DIMORPHISM IN *STROMBUS LUHUANUS* LINNAEUS 1758 (GASTROPODA: STROMBIDAE)

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*Abstract* Sexual size dimorphism of *Strombus luhuanus* was explored with Geometric Morphometrics. Using 39 landmarks and semi-landmarks, principal component analysis (PCA) showed possible separation of sexes with significant loadings on shoulder and the inner lip. Discriminant Function Analysis (DFA) cross-validated classification showed males and females can be identified using the generated shape variables with 83.72% and 71.43% accuracy respectively. Examination of the generated mean shape showed that males are slimmer while females are more bulbous with vector changes along the inner lip and shoulder. Sexual shape may be related to differential energy allocation of reproduction with males spending more on finding a mate and females devoting more on egg production. Statistically quantified shape dimorphism will facilitate better understanding of reproductive behaviour and natural population dynamics of this species.

*Key words* Sexual dimorphism, strombids, geometric morphometrics

## INTRODUCTION

Sexual dimorphism is an evolutionary result of differential selection among the sexes (Leonard, 2016; Fairburn and Roff, 2006; Shine, 1989) typically expressed in size, shape, and other morphological differences between male and female members of a species (Minton and Wang, 2011; Shine, 1989). Divergent evolution is primarily meant to maximise fecundity of the sexes. In this theoretical construct, males are primed for sperm competition favouring bigger body sizes (Shine, 1989; Blankenhorn, 2005). An alternative explanation to sexual dimorphism is intraspecific niche divergence where the sex-related habits selected for differentiated morphology that maximised survival probability (Shine, 1989; Leonard, 2016). Foraging success and feeding habits of the sexes determines a different development trajectory resulting in sexual dimorphism (Blackenhorn, 2005). Sex-related differences, for example of the radula in Archeogastropods and Neogastropods are related to their microhabitats (Shine, 1989). Sexual dimorphism thus can be used not only to infer reproductive biology of a species, but also behavioural ecology with both determinants interacting.

Phylum Mollusca is an interesting group for sexual selection and adaptive radiation studies (Schiltuizen, 2002) as shells record the ontogenetic history of the animal (Avaca *et al.*, 2013).

Among three species of Neritidae, Govendan and Natarjan (1972) reported that males are smaller relative to females in all aspects of size measurements i.e. shell length, width, and height. The voracious herbivore *Pomacea canaliculata* females were also found to have larger shell length measurements with wider shell opening relative to males (Stebenet & Cazzaniga, 1998). *Littorina variegata*, on the other hand, has females growing twice as fast as males and thus larger at sexual maturity (Riascos & Guzman, 2010). *L. zebra*, however is not dimorphic underlining variability in sexual size differentiation within genera. Among the strombids, several species had also been reported to exhibit sexual size dimorphism. *Lambis* males are usually smaller in size with shorter and flat spines while females have longer spines and a longer knob on the shoulder (Pastorino, 2007). Males of *Strombus pugilis*, on the other hand, have a larger aperture but with a smaller columellar angle, making it appear larger and more bulbous than females. Females of *Strombus canarium* are larger than males but the differences are not significant (Cob *et al.*, 2008). *S. raninus*, *S. alatus*, *S. costatus*, and *S. gigas* female members of the species are also larger in terms of shell length than males (Shawl & Davis, 2004).

Traditional morphometrics had been widely used to report dimorphism, but results are usually compounded size and shape variables resulting from different points chosen from

the shell surface (Caravajal-Rodriguez, Conde-Padín, and Rolán-Alvarez, 2005). Variability thus is reported to be very high. The molluscan shell is extremely plastic with shape and size affected by ecological factors such as wave exposure (Conde-Padín, Grahame, and Rolan-Alvarez, 2007; Hollander, Adams, and Johannesen, 2006; Cuña and Quesada, 2012), geographical location (Sepulveda & Ibañez, 2012), rate of predation (Bourdeau, 2012; Bourdeau, 2009; Márquez *et al.*, 2015), sexual isolation (Conde-Padín *et al.*, 2008), pollution impact (Márquez, González-José and Bigatti, 2011, Márquez, Primost and Bigatti., 2016) and imposex (Primost, Bigatti and Márquez, 2015). Shell morphology thus presents both a constitutive and inductive character controlled by genetics and environment (Bourdeau, 2012).

