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Article

Describing shell shape variations and sexual dimorphism of Golden Apple Snail, *Pomacea caniculata* (Lamarck, 1822) using geometric morphometric analysis

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Abstract

Pomacea caniculata or Golden Apple Snail (GAS) existed to be a rice pest in the Philippines and in Asia. Likewise, geographic location also contributes its increasing populations thus making it invasive among freshwater habitats and rice field areas. This study was conducted in order to describe shell shape variations and sexual dimorphism among the populations of *P. caniculata*. A total of 180 were randomly collected in the three lakes of Esperanza, Agusan del Sur (Lake Dakong Napo, Lake Oro, and Lake Cebulan), of which each lake comprised of 60 samples (30 males and 30 females). To determine the variations and sexual dimorphism in the shell shape of golden apple snail, coordinates was administered to relative warp analysis and the resulting data were subjected to Multivariate Analysis of Variance (MANOVA), Principal Component Analysis (PCA) and Canonical Variate Analysis (CVA). The results show statistically significant ($P<0.05$) from the appended male and female dorsal & ventral/apertural portion. While male and female spire height, body size, and shell shape opening also shows significant variations. These phenotypic distinctions could be associated with geographic isolation, predation and nutrient component of the gastropods. Thus, the importance of using geometric morphometric advances in describing sexual dimorphism in the shell shape of *P. caniculata.*

Keywords invasive species; phenotypic variations; relative wrap analysis; Lake Dakong Napo.

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1 Introduction

The *Pomacea caniculata* (Lamarck, 1822) also known as “Golden Apple Snail” was introduced in the Philippines from South America as the alternative protein source (Cowie, 2002, Thaewnon-ngiw, et al, 2004). Because of its increasing populations, it dominates freshwater habitats and far ahead became rice field pests in the Philippines and in Asia (Guerrero, 1989; Cagauan and Joshi, 2003; Joshi, 2005; Joshi et al., 2005; Cowie, 2006; Kho, 2011; Mahilum and Demayo, 2014). Dynamically, this type of snails thrives in different environments making it capable of forming new species (Dong et al., 2011). Likewise, the tropic vegetation favors the snail to perform closing off its spire entirely during the dry season; thus making them survive and the nutritional requirement also enhances its adaptive form due to the polyphagous ability; likely to eat detritus, animal matter, macro-phytophagous, submerged and floating plants (Estebenet and Martin, 2002). At present, this species causes serious problem to many farmers specifically in rice production due to its plant consuming and reproductive ability (Baldia, 1989). Moreover, the geographical location is also an important factor for this species to become more widespread. In the Philippines for instance, the types of snail are different from any regions in South America, in fact, study shows that they were behaving unlike in Argentina and Japan it took a longer period of time to reach maturity and hatch eggs (Dong et al., 2011). Therefore, allopatric populations that inhabit different types of regions may also result in ecomorphological differences (Alonso et al., 2006). The distribution pattern of each golden apple snail is an important factor of its morphological variations. The small-scale geographic pattern has been associated to effect shape differences within its populations (Balbosa et al., 2012). Accordingly, the fast growing and invasion of this species in different types of environment enhances its genetic variability expressing speciation i.e. genotype or phenotype (Tabugo et al., 2011). Because of its physiological, anatomical, and ecological traits the snail have been subjected by many studies in determining morphological analysis (Estebenet and Martin, 2002). However, the inconsistent environments of the organism establish to have phenotypic advances both biological and morphological forms which attributed to being more receptive and adaptive to changing ecological variations (Cazzaniga, 2002; Torres et al., 2013). Several studies shows that shell shape differences are also associated with sexual dimorphism, the outcome of sexual selection (Johnsen et al., 2003). Sexual dimorphism is evidently present in *P. caniculata* through shape differentiation which several studies accounted (Tabugo et al, 2010 & Torres et al, 2013). Shape variations and covariations are significant elements in determining morphological distinctions within the species of the same taxa (Cabuga et al., 2016).

In describing morphological variations in the shell shape of *P. caniculata*, geometric morphometric was employed. Geometric morphometric (GM) is a tool for describing shape and shape variances among biotic elements. The method proposed to distinguished and analyzed shape differences from the obtained coordinates assigned in the body shape of organisms and eliminating the effects of orientation, position and size. The X and Y Cartesian coordinates will analyze the shape variations among, between, and within the populations of organisms (Adams et al, 2004). GM has established the importance of determining quantitative shape analysis of different biological forms (Moneva et al., 2012). It is also an effective tool for determining sexual dimorphism among snail shell shape of *P. caniculata* (Galliguez et al., 2009; Minton and Wang, 2011). Several studies have utilized GM in order to quantify variances in the body shapes of aquatic organisms (Mahilum and Demayo, 2014). Indeed, GM contributes advances over traditional measurements of examining the effects dissimilarities in orientation, location, and position of the organisms (Bookstein, 1991; Chiu et al., 2002; Rohlf, 2003; Zelditch et al., 2004). This application is a breakthrough in science particularly in biology since biological forms are essential for evolutionary history (Cabuga et al., 2016). The geometric morphometrics plays an important instrument for showing shell shape variances (Samadi et al., 2000; Galliguez et al., 2009; Minton and Wang, 2011). Utilizing landmark-based methods was effective means of identifying subtle differences among biological structures (Ibañez et al., 2007). It allows illustrating phenotypic variances and transforming dissimilarities into an image shape thus proposing a three-dimensional shape of variations (Webster and Sheets, 2010). Hence, this study aims to identify sexual dimorphism in the shell shape of *P. caniculata* through geometric morphometric using landmark-based analysis.

2 Materials and Methods

This study was conducted at Lake Dakong Napo, Lake Oro, and Lake Cebulan, Esperanza, Agusan del Sur, Philippines. It geographically lies between 8^{0}55’92.94”N and 125^{0}52’22.32”E. The collection of the samples was done in the month of February and March 2017 with the aid of local fisherman.

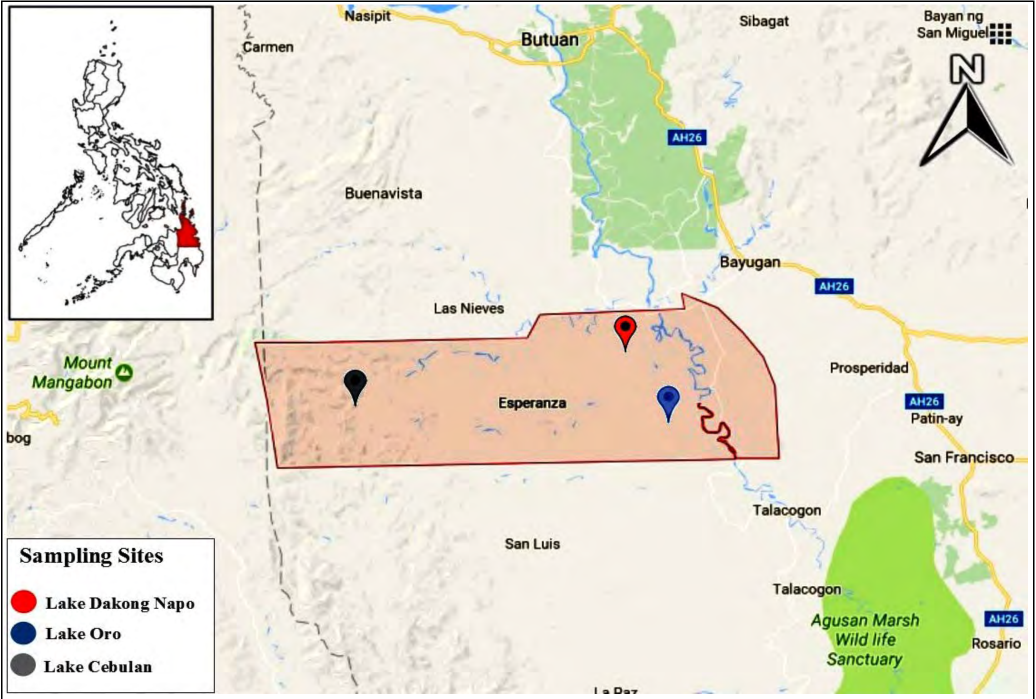

Fig. 1 Map of the study area Esperanza, Agusan del Sur, Philippines.

