



Evolutionary Perspectives for Analyzing Shell Shape Changes in Golden Apple Snail (*Pomacea canaliculata*) using Landmark-Based Geometric Morphometrics

Rose Chinly Mae H. Ortega^{1,*}, Chinee S. Padasas¹, Grapesy Pink Alsonado¹, Nazma D. Eza^{1,2}, Angeleth U. Taotao¹, Mark Anthony Torres¹

¹Mindanao State University-Iligan Institute of Technology, Iligan City, Philippines 9200

²Mindanao State University- Main Campus, Marawi City, Lanao del Sur, Philippines 9700

Key words: Ampullariidae, GM analysis, Golden Apple Snail, Relative warps, Species Concept.

<http://dx.doi.org/10.12692/ijb/21.1.102-114>

Article published on July 28, 2022

Abstract

Geometric morphometric (GM) analysis was used to carry out variations of the shell shape of *Pomacea canaliculata*. Freshwater golden apple snails were investigated to find out whether males and females vary in their dorsal and ventral shell orientation considering the fact that they adapt differently to their environment. Relative warps (RW) analysis was done using tpsRelw which corresponds to the Principal Components Analysis (PCA). For males and females, dorsal relative warps reveal 35.49% and 41.18%, respectively. These findings reveal that males have a larger bodies with longer and expanded spires, while females have compressed bodies in both extremes. Male accounts for 50.37% of ventral relative warps, which shows a broader body at the negative extreme and compressed at a positive extreme, while female account for 54.76%, which show expansion of the body part with the narrower spire at the negative extreme and narrower aperture with compression at the positive extreme. The differences could be due to environmental selection pressures, which could lead to genetic modifications and, as a result, phenotypic plasticity. Furthermore, it can aid in the preliminary comprehension of particular species concepts as well as the overall notion of evolution, which is concerned with changes in the genetic composition of populations of organisms across time.

*Corresponding Author: Rose Chinly Mae H. Ortega ✉ rosechinlyortega@gmail.com

Introduction

Pomacea canaliculata is a common freshwater golden apple snail that belongs to the *Ampullariidae* family. It is thought to be the most adaptable species in the group, with a wide range of distribution (Tripoli *et al.*, 2015). *P. canaliculata* was introduced from South America to Southeast Asia and eventually introduced in the Philippines as a dietary protein supplement (Cabuga *et al.* 2017) and used in commercial aquaculture (Hayes *et al.* 2008). However, this species has demonstrated rapid population growth that it can endure harsh environments and dominates freshwater habitats, becoming a rice field pest in parts of Asia and Africa (Buddie *et al.*, 2021). Furthermore, golden apple snails develop a variety of adaptations that allow them to endure a wide range of environmental situations, making this species a great eco-evolutionary model (Madjos and Anies, 2016). Due to its polyphagous ability, the tropic vegetation in the Philippines may provide a favorable habitat for the golden apple snail's survival and nutritional requirements (Estebenet and Martin, 2002).

The intra and inter-population variations in shapes and sizes of the golden apple snail are qualitatively recognizable (Mahilum and Demayo, 2014a). In the Philippines, the golden apple snail exhibits morphological characteristics variability in many populations such as shell colours, shape, sizes, angle of opening and banding patterns ranging from golden yellow, olive-yellow to dark olive with different numbers of small and large bands (Thaewnon-ngiw *et al.*, 2004; Tamburi and Martin, 2009).

In the adult stage, females of the golden apple snail are larger with their shell curves inward, while the male is smaller while its shell curves outward (Madjos and Anies, 2016) making this species a good model to explain the problem of its progression as pest and its evolution as an organism (Mahilum and Demayo, 2014b). Also, the type of golden apple snail in the Philippines is different compared to other regions in South America, and in Argentina and Japan, its development took a much longer period to be

matured (Estenebet *et al.*, 2013; Bae *et al.*, 2021). Thus, the distribution of the golden apple snail in different regions of the world could be an important factor in its morphological variations (Cabuga *et al.*, 2017) and may enhance genetic variability. Likewise, the small-scale geographic pattern has been associated with affecting shape differences within its populations (Balbosa *et al.*, 2012).

