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# Phenotypic variation in populations of *Pomacea canaliculata* (golden apple snail): a case of agroecotypes?

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**Abstract**. Evolutionary biology uses most preferably molluscan model such as *Pomacea canaliculata* (Lamarck) to demonstrate evolutionary adaptations to different ecological conditions. Peculiar adaptations of *P. canaliculata*, a dioecious golden apple snail have recently been recognized as important, albeit neglected, models for evolutionary ecology studies. Several studies have shown different interpretations about shell shape variation and sexual dimorphism on *P. canaliculata* however, this study sought to evaluate and determine the existence of phenotypic variation in the shells of golden apple snails using geometric morphometric techniques on geographically isolated ricefield's populations from three different geographical zones in Mindanao, Philippines. Three views of the shell shape were studied, which includes the ventral/aperture, dorsal and the top/whorl portion of the shell. Results show extremely significant phenotypic variations on the different shell part views (aperture, dorsal and whorl) in both male and female *P. canaliculata* collected from three different ricefields. Since phenotypic traits are assumed to correlate with similarity in habitat use, this phenotypic variation could possibly be a case of agroecotype, a phenomenon which needs to be further explored in this most proliferative rice pest *P. canaliculata* since this term is applied to a crop or organism being adapted to its environment. This population studies could provide significant biological information for further management strategies against this invasive pest.
**Key Words**: sexual dimorphism, geometric morphometrics, population studies, invasive species.

**Introduction**. Phenotypic variation can be defined as morphological differences exhibited by organisms in response to its ecological conditions (Gilbert 2000; Relyea 2002). The interplay of biotic and abiotic aspects of their environments may cause animal populations to exhibit different reaction norms in response to salinity, reduced resources and predators especially in managed ecosystems such as the rice fields (Miner et al 2005).

*Pomacea canaliculata* (golden apple snail) is one of the major pests in rice fields. It was originally introduced in the Philippines from South America for human consumption purposes however; it has become one of the most destructive pests of lowland rice and has been listed as one of the 100 Worst Invasive Species (Burela & Martin 2011; IUCN 2014). Wackernagel & Rees (1997) estimated that yield tosses owing to this pest ranged from 1% to 40% of the planted area in the Philippines, resulting in huge production loss. Of the 3 million (M) hectares of rice lands in the Philippines, 1.2-1.6 M hectares are infested with golden snail. In 1990, P212M was spent to control this pest (Kenji 2003; Moneva et al 2012a). The first account that it had become a major pest was recorded in 1986 when about 300 hectares of irrigated rice farms in Region 2 (Cagayan Valley) were heavily damaged. Since then, rice area infested with this pest has been increasing until it became a national menace (IRRI 2014).

To minimize this loss, an integrated approach consisting of cultural (proper farming practices), biological (inducing predators such as ducks) and chemical components (application of molluscicides) was employed (Teo 2001a, b; Integrated Golden Apple Snail (Kuhol) Management DA/FAD 1989). Paddy farmers practice proper crop husbandry to counteract this snail attack. However, the problem of its rampant proliferation is still a big problem in agriculture, especially in rice fields. Ricefields are type of managed, agricultural ecosystems characterized by extreme instability resulting from frequent destruction of the environment by farming practices (Figueroa et al 2014; Joshi 2010).

According to Relyea (2002), the ability of an organism such as *P. canaliculata* to produce alternative phenotypes in response to environmental change is often an adaptive strategy to minimize loss of fitness in a harsher environment (e.g. managed ricefields) or to maximize fitness in a favorable environment. Thus, phenotypic variation responses are thought to evolve due to disruptive selection for alternative phenotypes across temporally or spatially heterogeneous environments. If individuals are organized into distinct populations that differ in the environmental homogeneity that they experience and there is low dispersal among populations, then the evolution of population-specific norms of reaction is observable.

Agroecotype is defined as a crop-plant type or organism adapted to a particular environment such as agricultural lands (Basafa & Taherian 2009; Piergiovanni et al 2004). Phenotypic traits are assumed to correlate with similarity in habitat use. Chiba (2009) emphasized that the possible existence of different strategies to serve the same function, or of adaptive changes to use the same resource or habitat, may cause divergence of such phenotypes. In the case of *P. canaliculata*, certain adaptive measures in a managed ricefield ecosystem are evident to maximize its fitness despite being faced with extreme instability of their environment. This phenotypic variation in the shell shape of the golden apple snail could be a case of agroecotype.