2.1 Sample processing

The samples of *P. caniculata* were collected from the three different lakes in Esperanza, (Lake Dakong Napo, Lake Oro and Lake Cebulan) Mindanao, Philippines (Fig. 1). About 180 individuals were randomly sampled, each consists of 30 males and 30 females per study area. Preliminary methods were applied to the shell; it was boiled with water and cleaned with running water while extracting the meat using forceps. After which, shells with eroded and cracks spire were excluded (Mahilum and Demayo, 2014). The images of the shell were captured by a digital camera (Lenovo, 13 megapixels). The position of the shell was in the columella at 90^{o} in order to emphasize the aperture view or the apex is visible (Moneva et al., 2012). To eliminate measurement error, the samples were tri-replicated and captured with the same position.

2.2 Landmark selection and digitization

From the obtained images, it was sorted according to its sexes and transferred into tps files using tpsUtil. The samples were then digitized using the landmarking method of the tpsDig version 2 (Rohlf, 2004). A total of 17 and 21 anatomical landmarks points in dorsal and ventral aperture were used in this study (Fig. 2).

2.3 Shape analysis

The converted tps files with corresponding anatomical landmarks were subjected to tpsRelw to acquire the relative warp analysis (RWA) and to get the X and Y Cartesian coordinates. In comparing patterns of sexual dimorphism, histograms were utilized to describe and represent variations between the sexes. The resulting relative warp scores of the shell shapes were analyzed and computed using the Paleontological Statistics Software (PAST). This provides significant details on the dissemination of the collected data from mean over the range of the variable. The accumulated coordinates were then subjected to MANOVA, Principal Component Analysis (PCA), and Canonical Variance Analysis (CVA) (Hammer et al., 2009).

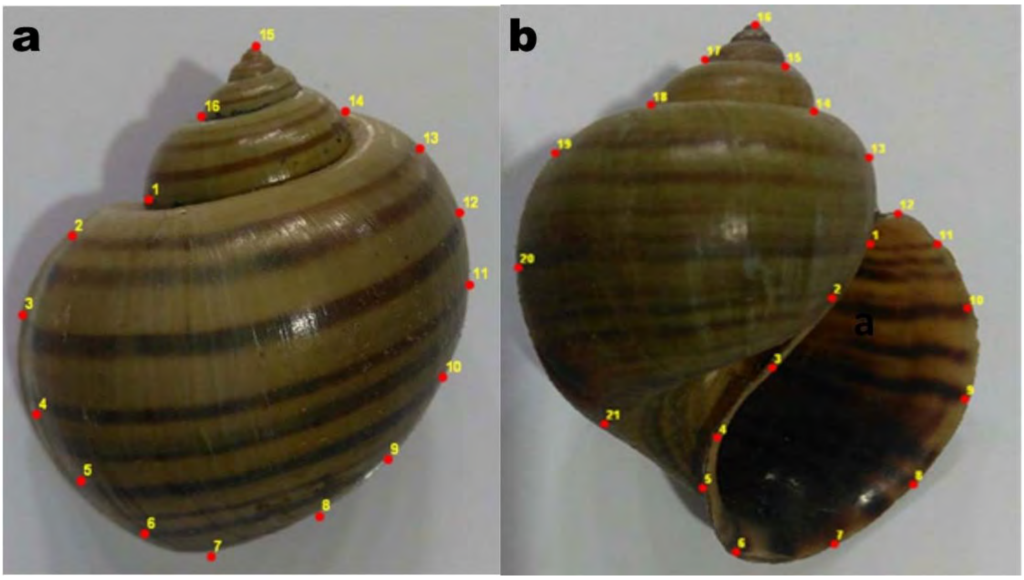

Fig. 1 Landmark points of the shell shape of *P.caniculata* (**a**) dorsal (**b**) ventral / apertural view.

3 Results and Discussion

The obtained results show a high significant value of ($P<0.05$) in the appended female and male dorsal and ventral/apertural portion from Lake Dakong Napo and Lake Oro. Similarly, the appended female and male dorsal portion from Lake Cebulan also shows a high significant value, however the appended female and male ventral/apertural portion shows a non-statistically significant (Table 1).

Table 1. Results of MANOVA test for pooled female and male dorsal & ventral/apertural portion.

FACTORS	WILK'S LAMBDA	DF1	DF2	F	P-VALUE
		Lake Dakong Napo			
Female & Male Dorsal	0.6597	5	174	17.95	2.464E -14**
Female & Male Ventral	0.8082	5	174	8.26	5.045E -07**
		Lake Oro			
Female & Male Dorsal	0.8165	4	175	9.835	3.362E-07**
Female & Male Ventral	0.7198	5	174	13.55	3.694E-11**
		Lake Cebulan			
Female & Male Dorsal	0.8607	4	175	7.081	2.636E -05**
Female & Male Ventral	0.333	4	175	87.63	9.694E -41^{ns}

*** highly significant, ns-non-significant.*

Table 2 Description of the observed variations in the dorsal shell shape of *P. caniculata* as produced by five significant relative warps.

RW	MALE	FEMALE
1	The first relative warp (RW1) demonstrates the dissimilarities of the shells body and spire. The observed variations found along the samples at negative axis which more pronounced and longer apical spire rather than a short and wider spire at the positive axis.	The RW1 shows the differences of the shells body and spire. Samples at positive axis have a wider body and less pronounced apical spire while the negative axis has a narrower body size but longer & pronounced apical spire.
2	The second relative warp (RW2) illustrates the variation of the shells body and spire. It was detected that samples at negative axis apical spire were pronounced while at positive axis apical spire was shorter.	The RW2 displays unlikeness in the apical spires. Samples at negative axis were wider apex leading to a shorter spire. While samples at positive axis have a longer apical spire.
3	The third relative warp (RW3) explains the differences in the body size and spire. Samples at negative axis tend to have a wider body and shorter spire. When compared to the samples at positive axis it has a narrowed body but defined spire.	The RW3 suggests variations in its spires height and body. Samples at positive axis show a longer apical spire while negative axis has a shorter apex. On the hand, the body of the samples at negative axis was wider when compared to a positive axis which is narrower.
4	The fourth relative warp (RW4) shows the differences in the shell and spires height. Samples at negative axis have a wider body and less pronounced spire. On the contrary, samples at positive axis have a narrowed shell leading to a more pronounced spire height.	The RW4 illustrates variances in the shell size. Samples at negative axis have a wider body size while samples at positive axis have a narrower body size.
5		Lastly, RW5 suggests a wider shell size leading to a shorter apical spire at negative axis when compared to a positive axis which is narrowed suggesting a well-formed apical spire.

Table 3 Description of the observed variations in the ventral shell shape of *P. caniculata* as produced by five significant relative warps.

RW	MALE	FEMALE
1	The first relative warp represents variation in the shell size opening. Samples at negative axis have slimmer/narrower size opening while samples at positive axis have a wider/globose shell opening.	The RW1 depicts unlikeness in the shell and shape of the opening. Samples at negative axis have a wider shell and narrower shape opening. While samples at positive axis have a slimmer body but wider shell shape opening.
2	The second relative warp denotes differences in the shell & shape opening. Those samples at negative axis have a shorter body size and narrower openings when contrast to the positive axis which has a wider/more globose opening.	The RW2 indicates shape and size differences in the body and shell opening. Samples at negative axis show wider body size leading to a narrower shell opening. While samples at positive axis have slimmer body size making the shell opening more pronounce/globose.
3	The third relative warp explains the dissimilarities of the shells body and shape/size opening. Samples at negative axis have a wider body size and narrower shape opening. While samples at positive axis have a narrower body size but wider shape opening.	The RW3 illustrates variances in the shells shape/size opening. Samples at positive axis have a wider/globose shape opening when compared to a negative axis which has a narrowed shape opening.
4	The fourth relative warp shows differences in the shell body and size opening. Samples at negative axis have a wider body and narrower shape opening. In contrast, samples at positive axis have a narrower body but wider shape opening.	The RW4 shows unlikeness of body size and shape opening. Samples at negative axis have a wider body and narrowed shape opening when compared to a positive axis which had a longer body and wider shape opening.
5		The fifth relative warp (RW5) explains variation in the shells body shape and size. Samples at negative axis have a wider body size and narrowed shape opening while positive axis had a narrowed/longer body and wider shape opening.