With these, several studies have been conducted and subjected to determining the morphological analysis of the golden apple snail (Estebenet and Martin, 2002). Such studies are on the shell shape differences which have been shown that it is associated with sexual dimorphism (Johnsen *et al.*, 2003) and this shape variation is a significant element in determining morphological distinctions within the species of the same taxa (Cabuga *et al.*, 2016). There are also studies that used qualitative descriptions as references to observe the morphological variation within species in the population, but it is suggested that an advanced quantitative tool should be used in determining the morphological variation among the population (Mahilum and Demayo, 2014a). Geometric morphometric (GM) is one of the advanced tools for describing the sizes, shapes, and shape variances of an organism such as *P. canaliculata* (Ordynets *et al.*, 2021) and it gives an accurate and precise result in obtaining the desirable exact shape among biotic elements (Thelwell *et al.*, 2022). It is also an effective method of determining the sexual dimorphism among organisms (Minton and Wang, 2011). Furthermore, it was debated that the shell shape of *P. canaliculata* could be influenced genetically and environmentally and the differences in the shape of this species acquire may provide useful information to solve this issue (Mahilum and Demayo, 2014b). Hence, this study aims to examine and describe the shell shape variation of *P. canaliculata* collected from rice fields through geometric morphometric tools, specifically landmark-based analysis.

Materials and methods

Study area

Golden Apple snails (*Pomacea canaliculata*) were

collected from an irrigated rice field at Steeltown Brgy. Sta. Elena, Iligan City (Fig. 1). A total of 90 adult snail samples comprising 45 males and 45 females were randomly selected and placed in clean plastic containers. Environmental (abiotic) factors

such as relative humidity, salinity, pH, and water temperature were measured. Samples collected were then transported to the main laboratory of the Department of Biological Sciences, MSU-IIT for the photograph and morphological analysis.