Because of these golden apple snails' low mobility, their populations tend to reflect phenotypic variations. In a study by Torres et al (2011), *P. canaliculata* collected from different ecological situations (managed and unmanaged ecosystems) showed variability within and among populations and that geography alone could not explain such variability. Mahilum & Demayo (2014a) further stressed that the observations of intraspecific variations in many populations make this species a good model for inferring the mechanisms behind population differentiation as this organism is believed to have evolved into a complex of morphologically divergent populations in response to ecologically diverse habitats.

Evolutionary adaptations as defined by Crispo (2008) are traits that increase fitness, the driving force for natural selection. Traits that increase the survival rate of a species contribute to an animal's fitness, but selection will only favor such traits insofar as survival improves the reproductive success of the organism.

Evolutionary ecological studies focusing on *P. canaliculata* populations such as this are significant in integrated pest management. As to the question whether this is a case of agroecotypes will further be explored and given light. Such implication is inferred in this study. These geometric morphometrics analysis study sought to quantify morphological characters that have adaptive significance in this evolutionary successful ricefield pest.

**Material and Method**. Adult *P. canaliculata* snails were handpicked purposively along the 3 geographically isolated rice fields. Figure 1 shows the three geographical locations of the rice fields where *P. canaliculata* were collected in Mindanao, Philippines: a) Butuan City - Region XIII, b) Iligan City - Region X, and c) Pagadian City - Region IX.

Thirty (30) males and 30 females were randomly chosen as a representative for the population of each, having a total of 180 specimens. Sex identification was based on the works of Torres et al (2011) and Yanes et al (2010) wherein female's aperture accordingly curves inward while the male shell curves outward.

Geometric morphometrics (specifically landmark-based methodology) was then employed. This quantitative representation and analysis of morphological shape uses geometric coordinates instead of measurements. Phenotypic plasticity in morphometric traits could be observed using this approach (Ginter et al 2012; Addis et al 2010; Rohlf 2001).

In coordinate acquisition, each snail from each population was photographed on its dorsal, ventral and top view parts using WG1 Pentax camera (14 megapixel, optical zoom 10x). The same light exposure was employed all throughout. Images of the shell were oriented in the same position with the columella at 90° of the x-axis in the aperture view or in the orientation in which the apex is visible. The digital photographs were then processed using TpsDig 2.10 software (Rohlf 2006) for landmark acquisition. Seventeen (17) landmarks were identified on the dorsal part; 21 landmarks on the ventral part and 13 landmarks on the top view showing the whorl. Figure 2 shows the landmarks acquired in the 3 different views of the shell.