Geometric morphometrics (GM) uses the Kendall's space theory to describe a shape in terms of coordinates which may be statistically compared using multivariate analysis to show inherent shape differences exclusive of other compounding factor (Slice, 2007; Zelditch *et al.*, 2012). Using the tool, Hollander, Adams, and Johannesen (2006) were able to show that different morphs of *Littorina saxatilis* are significantly affected by habitat, sex, and size as well as their interactions. Sexual shape dimorphism of the species results from different growth trajectories with adult males from wave-exposed sites exhibiting isometry and females shifting from isometric growth in juveniles to allometric growth when adult. Allometry is the change in shape relative to change in size (Mitteroecker & Gunz, 2009). Females on boulder shores where predation rate is high were found to have allometric growth emphasising that shift in growth trajectory is in relation to reproductive needs affected by habitat covariate. Females are consistently larger giving a fecundity advantage. Females of *Buccinapops globulosus* are also significantly larger across three populations (Avaca *et al.*, 2013).

The *Strombus luhuanus* (locally called in the Philippines as *liswi* (Tagalog) and *sikad* (Visayas)) is a strombid that is widely distributed and exploited for both meat and shell in the Indo-West Pacific region (Thomas, 2007). The species is recognisable from its congeners by its orange to reddish aperture and from cone shells by the presence of the strombid notch. As most members of Strombidae, it is gonochoric with

sexual dimorphism only observed by the presence of a spade-like penis observed on the right dorsal side of the foot (Kuwamura *et al.*, 1983). Instead of a penile organ, females have genital groove with or without a bilobed or simple projection that is much more reduced than penis. *S. luhuanus* form permanent age-specific aggregations among sandy-seagrass habitats (Poiner & Caterall, 1988). Dimorphism in the species had never been reported, although Poiner and Caterall (1988) and Kuwamura *et al.* (1983) indicated that adult females are longer than males. This study investigated if there is sexual shape difference in *S. luhuanus*. Demonstration of sexual dimorphism using GM would facilitate population dynamics studies of aggregations as well as exploration of ecological factors affecting populations.

## MATERIALS AND METHODS

Adult specimens of *S. luhuanus* were collected from sub-tidal populations in Mansalay, Oriental Mindoro, Philippines (N12° 26' 51.48", E 121° 24' 47.18") from two proximate aggregations about 30-meters from each other. Care was taken that samples were all adults by choosing individuals with thickened and flared lips. Growth of the species is determinate with maximum length attained two years from when thickening and flaring of lips occur (Poiner & Caterall, 1988). Gathered specimens were boiled to facilitate extraction of the soft tissues and sexing was done by physical observation of penile organs in males. Shells for each sex were separated with 78 males and 78 females composing the study sample. Shell images were acquired with Canon S95 digital camera with grid settings on to assist in focusing the images. Each shell was positioned on its adaxial side fixed using clay dough to prevent rolling and toppling. Positioning was standardised by making the suture between the lip and the body whorl constantly visible on the image and the central portion of the shell filling the center quadrat of the camera grid.

Twelve (12) type II and 27 type III landmarks (LM) were digitised along the surface of the captured images (Fig. 1) using TPSDig (Rohlf, 2005). LM1 was located at the extreme anterior part of the body whorl. LMs 2–10 were equidistant points distributed along the outer side of the body whorl with LM10 located at the suture of the outer body



**Figure 1** Positions of landmarks (blue circles) and semilandmarks (yellow circles) superimposed on the shell of *Strombus luhuanus*.

whorl and the spire. Equidistant points on the left and right side of an imaginary median line along the apex are LM11, LM12, LM14 and LM15. The apex was LM13. LM16 was located on the suture of the apex and the inner body whorl. LM18 was the most posterior point of the posterior canal where the lip was attached to the body (anterior part of columella). LM20 was located anterior to LM18 where the lip is anteriorly attached to the body. LM17 and LM19 are midway points of LM18 and LM20 and LM18 and LM20. LM21 to LM28 were equidistant points along the inner side of the body whorl/columella while LM29 was the lowest point of the anterior canal. Between the anterior canal and the strombid notch LM30 was located at the curvature of the shell. All landmarks between the LM31-LM35 and LM35-LM39 were equidistant points along the inner and outer lip margin. LM35 was the most proximate point of the flared outer lip.

The semi-landmarks were allowed to slide on the shell surface using the landmark sliders function of TPSRelW (Rolf, 2003) with  $\alpha=0$  and sliding method set at Chord-min BE which minimises the bending energy during Procrustes superimposition (Rohlf, 2004; Sheets *et al.*, 2006; Mitteroecker and Gunz, 2009).

Principal Component Analysis (PCA) was used to explore the morphospace defined for the possible separation of sex classes. To test and correct allometric contribution, shape variables were regressed with centroid size (a proxy for size) (Klingenberg, 1996). While the GPA algorithm fitted individuals to a mean shape effectively removing the factor of size and rotation of sample in the analysis, it is of biological importance to know of the total contribution of size in changing the shape and should be isolated to standardise shape variables. Residuals of the regression analysis were used for the subsequent Discriminant Analysis (DA) to statistically discriminate the sexes.