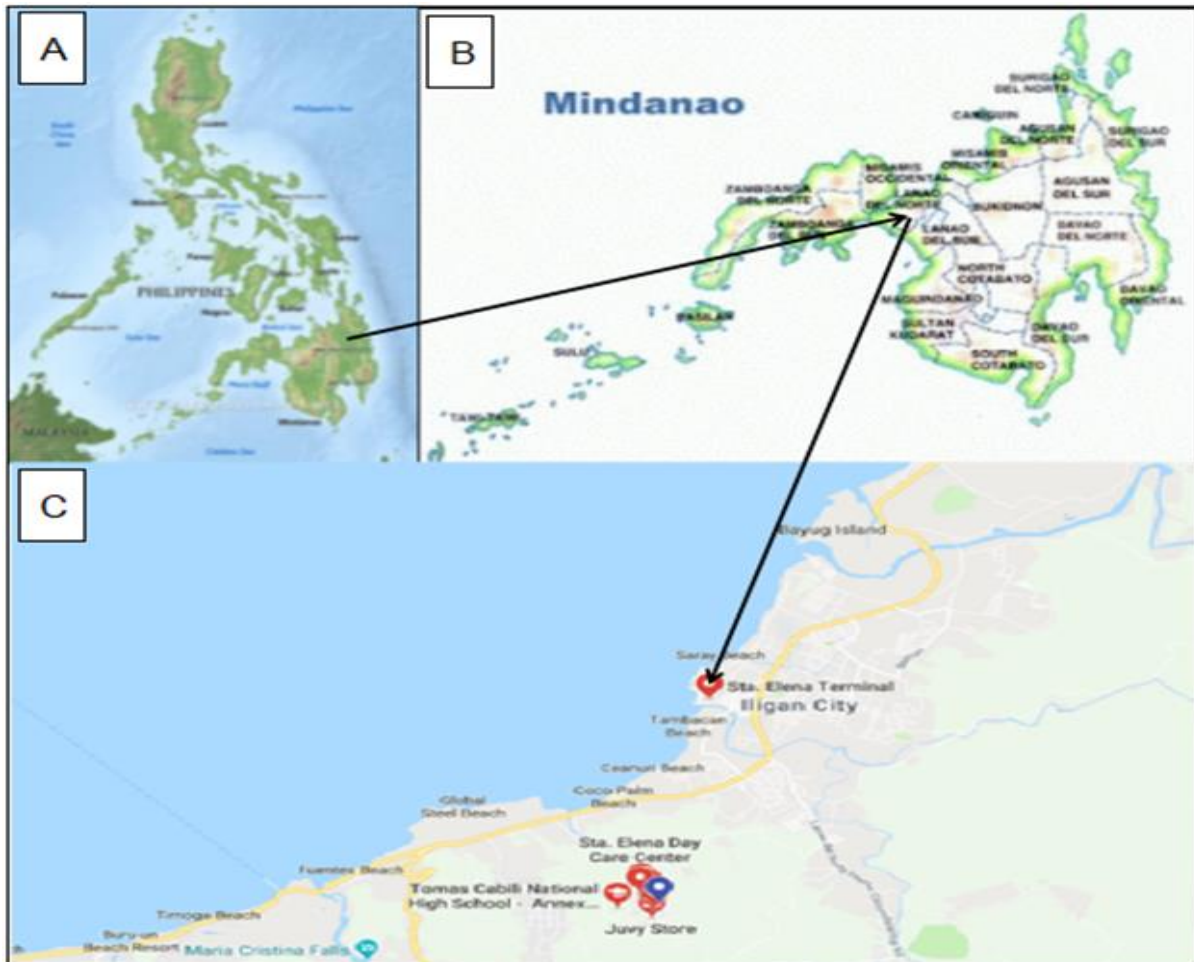


Fig. 1. (A) Map of the Philippines (freeworldmaps.net), (B) Mindanao (printable-maps.blogspot.com), and (C) Brgy. Sta. Elena (google.com) where the sampling site is situated.

Landmark selection

Shape analysis was done according to the protocol described by Mahilum and Demayo in 2014. Dorsal and ventral orientations of Golden Apple Snail (GAS) shell were photographed for landmark and outline data acquisition using tpsDig232 and were tri-replicated to exclude measurement error. TpsDig facilitated the geometric morphometric analysis by making it easier to collect and maintain landmark data from digitized images (Mahilum and Demayo, 2014) as well as some of the points represented conserved topological positions relative to other landmarks and junctions of the other structures

(Torres *et al.*, 2011). Landmarked images were then further analysed using tpsUtil to create a geometric morphometric thin-plate spline file with the X and Y coordinates outlined landmark points along the contour of the shell (Mahilum and Demayo, 2014) and relative warps analysis was also done using tpsRelw which corresponds to the Principal Components Analysis of the covariance matrix of the partial warp scores which are different scales of a thin-plate spline transformation of landmarks and which are the main components of the matrix that combine partial warps and uniform components (Torres *et al.*, 2013).

Data analysis

The resulting relative warp scores of the shell shapes were analyzed and computed using the Paleontological Statistics Software (PAST). A histogram was then employed to visualize the distribution of the shape variation among the population of Garden Apple Snail.

Results and discussion

Relative warps (RW) analysis can be viewed as variations of the shape of the shell structure that are mutually uncorrelated (Avaca *et al.*, 2013). In this study, the evaluation of relative warps individually

with the support of the relative coefficients is evidence of the landmarks' movement. Relative warp shows variation among males and females from dorsal and ventral views, respectively. Relative warps were reduced with a reported value of greater than or equal to 5% from 17 dorsal landmarks and 21 ventral landmarks. A total of nine relative warps were displayed having the most percent variation based on the report scores in the dorsal view with five relative warps for males and four relative warps for females, while seven relative warps were displayed in the ventral view with four relative warps for male and three relative warps for female.

Table 1. RW-implied deformation for individual variation in the male and female shell in dorsal view.

Male		Female	
RW1 (35.49%)	The spire of the shell at the negative extreme is longer and the body is stretched widening the body part.	RW1 (43.18%)	Variations are visible on the body as it is displaced upward making the spire narrow on the positive extreme.
RW2 (26.60%)	The spire of the shell at the negative extreme is longer and the body is stretched upward narrowing the body part. At the negative extreme, the body is expanded.	RW2 (18.37%)	Variations in the body are visible on the lower part as it narrows making it pointed on the negative extreme which a wider spire. A slight widening of the body in the positive extreme.
RW3 (13.48%)	Variation at the positive extreme is visible as the body narrows and is compressed.	RW3 (14.45%)	The body is displaced inward making it narrow on the negative extreme. Expansion of the body part made it wider on the positive control.
RW4 (6.62%)	The spire at the negative extreme is bigger and the body is wider. A slight upward stretch of the body at the positive extreme makes it narrower.	RW4 (6.65%)	Visible expansion of the body part making it wider at the positive extreme.
RW5 (5.61%)	The variation that is visible from the body displaced the spire to be unrecognizable having a wide stretch at the negative extreme. At the positive extreme, the body is slightly narrowed because of the inward displacement.		