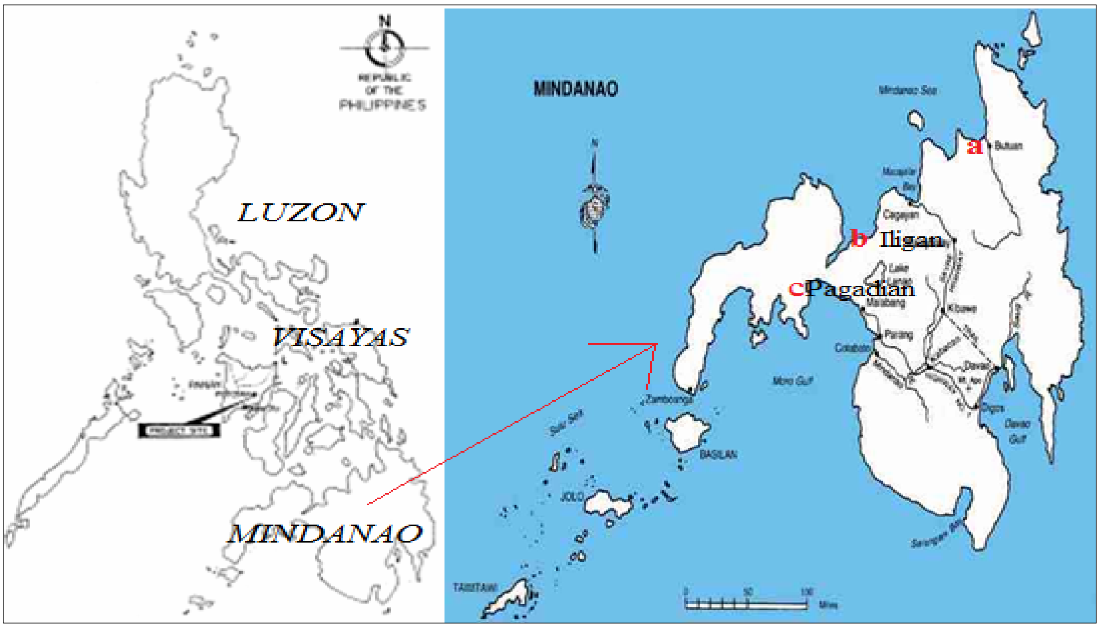
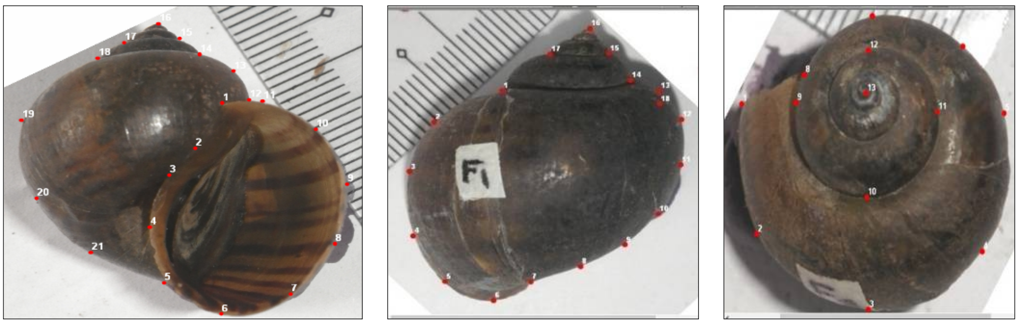



Figure 1. Map showing the geographical location of the 3 rice fields sites; (a) Butuan City, (b) Iligan City and (c) Pagadian City)-taken from google map.com images.



a b c

Figure 2. Landmark acquisition of *P. canaliculata* showing the (a) apertural view; (b) dorsal view, and (c) whorl.

***Transformation and multivariate analyses***. Each set of coordinates were submitted separately to a Generalized Procrustes Analysis (GPA) available in the tpsRelw software (Rohlf 2001). This procedure translated, rotated and scaled the original configurations in order to achieve the best superimposition of all shapes. The size of each specimen is represented by the "centroid size", a measure that is able to estimate the size in all directions in a body better than is possible by using univariate measures such as maximum length. After this superimposition, the software breaks down the morphological difference into a series of non-uniform components, described as partial warps. The scores of the specimens on the partial warp axes constituted the shape variables that were used in the subsequent statistical analyses (Rohlf 2007). The software was used to introduce shape variables into a Principal Component Analysis (PCA), and to visualize the warping associated with the various principal components (PCs). These components represent relative warps in the context of a TPS (thin-plate spline) approach (Burela & Martin 2011). PCAs can identify any regularity within the sample. In a morphometric analysis, these regularities correspond to simultaneous displacements of anatomical points that are often observed in the specimens. A value is assigned to each relative warp and is expressed as a percentage, reflecting the proportion of the variation accounted for by this component. PCA automatically classifies the relative warps in decreasing order of their specific values. The greatest variations, generally attributable to biological factors, occur in the first few relative warps. The morphological warps associated with each component are visualized by observing the conformations corresponding to the points located at the ends of the axes. The changes in shape are illustrated by a potentially warpable grid, which represents the warps corresponding to a consensus (an average individual).