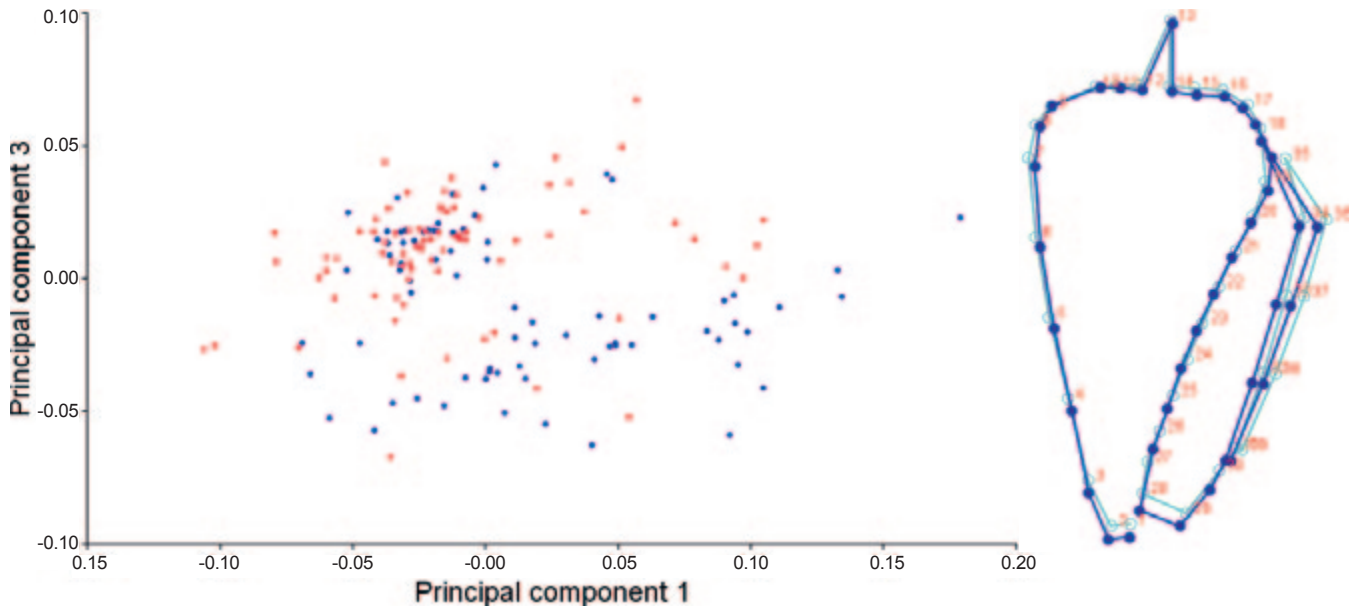
All analyses were done in MorphoJ1.06d (Klingenberg, 2011).

## RESULTS AND DISCUSSIONS

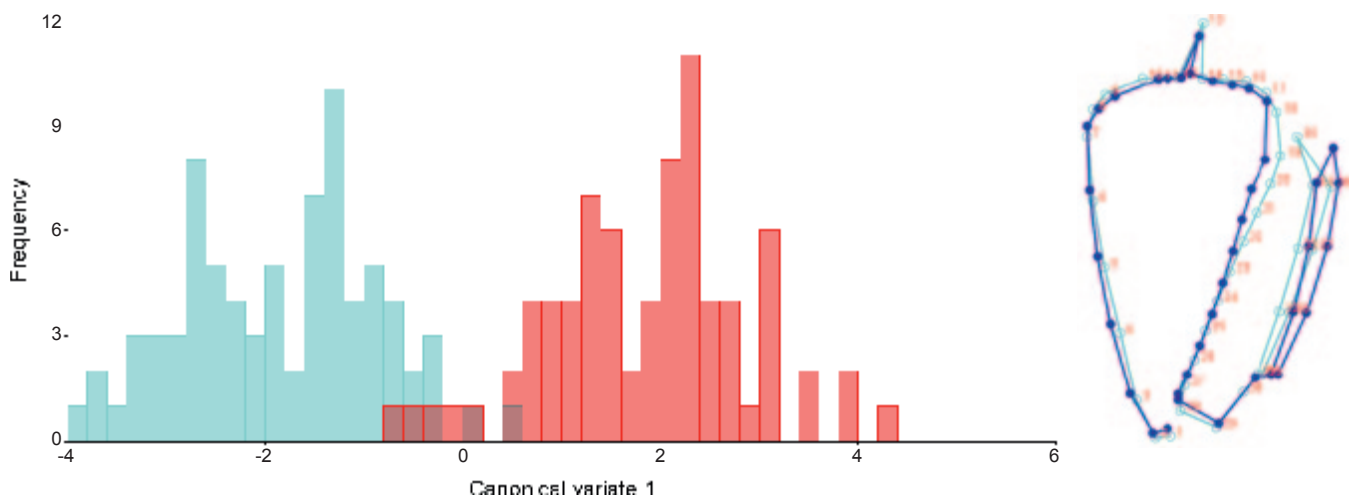
The first three principal axes of the PCA separates the sexes with the upper left quadrant of the plot occupied by males and majority of the females occupying the negative quarters (Fig. 2). Overlap of the points is significant but may be attributed to the compounding effect of age of samples used (allometry  $p=0.0017$ ). Shape differences between the sexes are on the inner lip portion as well as the inner shoulder. The shoulder in females is broader resulting to the narrowing of the aperture. Males have wider aperture relative to females.