Relative warps were reduced with a reported value of greater than or equal to 5% from 17 dorsal landmarks and 21 ventral landmarks. A total of nine relative warps were displayed having the most percent variation based on the report scores in the dorsal view with five relative warps for males and four relative warps for females, while seven relative warps were displayed in the ventral view with four relative warps for male and three relative warps for female. For the dorsal relative warps of the male (Fig. 2), RW1

comprises 35.49% and shows displacement in the body making it broader with a longer and more expanded spire. RW2 with 26.60% shows displacements on the negative extreme which made the body narrower with the upward displacement and compression at the middle part. RW3 having 13.48% have pronounced variation on the negative extreme with a wider body and narrower on the positive extreme. RW4 comprises 6.62% displays variation of the broadening of the shell body for both positive and

negative extremes. RW5 with 5.61% variation has displayed outward displacement of landmarks in the negative extreme and inward displacements on the positive extreme in the spire adjacent to the body making the body broader for both extremes. For female relative warps (Fig. 2), RW1 with 41.18% shows upward displacement of landmarks making the body a little compressed in both extremes. RW2 comprises 18.37% showing compression on the upper part of the body and expansion on the lower part at

the positive extreme. At the negative extreme, there is an expansion on the upper part and a more compressed lower part of the body. RW3 having 14.45%, shows a broader body with expanded deformities at the positive extreme and a narrower body from a slight compression at the negative extreme. RW4 comprises 6.65 and shows slight body compression for both extremities. All landmark displacements shown in Figs 2 & 3 are summarized in Table 1.

Table 2. RW-implied deformation for individual variation in the male and female shell in ventral view.

Male		Female	
RW1 (50.37%)	Outward displacement of the landmarks in the body makes the body wider in the negative extreme and narrower in the positive extreme.	RW1 (54.76%)	Variations are visible on the body. The lower part of the body (operculum) in the positive extreme is compacted.
RW2 (18.09%)	Variations in the body of the positive extreme make the body wider and compressed on the negative extreme.	RW2 (15.88%)	The spire on the negative extreme is smaller with inward bending of the body making it narrow. The negative extreme possesses a slightly wider body.
RW3 (8.72%)	The spire on the positive extreme is narrow with a wider body, the spire on the negative extreme is wider and the body is stretched.	RW3 (8.90%)	Variations are visible on the upper part of the body adjacent to the spire of the negative extreme; it displaced upward making the spire expand.
RW4 (7.22%)	At both extremes the body are stretched making it wider.		

For ventral relative warps, male (Fig. 4) RW1 comprising 50.37%, the body at the negative extreme is broader and at the positive extreme, the aperture area is compressed. RW2 with 18.09% shows displacements that make the body broader and the spire is thinner. RW3 having 8.72%, shows slight body compression at the negative extreme. RW4 comprises 7.22% have narrower aperture area and bent spire at the positive extreme and slight broadening of the body at the negative extreme. For females (Fig. 5), RW1 having 54.76% shows the expansion of the body part with a narrower spire at the negative extreme and narrower aperture with slight compression of the body at the positive extreme. RW2 comprises 15.88% have narrowed aperture, wider body and spire at the negative extreme. Positive extremities have shown a slightly compressed body. RW3 comprising 8.90%, shows a slight widening of the body for both extremities. All

landmark displacements shown in Figs 4 & 5 are summarized in Table 2.

The spire, body and aperture parts of the Golden Apple snail shell are integrated with each other because they develop, function and evolve jointly (Mahilum and Demayo, 2014c). The relative warp analysis results showed that the shape of the shell of the male and female *P. canaliculata* differ based on the compression and expansion of the spire and body. These can be described using the consensus morphology superimposition (Figs 2-5).

The compression of the male dorsal shell in the middle part and the expansion of the female dorsal shell in the lower part could be attributed to reproductive physiology or feeding rates (Ponder *et al.*, 2019). The sexual dimorphism of growth efficiencies and ingestion rates explain why females

tend to reach a larger adult size than males, a pattern probably explained by the development of the testicle and correlated reduction of mid-gut gland size (Tamburi and Martin, 2009). Furthermore, a relationship between size-corrected shape and food

availability was found in females, which were more globose and had a larger aperture when grown under high food availability. The best-nourished females' reproductive organs grow faster and their thinner shells (Tamburi and Martin, 2009) explain this result.