These morphological differences could be associated with the shell shape/size opening and height of the spire (Moneva et al, 2012). While variations among lake populations are the distinction of spires length/height, narrow/wide body, and or narrow/wide aperture (Mahilum and Demayo, 2014). Divergences in the shell shape opening could also be related to its predatory defense against decapods as the main predator of the freshwater snails (DeWitt, 2000). The spires height is also relatively important as a physical mechanism for defense and survival (Borra, 2006). Narrowed aperture possibly acts as a protection in the freshwater habitat against environmental alterations. On the contrary, wider aperture implies susceptibility to predation (Williams, 2005). Predators are natural entities that could harm shell shape opening or aperture (Mahilum and Demayo, 2014). These would create changes on the shells aperture, body, and spire. The influence of lake temperature has been associated to affects morphological trait and it is reasoned that temperature acts as a direct factor affecting the shell growth (Mahilum and Demayo, 2014). The observed variations in the male and female dorsal and ventral/apertural portions were presented in (Table 2 & 3). This shows the differences of spires height, body size and shell shape openings from the collected samples of *P. caniculata*.

The discrimination on the consensus morphology was established using relative warp analysis and found out that the collected populations of *P. caniculata* in the three lake indicate differences of their body size, shell shape opening, and spires height (Fig. 3 & 4). The collected samples in Lake Dakong Napo, males generated four relative warp (RW) scores accounted for 75.88% (dorsal) and 77.53% (ventral). While females generated five relative (RW) scores accounted for 78.87% (dorsal) and 79.69% (ventral). Meanwhile, the collected samples in Lake Oro, males generated four relative warp (RW) scores accounted for 80.27% (dorsal) and 79.90% (ventral). The females generated four relative warp (RW) scores accounted for 73.44% (dorsal) while the ventral portion generated five relative warp (RW) scores which are 81.77%.Finally, the collected samples in Lake Cebulan males generated three relative warp (RW) scores accounted for 75.88% (dorsal) while for ventral portion generated four relative warp scores (RW) accounted for 72.87%. The females generated three relative (RW) scores accounted for 75.10% (dorsal) while the ventral portion generated five relative warp (RW) scores accounted for 78.51%. In general, if we ranked the generated relative warp scores of the male and female dorsal/ventral portion of the three lakes, the resulting highest relative warp scores obtained from Lake Oro followed by the Lake Dakong Napo and Lake Cebulan. The variations between sexes and geographical distribution could also develop variations in the spires height, wide/narrowed body and narrow/wide shell shape opening (Mahilum and Demayo, 2014). Thus, it is interesting to know that even organisms of the same taxon variations are possible and could only be identified through discriminating size and shape between sexes.

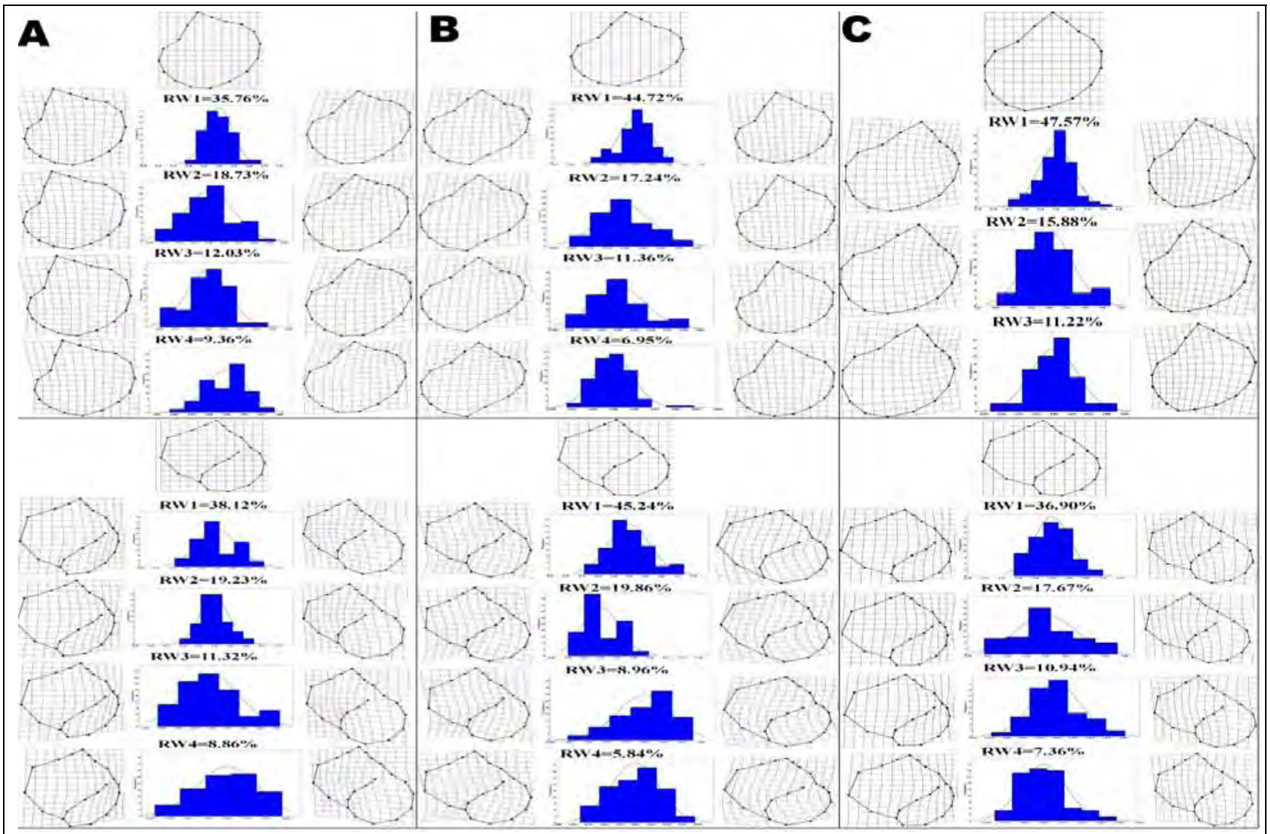

Fig. 3 Summary of the geometric morphometric analysis showing the mean shape and variation in dorsal/ventral of male populations of *P. caniculata* generated by Relative warps **A**. Lake Dakong Napo **B**. Lake Oro **C**. Lake Cebulan.