Optimisation of group classification through Discriminant Analysis (DA) showed a  $p$ -value of 0.0001 for the differences between male and female (Fig. 3). Cross-validated identification significantly discriminated females from males at the rate of 80.52% and 75.32% respectively.

Geometric morphometrics demonstrated that sexual shape dimorphism exists in *S. luhuanus*. Sexual dimorphism in size has been reported in other strombids (Cob *et al.*, 2008; Shawl and Davis, 2004), but this is the first time that differences in shape have been documented and statistically quantified. Females of the species are more



**Figure 2** Principal component analysis of males (●) and females (●) from covariance matrix of the Procrustes coordinates of the aligned individuals. Inset figure represents the displacement vector (blue) from mean shape (light blue) to the positive extreme shape for a scale factor 0.1x



**Figure 3** Discriminant analysis (DA) identification of the samples showing significant ( $p < 0.0001$ ) separation of males (●) and females (●). Inset figure represented shape difference between male and female means magnified 2x

globose with the inflation of points along the columella. The flaring on the inner lip area narrows down the aperture in adult females where the thickened outer lip curls toward the inner lip. As opposed to male, the relatively depressed inner lip margin gives an appearance of being slender. As a result, the aperture appears wider as the thickened outer lip retains significant clearance from the inner lip among adult males.

The sexual shape difference in *S. luhuanus* conforms to the results of studies among gonochoric gastropods where females are larger than males

because of the brood sac (Minton and Wang, 2011; Riascos and Guzman, 2010; Pastorino, 2007; Mathews-Cascon, *et al.*, 2005). A more globose shell allows for larger volume of sex tissue which gives the female an advantage in the reproduction role. Strombids lay egg capsules contained in a gelatinous string (Kuwamura *et al.*, 1983) and sands are incorporated into the string to form a mass with each mass containing about 100,000–300,000 eggs. Most gonochoric marine snails that registered sexual shape dimorphism have females with a wider aperture (Riascos and

Guzman, 2010; Son and Hughes, 2000; Avaca *et al.*, 2013). Apparently, *S. luhuanus* egg capsules do not require much apertural space to spawn. Penile organs, on the other hand, may need wider apertural space for extrusion as they may be extended up to 4cm (Kuwamura *et al.*, 1983). The more slender males may be an ecological adaptation for agility to increase reproductive success (Blackenhorn, 2005). In the field, aggregations of 3–4 adults were commonly observed. Kuwamura *et al.* (1983) indicated that these aggregations are actually copulating aggregations and are formed when a copulating pair is approached by another male, which may also mount the female. Antagonistic behaviour is sometimes displayed by males by flexing the operculum or waving the proboscis. Active mate search and antagonistic behaviours are both indicators of agility that are advantageous for reproductive success (Blackenhorn, 2005). Field sex ratio of observed patches showed that in 57% of patches, there are fewer males than females with ratios ranging from 1.0:0.1 to 1.0:0.8 (unpublished data). Multi-male aggregations may be expected when the sex ratio is biased for females but it is rare that two males copulate a female simultaneously (Kuwamura *et al.*, 1983) indicating that males prefer to singular mate. Coupling of any given pair lasts for about 2 hours any time of the day. The coupling duration and the frequency, as well as mate search and female guarding behaviours are favoured by more slender body size where more energy is spent for reproductive activity rather than growth. The quantified difference in the shape in the female and male *S. luhuanus*, provides new insights into differences in reproductive allocation of males and females.

The demonstration of sexual dimorphism using GM on the species must be utilised on field studies to minimise collection and to hasten data gathering related to population dynamics.

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