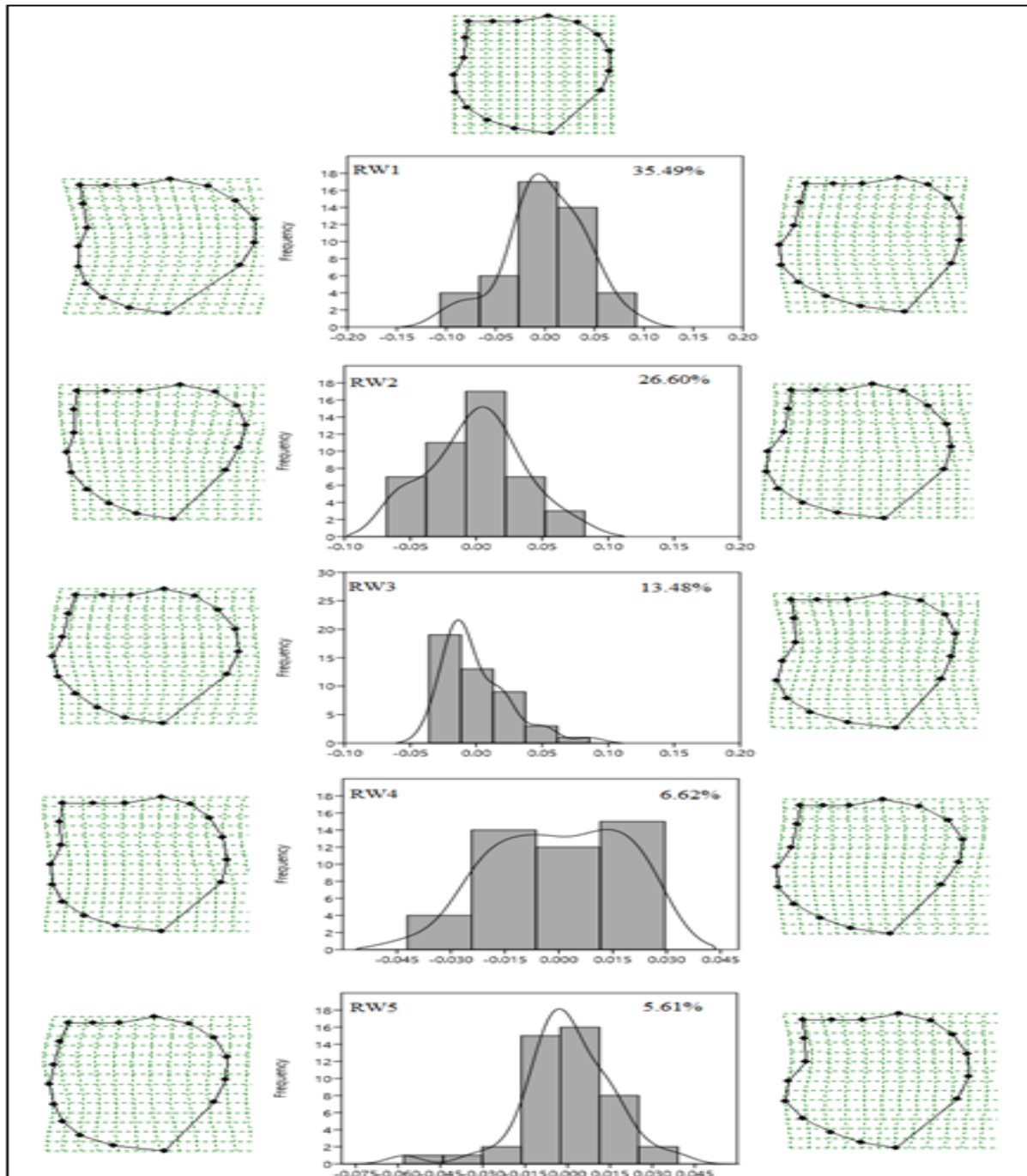


Fig. 2. Summary of the geometric morphometric analysis showing the mean shape and variation in dorsal of male populations of *Golden apple snail* generated by relative warps.

Tripoli *et al.* (2015) concluded that there is no shape variation between sexes based on dorsal, ventral, or whorl morphology, and Torres *et al.* (2011) observed

that there are no apparent differences in average forms between two sexes per population. However, in this study, differences between sexes are noticeable in

variations in spire length, wide and/or narrow body-wide and/or narrow aperture and either long or short aperture height, the same result observed in the study of Mahilum and Demayo, 2014a. Mahilum and Demayo (2014b) found out that variations exist in the shape of dorsal and ventral portions of the shell between male and female individuals based on landmark-based analysis suggesting the presence of

shell shape sexual dimorphism within the populations. Phenotypic variations among snails are associated with predation and nutrient requirements of the gastropods (Cabuga *et al.*, 2017). Meanwhile, in most cases, an intersexual pattern of growth shows that males are smaller and generally grow more slowly (Riascos and Guzman, 2010), but the sizes referred to might not be a factor for sexual variation.