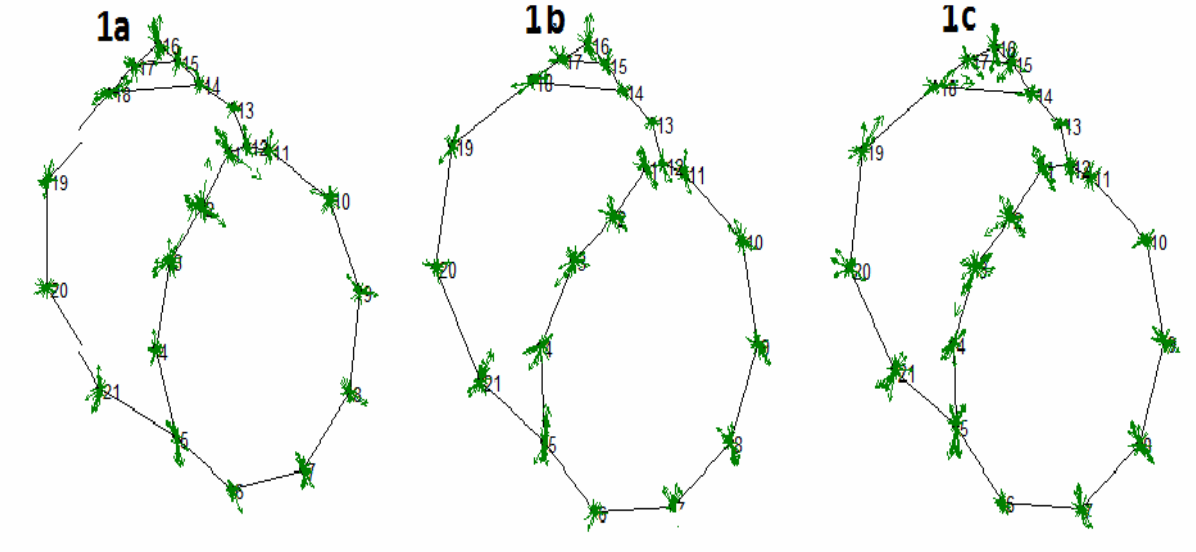
In the presentation of the results, the following statistics were used: (1) Descriptive Statistics using TPS; (2) Exploratory Statistics using Canonical Variate Analysis (CVA) and (3) Confirmatory Statistics using Multiple Analysis of Variance (MANOVA).

## Results and Discussion

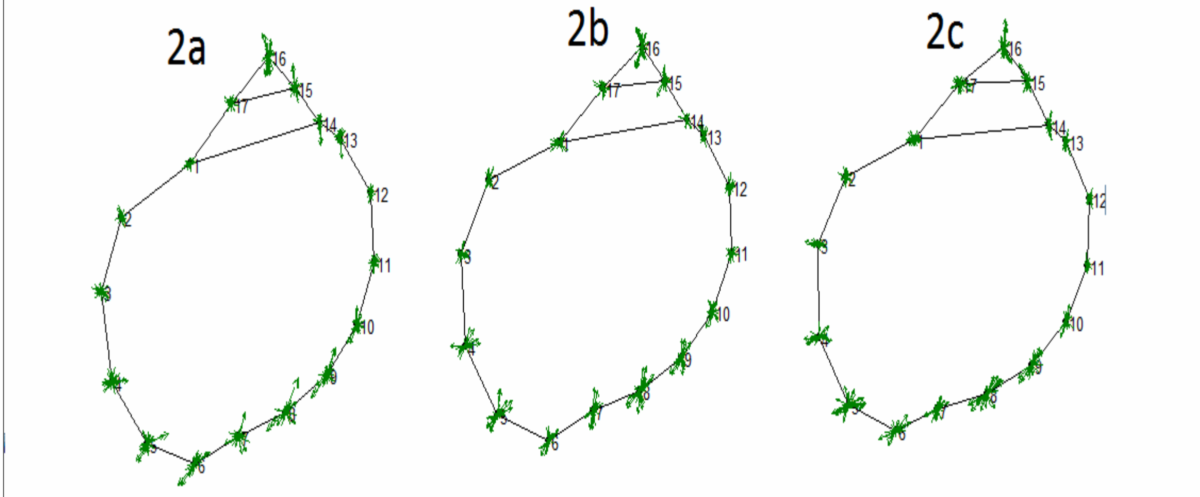
***Descriptive statistics presentation on the phenotypic variation of the P. canaliculata using Thin-Plate Spline (TPS) approach****.* Figures 3 and 4 show the visual presentation of the phenotypic variations on the different views of male and female *P. canaliculata* snails in three geographic locations.

It can be seen that the samples collected from Butuan ricefields (1a) have longer and wider aperture when compared to the ricefield populations of Iligan (1b) and Pagadian (1c) which have smaller-sized aperture. The 3 populations also vary with respect to the maximum width of the shell (ventral view). The shells collected from the ricefields of Iligan had shown relatively bigger shells than those found in Butuan and Pagadian (2a-c).