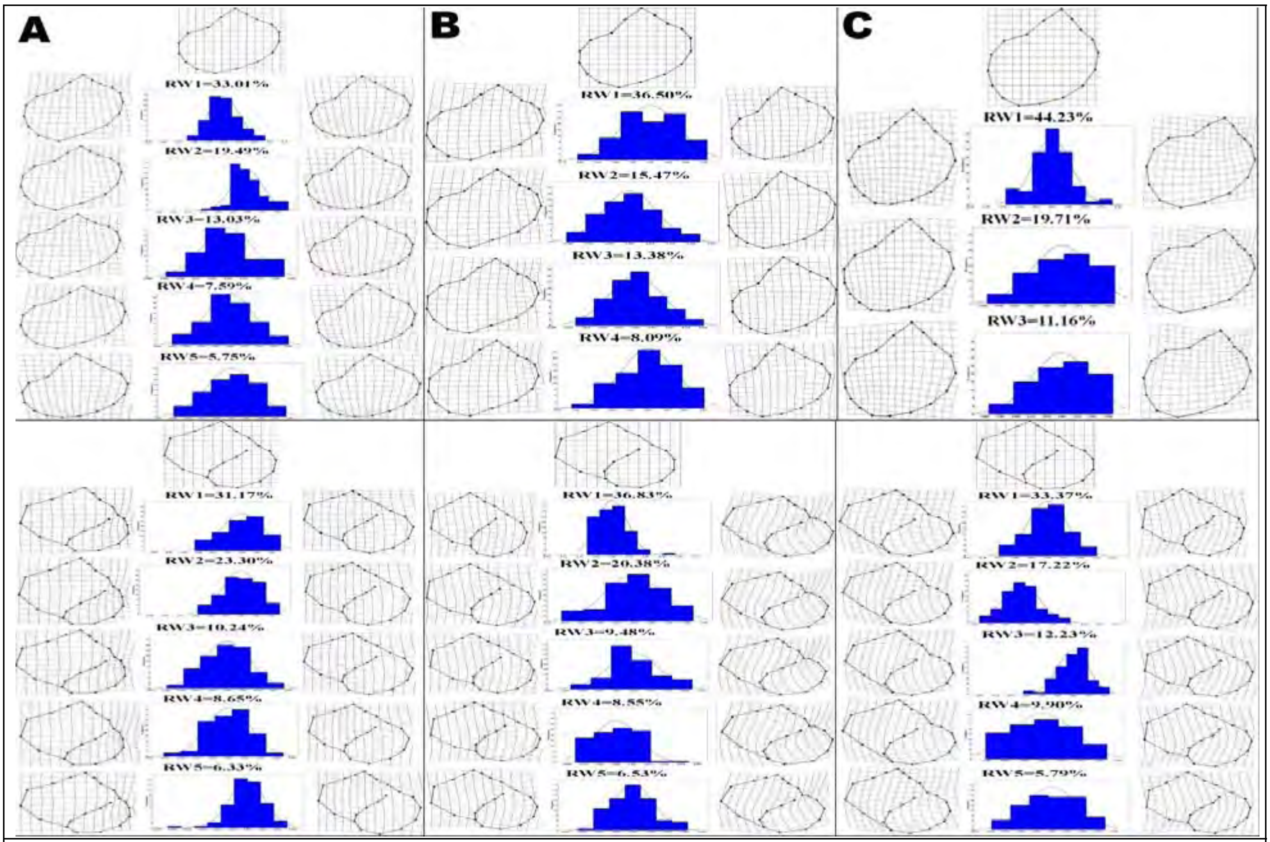

Fig. 4 Summary of the geometric morphometric analysis showing the mean shape and variation in dorsal/ventral of female populations of *P. caniculata* generated by Relative warps **A**. Lake Dakong Napo **B**. Lake Oro **C**. Lake Cebulan.

To identify sexual dimorphism based on shell shape from the collected samples in three lakes, populations, Principal Component Analysis (PCA) (Figs 5, 6, 7, 8, 9 & 10) and Canonical Variate Analysis (CVA) (Figs 11, 12, 13, 14, 15 & 16) was implemented. These illustrate sexual dimorphism based on the scatter plot provided by the analysis. The overlapping condition states the evidence in which two sexes shared phenotypic traits. Indeed, sexual dimorphism existed to determine the size and shape dissimilarities within the snail populations where females are larger than male (Estebenet and Cazzaniga, 1998). It is also believed that sexual dimorphism (SD) works as a compensatory mechanism for reproductive process, sexual selection and environmentally adaptive (Kokko and Brooks, 2003; Rankin and Arnqvist, 2008). Qualitative examination of *P. caniculata* showed that female operculum and aperture much broader when compared to male (Cowie, 2006). Environmental component establishes phenotypic variability and sexual dimorphism in which organism utilizes for survival and adaptation. Further, morphological variations in the shell shape have been associated with nutrient availability, habitat and climatic condition (Cabuga et al., 2017).

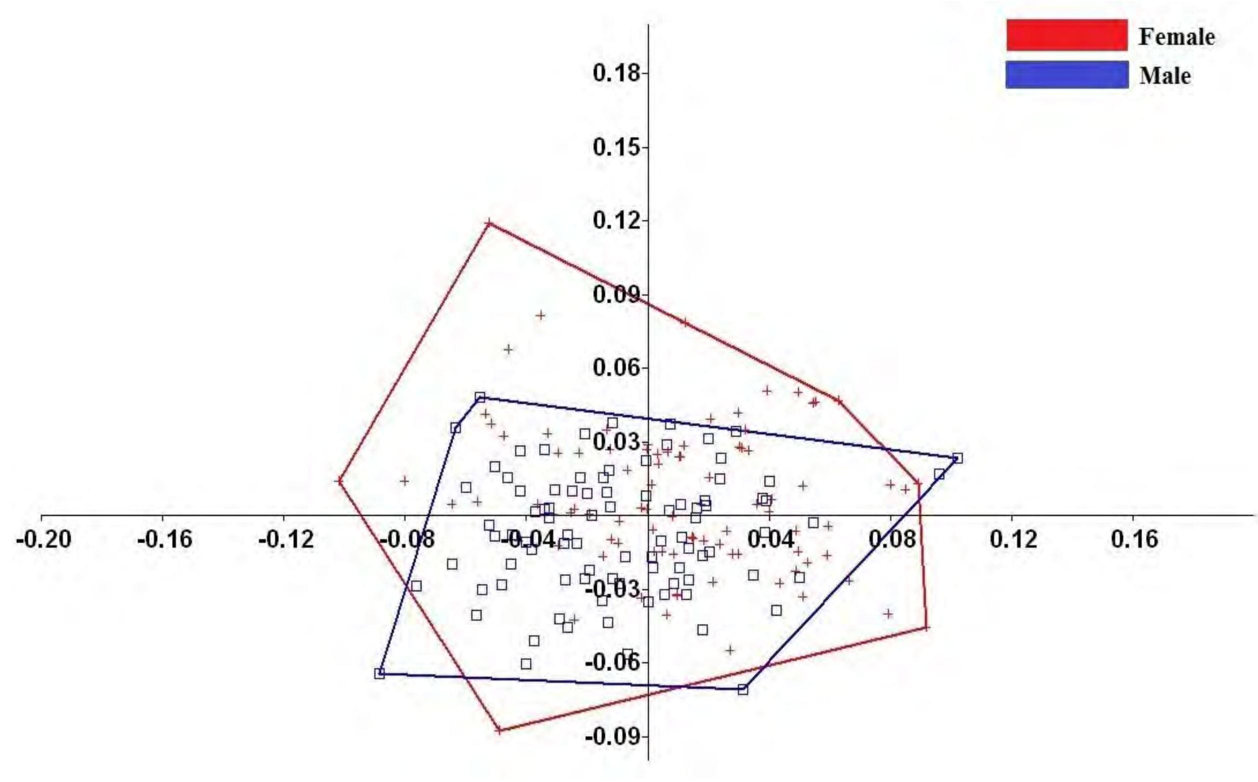

Fig. 5 PCA scatterplot showing appended female and male dorsal portion of *P. caniculata* collected at Lake Dakong Napo.

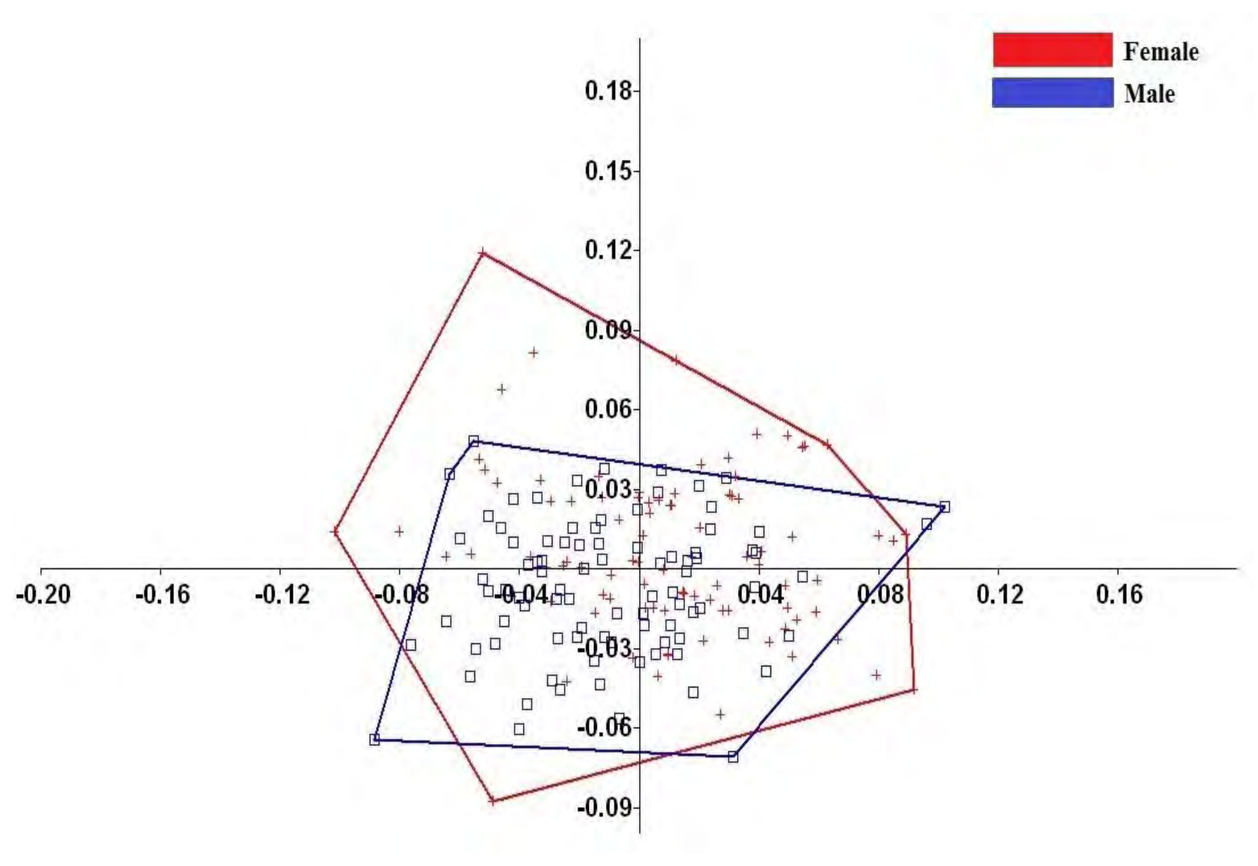

Fig. 6 PCA scatterplot showing appended female and male ventral portion of *P. caniculata* collected at Lake Dakong Napo.

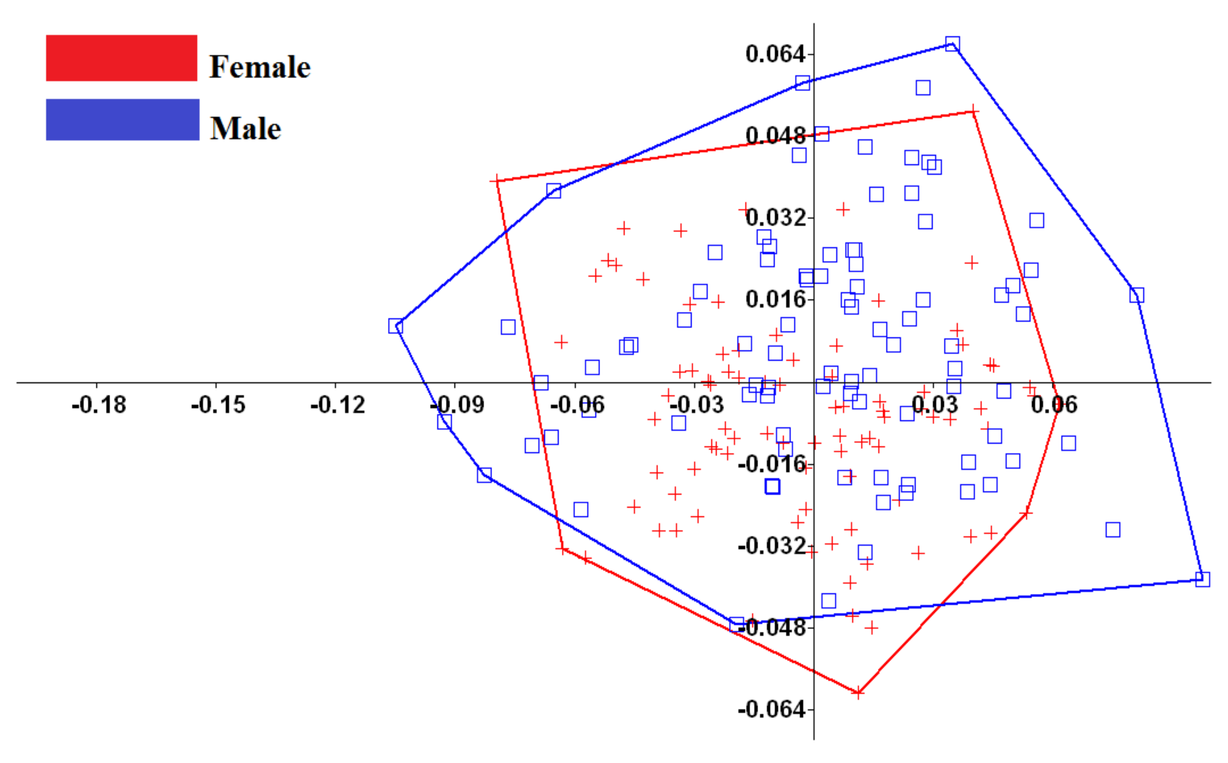

Fig. 7 PCA scatterplot showing appended female and male dorsal portion of *P. caniculata* collected at Lake Oro

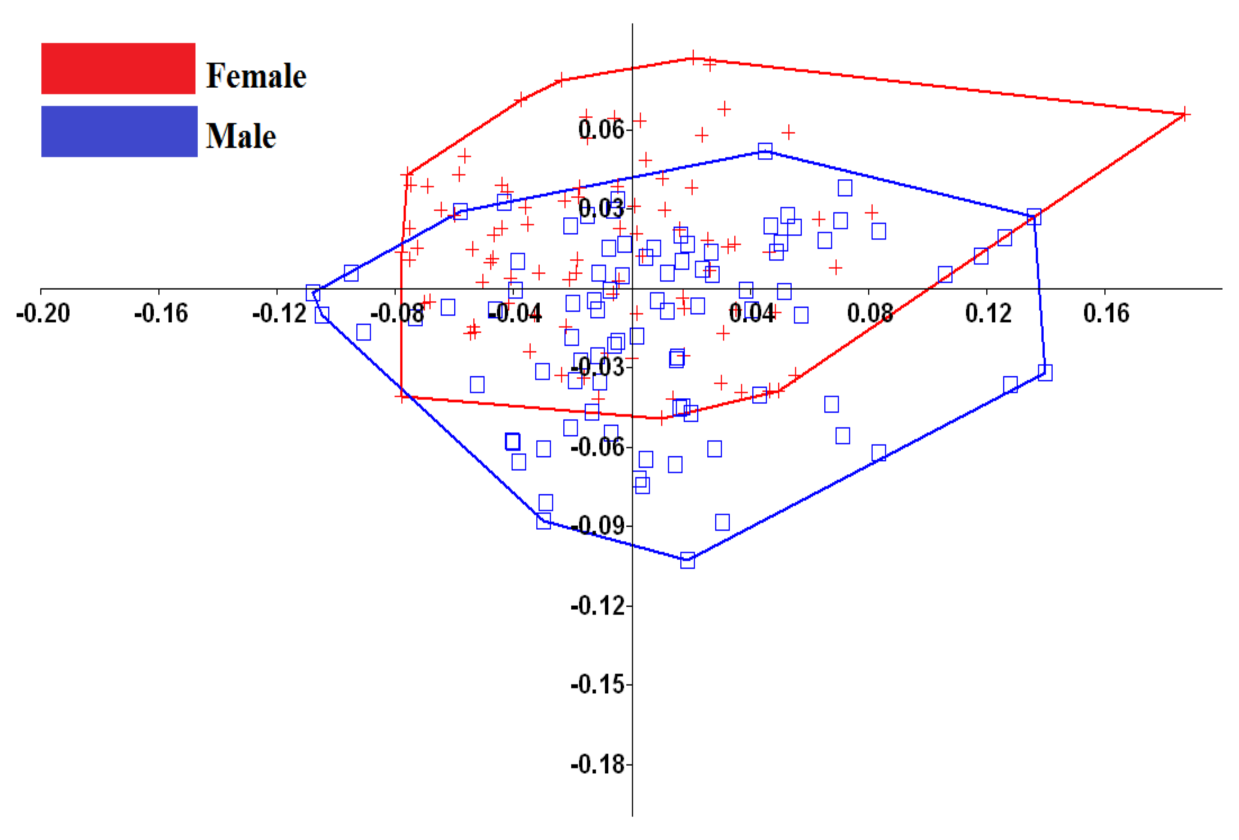

Fig. 8 PCA scatterplot showing appended female and male ventral portion of *P. caniculata* collected at Lake Oro.