These scientific observations are supported by the work of Chiba (2009) which states that phenotypic traits can be diverse, even if they result from a common adaptation to the same managed environment such as in the case of ricefields. In his experiment, an increase in the aperture and width expansion was found to be adaptive responses to the same burrowing lifestyle. Based on interviews from the local farmers, Butuan ricefields are treated with strong molluscicides while Pagadian ricefields are treated with biological measures by inducing ducks before seedling transplantation. Iligan ricefields are treated with both. This practice to counter-attack snail attack as well as geographical variations could be some factors in contributing to the snail's population phenotypic diversity.

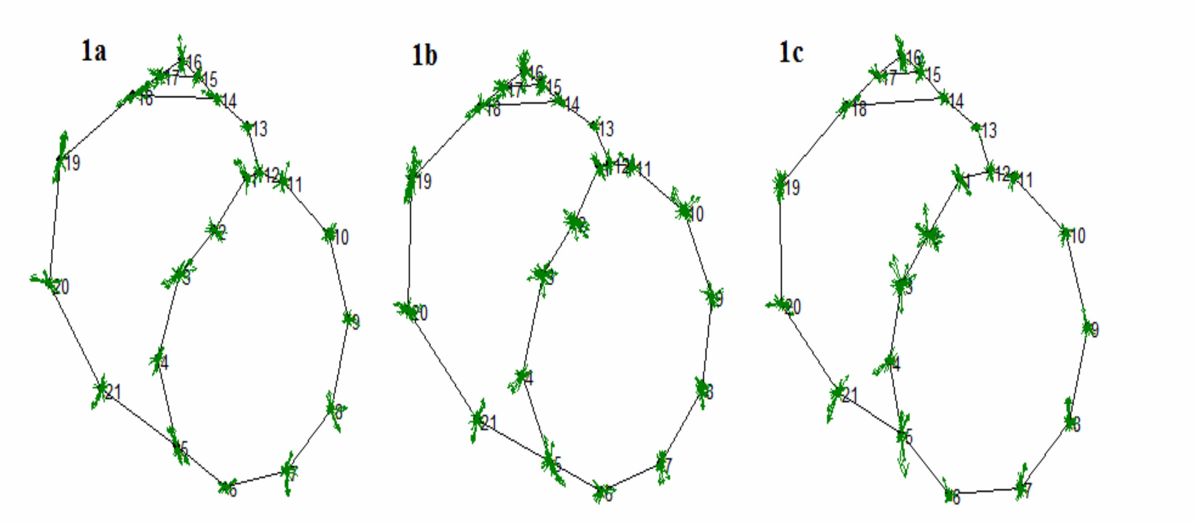



Ventral part of male *P. caniculata* showing apertural variations - (1a) Butuan ricefields; (1b) Iligan ricefields and (1c) Pagadian ricefields