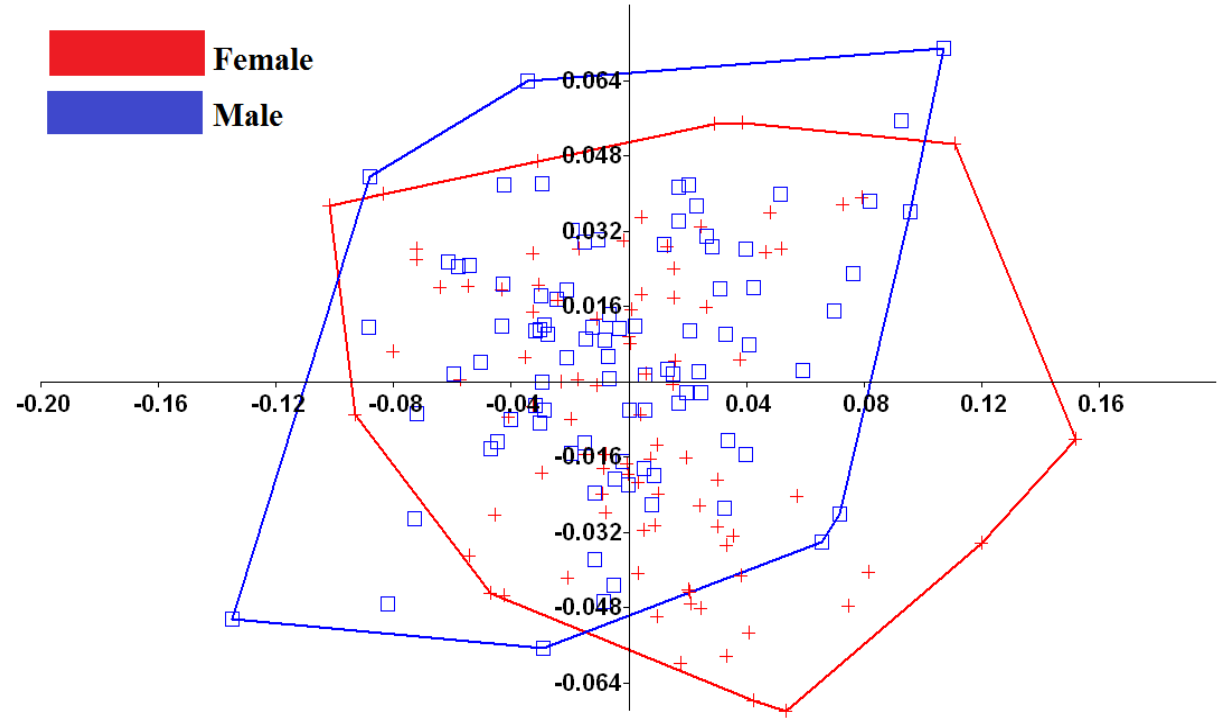

Fig. 9 PCA scatterplot showing appended female and male dorsal portion of *P. caniculata* collected at Lake Cebulan.

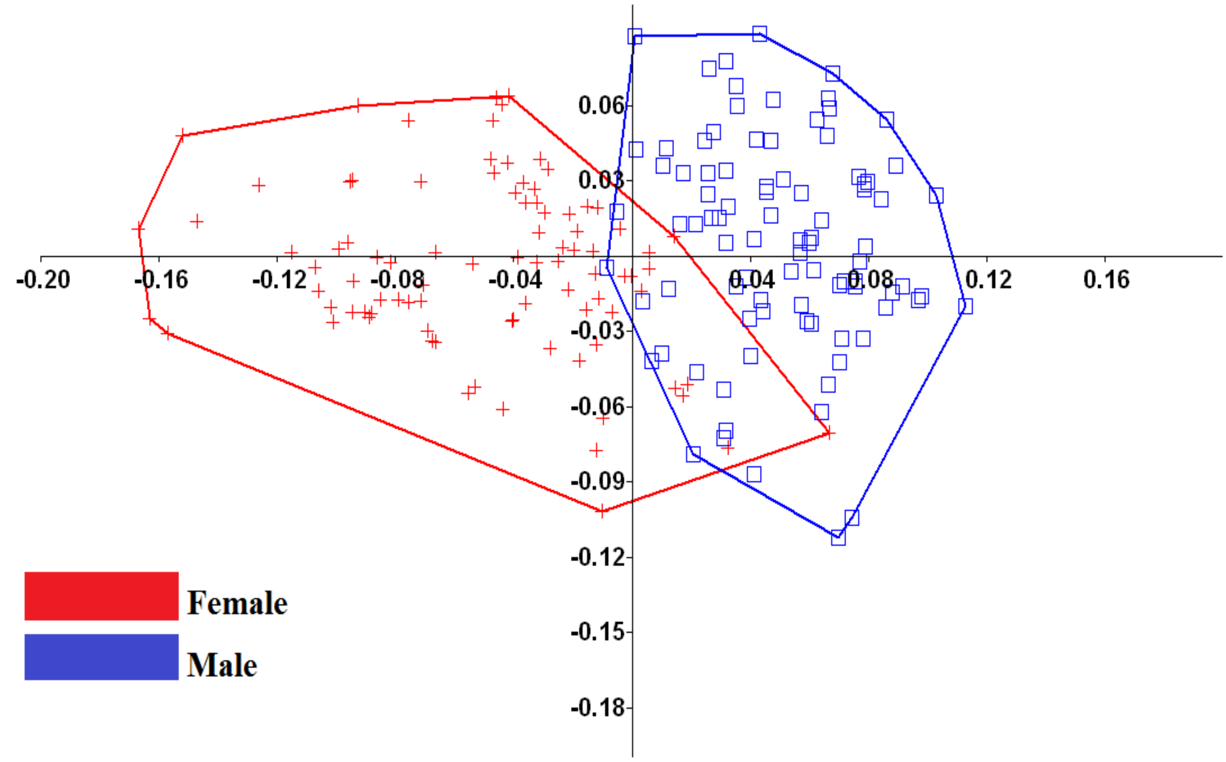

Fig. 10 PCA scatterplot showing appended female and male ventral portion of *P. caniculata* collected at Lake Cebulan.

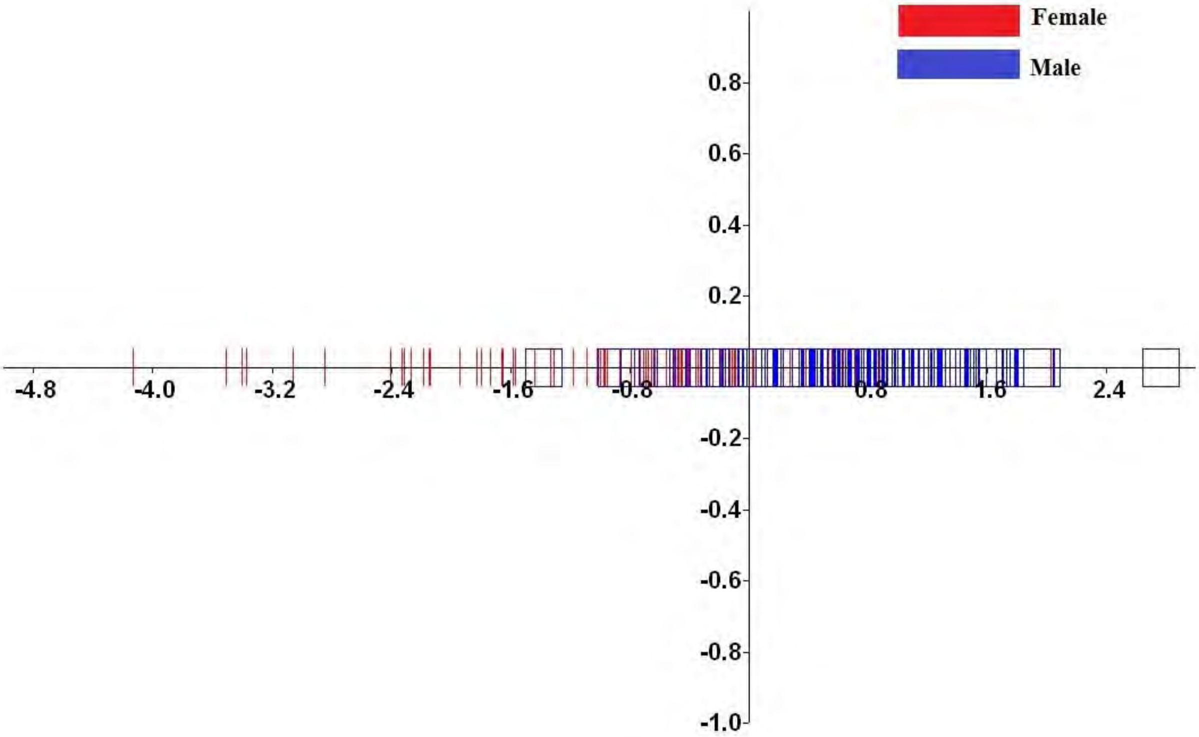

Fig. 11 CVA scatterplot showing appended female and male dorsal portion collected at Lake Dakong Napo.