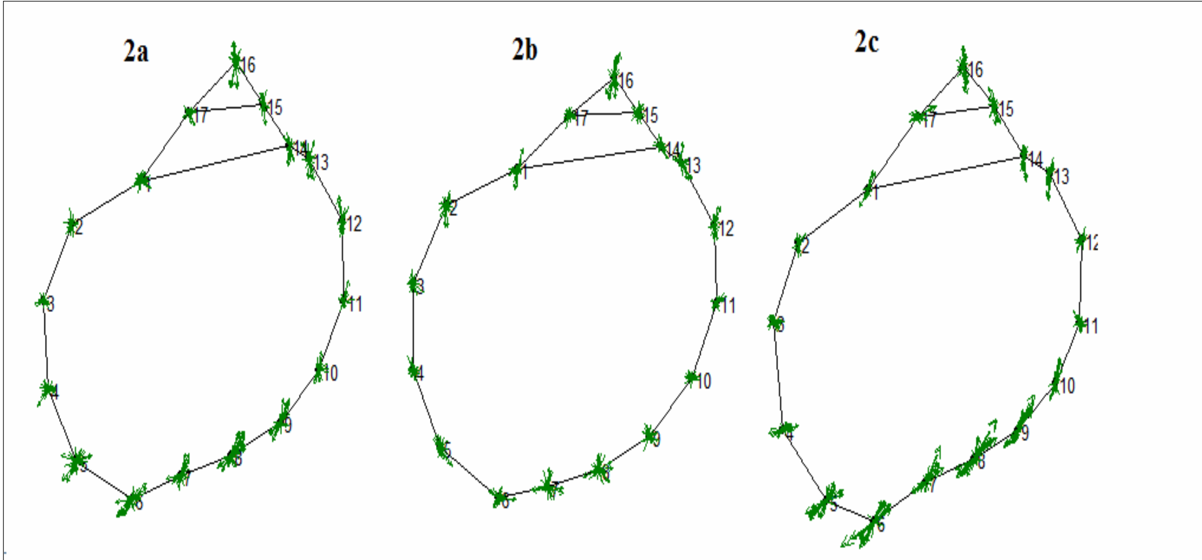



**Dorsal part variations of male *P. caniculata* collected from three diffrect ricefields - (2a) Butuan; (2b) Iligan and (2c) Pagadian**

Figure 3. Apertural and dorsal variations of male *P. canaliculata* using descriptive statistics (TPS).




Ventral part of female *P. caniculata* showing apertural variations - (1a) Butuan; (1b) Iligan and (1c) Pagadian




Dorsal part variations of female *P. caniculata* collected from 3 different ricefields - (2a) Butuan; (2b) Iligan and (2c) Pagadian

Figure 4. Apertural and Dorsal Variations of female *P. canaliculata* using Descriptive Statistics (TPS).

***Exploratory statistics using cluster plot variations on the different views of P. canaliculata***. The topology and the evolution of the geological land masses are some of the factors in the variability of both floral and faunal species. Among the three sites, Butuan has different geologic evolution which was formed as a deltaic plain from the deposition of river sediments flowing through the Agusan River, the third longest river of the Philippines. The Agusan River and its tributaries provide the valley with rich soil from periodic floods (ESSC 2002). On the other hand, Iligan and Pagadian were part of the mainland of Mindanao. As shown in cluster analysis (Figures 5-7) all views (aperture, dorsal and whorl) of the male and female *P. canaliculata*, Butuan had more different clusters compared to Iligan and Pagadian which have an overlapping clusters. Further, different views of the female snail have a little overlapping compared to males. This could be an indication that female phenotypic variations are greater than males.

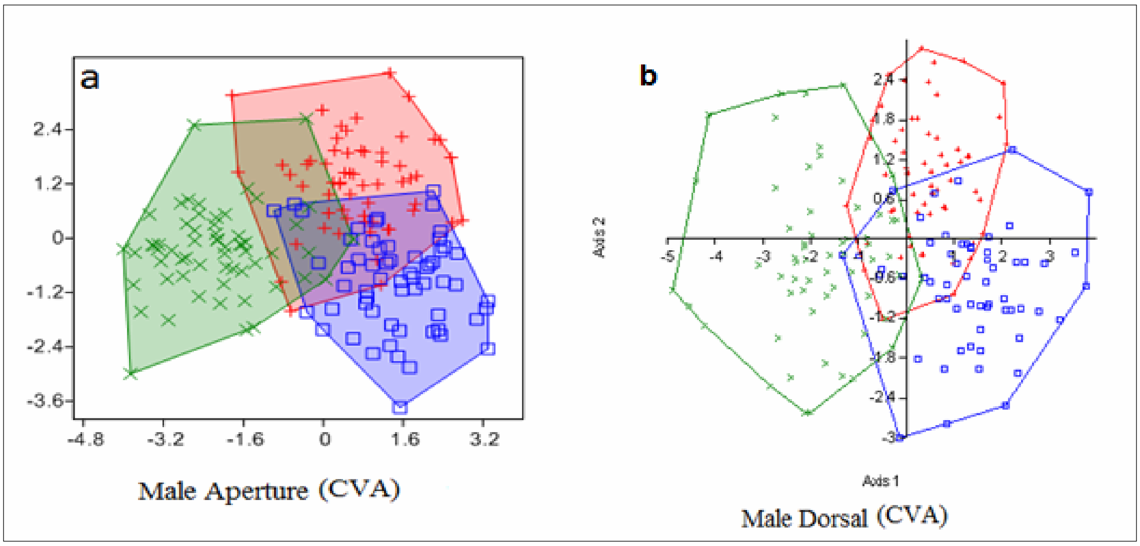



Figure 5. Cluster plot showing apertural (a) and dorsal variations (b) of male *P. canaliculata* using CVA.

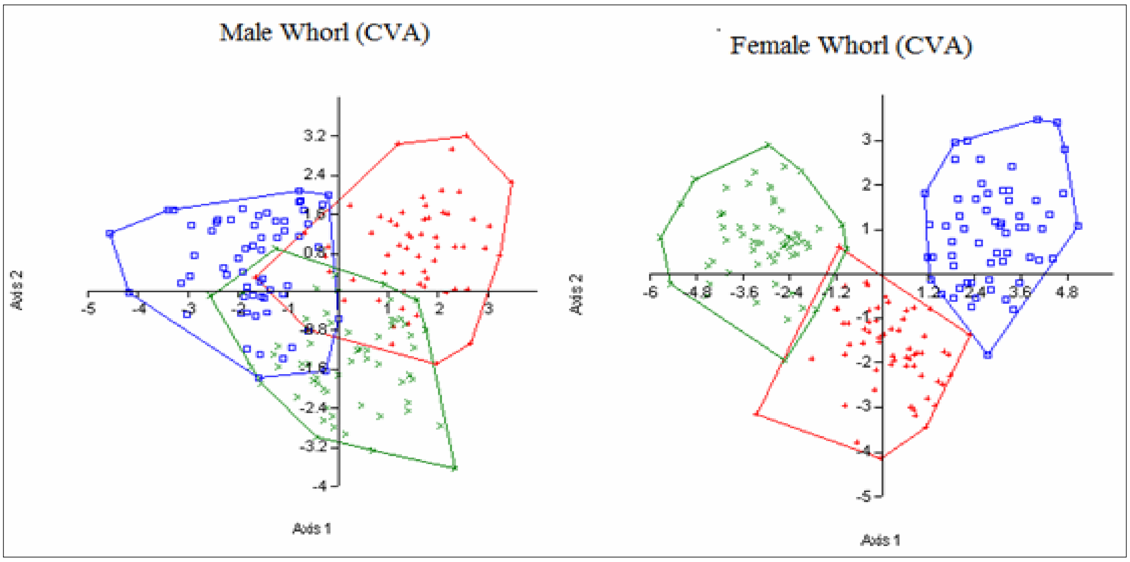



Figure 6. Whorl variations of male and female *P. canaliculata* using CVA.

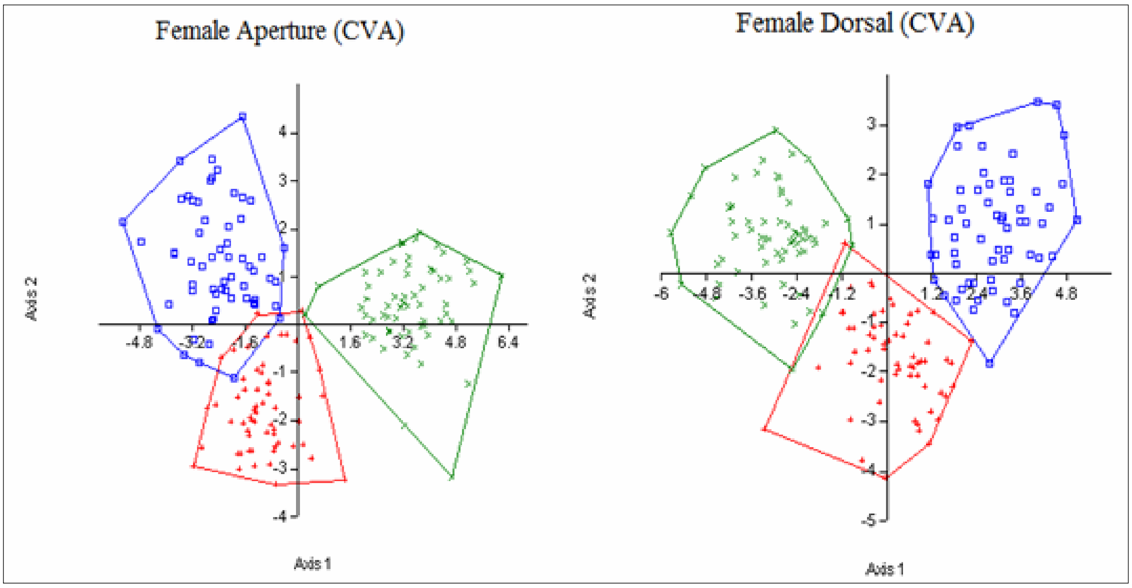



Figure 7. Cluster plot showing apertural and dorsal variations of female *P. canaliculata* using CVA.