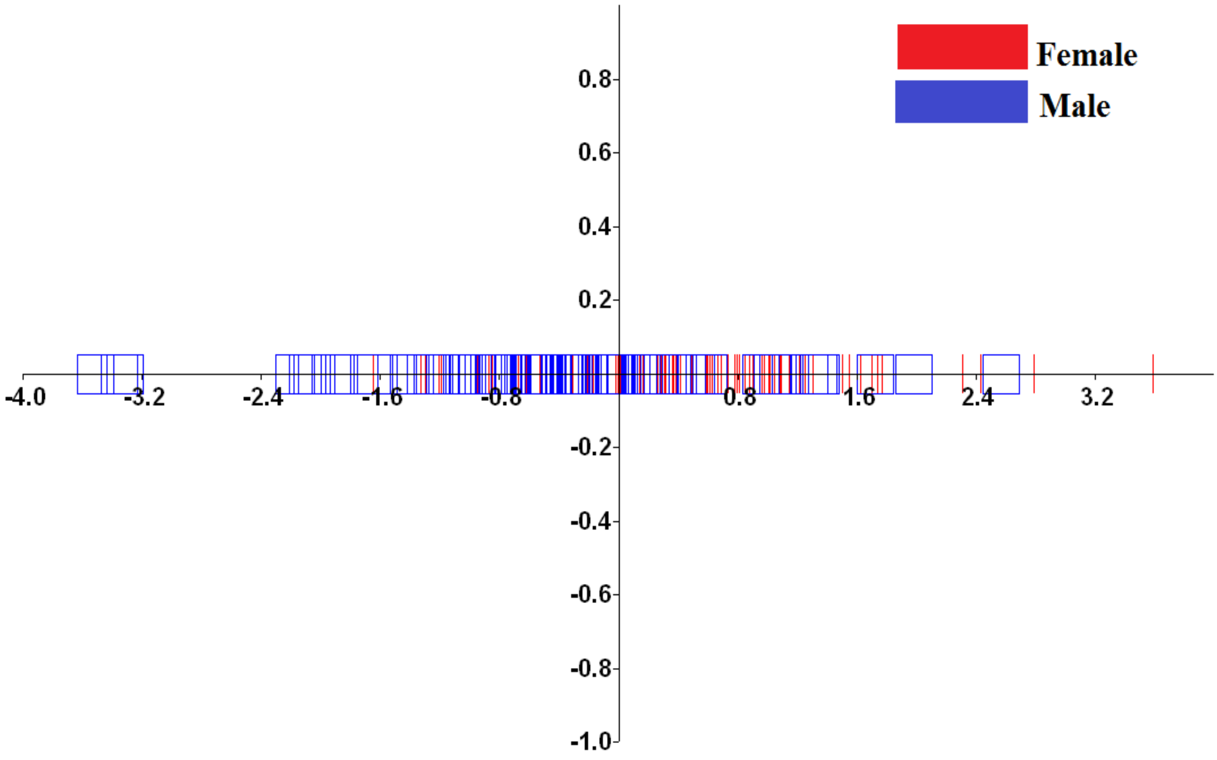

Fig. 12 CVA scatterplot showing appended female and male ventral portion collected at Lake Dakong Napo.

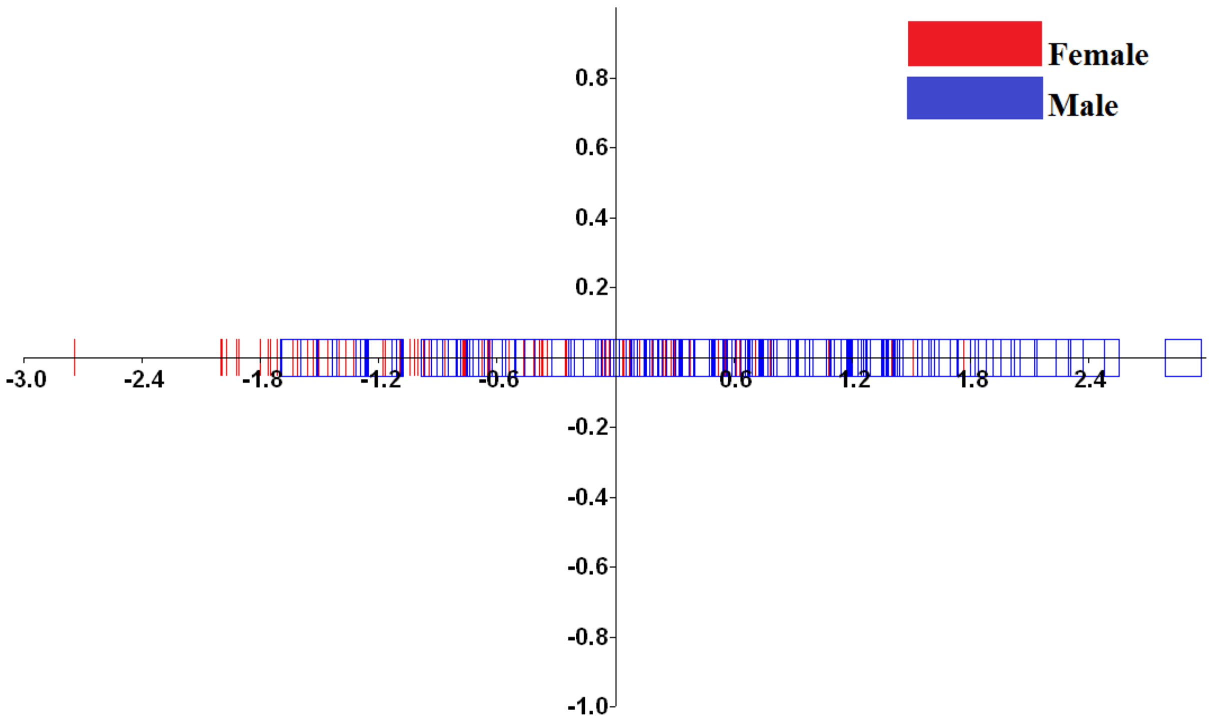

Fig. 13 CVA scatterplot showing appended female and male dorsal portion collected at Lake Oro.