These exploratory statistical data indicated that there is really variability of the morphometrics among the populations of *P. canaliculata* snail in these three sites of study. Further, male and female *P. canaliculata* also exhibits variation in their shell morphology which is supported in the studies of Moneva et al (2012b), Torres et al (2011), Mahilum & Demayo (2014a, b) and Galan et al (2015). Shell shape variation in size, whorl, aperture and even shell band pattern are recorded and indicates the occurrence of sexual dimorphism within species of golden apple snails accordingly.

***Confirmatory statistics using MANOVA***. Finally, phenotypic variations are confirmed by using MANOVA. In this statistical analysis, p-values less than 0.05 are considered significant. Table 1 shows the statistics values of the phenotypic variations exhibited by *P. canaliculata* using MANOVA.

Table 1

Confirmatory statistics values of the phenotypic variations exhibited by *P. canaliculata* using (Multivariate Analysis of Variance (MANOVA)

| *Sex* | *View* | *Pairwise comparison* | *p-value* | *Remarks* |
|---|---|---|---|---|
| Male | Aperture | Butuan vs. Iligan | $3.2 \times 10^{-5}$ | Extremely significant |
| | | Butuan vs. Pagadian | $5.2 \times 10^{-11}$ | Extremely significant |
| | | Iligan vs. Pagadian | $4.1 \times 10^{-14}$ | Extremely significant |
| | Dorsal | Butuan vs. Iligan | 0.0010 | Very significant |
| | | Butuan vs. Pagadian | $1.40 \times 10^{-8}$ | Extremely significant |
| | | Iligan vs. Pagadian | $6.33 \times 10^{-13}$ | Extremely significant |
| | Whorl | Butuan vs. Iligan | $5.47 \times 10^{-11}$ | Extremely significant |
| | | Butuan vs. Pagadian | $3.07 \times 10^{-12}$ | Extremely significant |
| | | Iligan vs. Pagadian | $4.1 \times 10^{-14}$ | Extremely significant |
| Female | Aperture | Butuan vs. Iligan | $1.05 \times 10^{-10}$ | Extremely significant |
| | | Butuan vs. Pagadian | $5.86 \times 10^{-20}$ | Extremely significant |
| | | Iligan vs. Pagadian | $9.2 \times 10^{-25}$ | Extremely significant |
| | Dorsal | Butuan vs. Iligan | $8.96 \times 10^{-17}$ | Extremely significant |
| | | Butuan vs. Pagadian | $6.88 \times 10^{-19}$ | Extremely significant |
| | | Iligan vs. Pagadian | $1.02 \times 10^{-29}$ | Extremely significant |
| | Whorl | Butuan vs. Iligan | $8.96 \times 10^{-17}$ | Extremely significant |
| | | Butuan vs. Pagadian | $6.88 \times 10^{-19}$ | Extremely significant |
| | | Iligan vs. Pagadian | $1.02 \times 10^{-29}$ | Extremely significant |

Most of the views (aperture, dorsal and whorl) in both male and female *P. canaliculata* from 3 different ricefield locations exhibit extremely significant phenotypic variations. Only Butuan vs. Iligan male dorsal pairwise comparison shows very significant value (0.0010). Overall, phenotypic variation is evident among populations collected from three different ricefields.

**Conclusions**. This contribution made by morphometrics has shown extremely significant differences in the body shape of *P. canaliculata* collected among three different rice fields in Mindanao Island, Philippines. These differences should be further investigated in order to clarify their source. Variability and differences of the shell in the *P. canaliculata* snail are due mostly to cumulative environmental effects in different views (aperture, dorsal and whorls). The study clearly proved that geologic distance is a factor of phenotypic variations. As to the question whether this is a case of agroecotype should be further explored by looking into abiotic components of the ricefields as well as other components of this managed ecosystem. Studies on the extent of variability of this invasive species are very important as these organisms contribute to the continuing decline in biodiversity and are considered to be proliferative pests of agricultural systems.

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