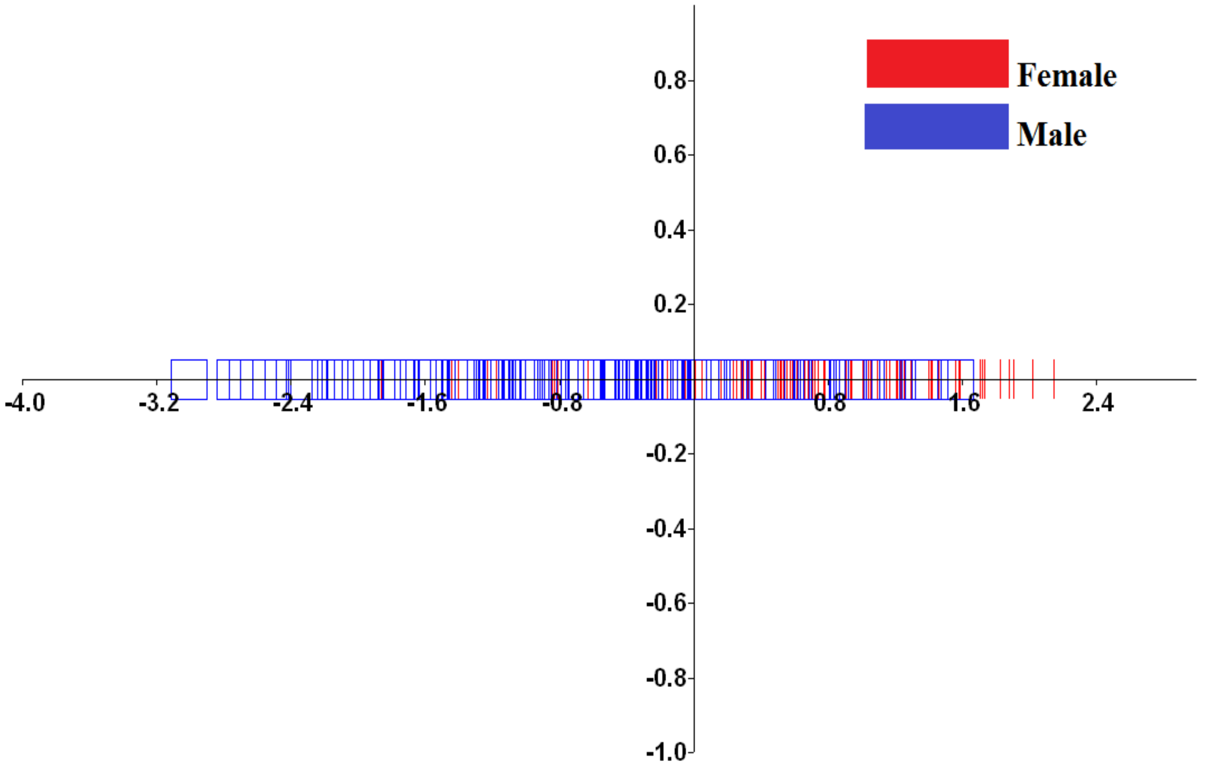

Fig. 14 CVA scatterplot showing appended female and male ventral portion collected at Lake Oro.

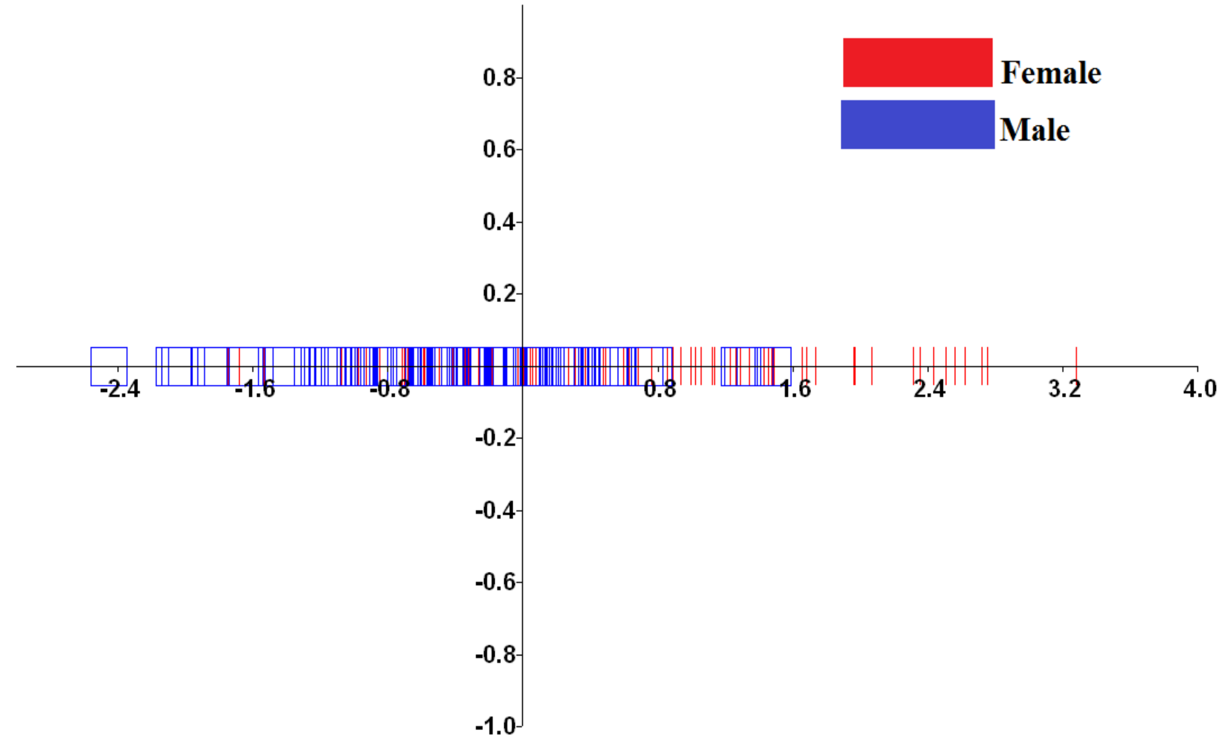

Fig. 15 CVA scatterplot showing appended female and male dorsal portion collected at Lake Cebulan.

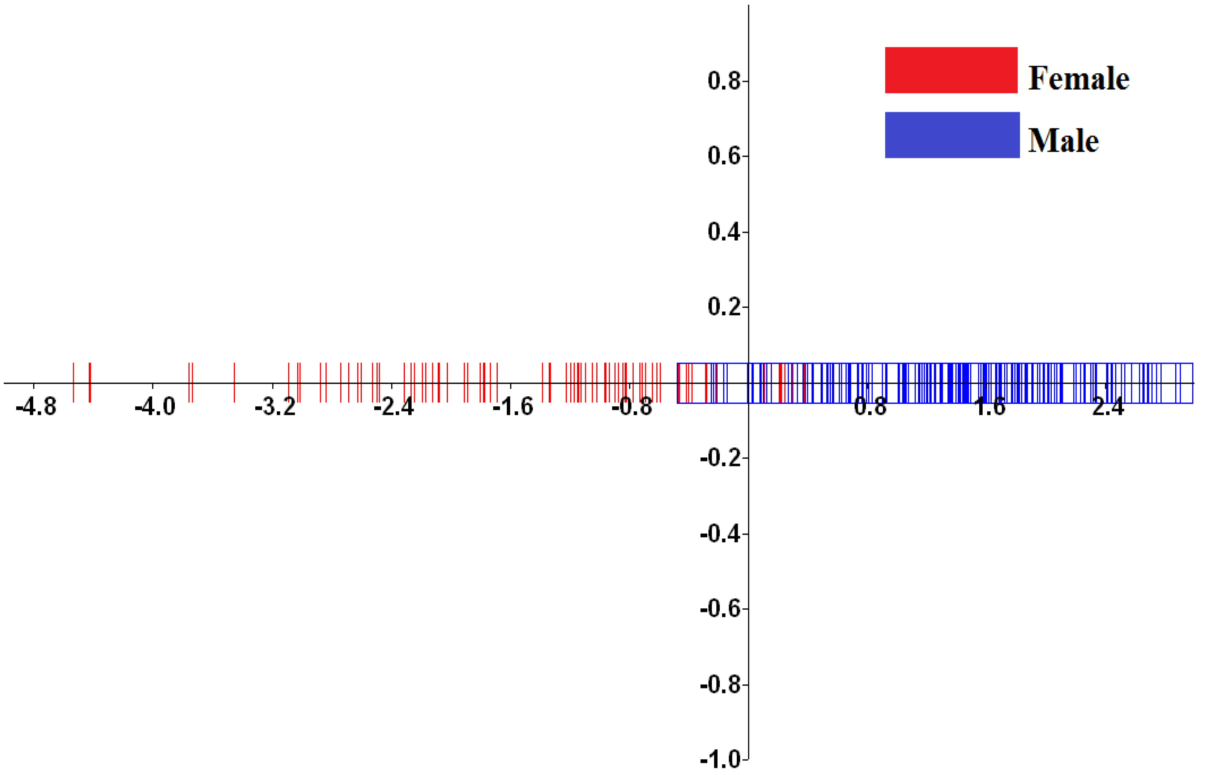

Fig. 16 CVA scatterplot showing appended female and male ventral portion collected at Lake Cebulan.

4 Conclusion

Relative warp analysis was applied to discriminate shell size and shape variations between sexes of *P. caniculata*. In MANOVA the obtained result shows statistically significant (P<0.05) in the appended male and female dorsal and ventral portion of the three lakes population while Principal Component Analysis (PCA) and Canonical Variate Analysis (CVA) identifies sexual dimorphism in the male and female samples. It indicates that phenotypic variations among snails are prevalent and these associated with geographical isolation, predation and nutrient requirement of the gastropods. Furthermore, the importance of using geometric morphometrics (GM) provides subtle details in performing quantitative shape analysis in the shell shape and utilizing GM is an efficient tool to identify morphological variations could establish shape differentiation among or within species belonging to the same taxon.

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