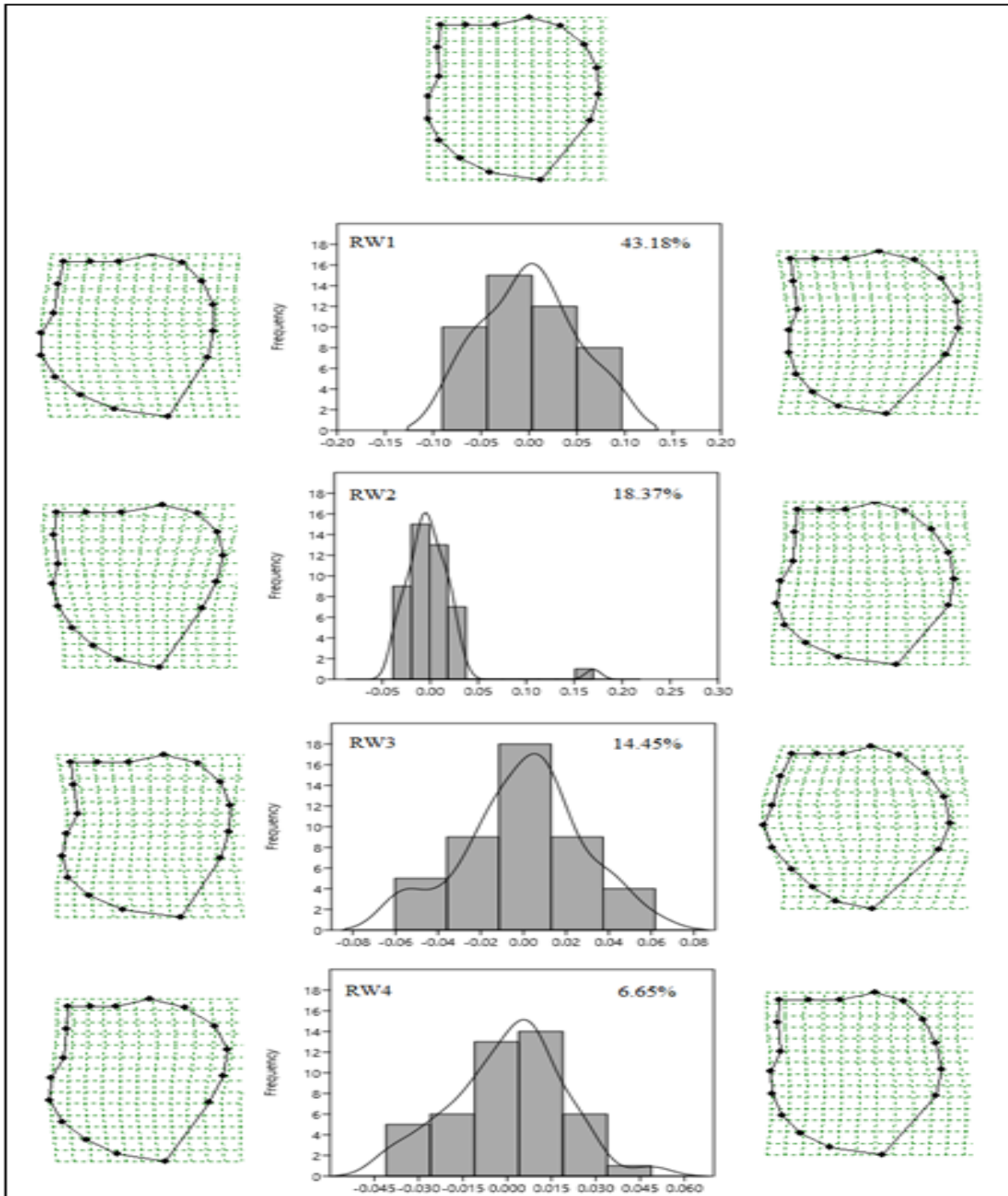


Fig. 3. Summary of the geometric morphometric analysis showing the mean shape and variation in dorsal of female populations of *Goldenapple snail* generated by Relative warps.

The variations and sexual dimorphism can be brought by either possible different environmental factors like hydrological factors and substrates (Torres *et al.*, 2013) and the temperature was argued to be one of the factors that affect shell formation or genotypic

activity that leads to phenotypic plasticity in the invasive species (Mahilum and Demayo, 2014a) which can affect its response to selection and may modify the end result of genetic diversity evolution at the population level (Grenier *et al.*, 2016).

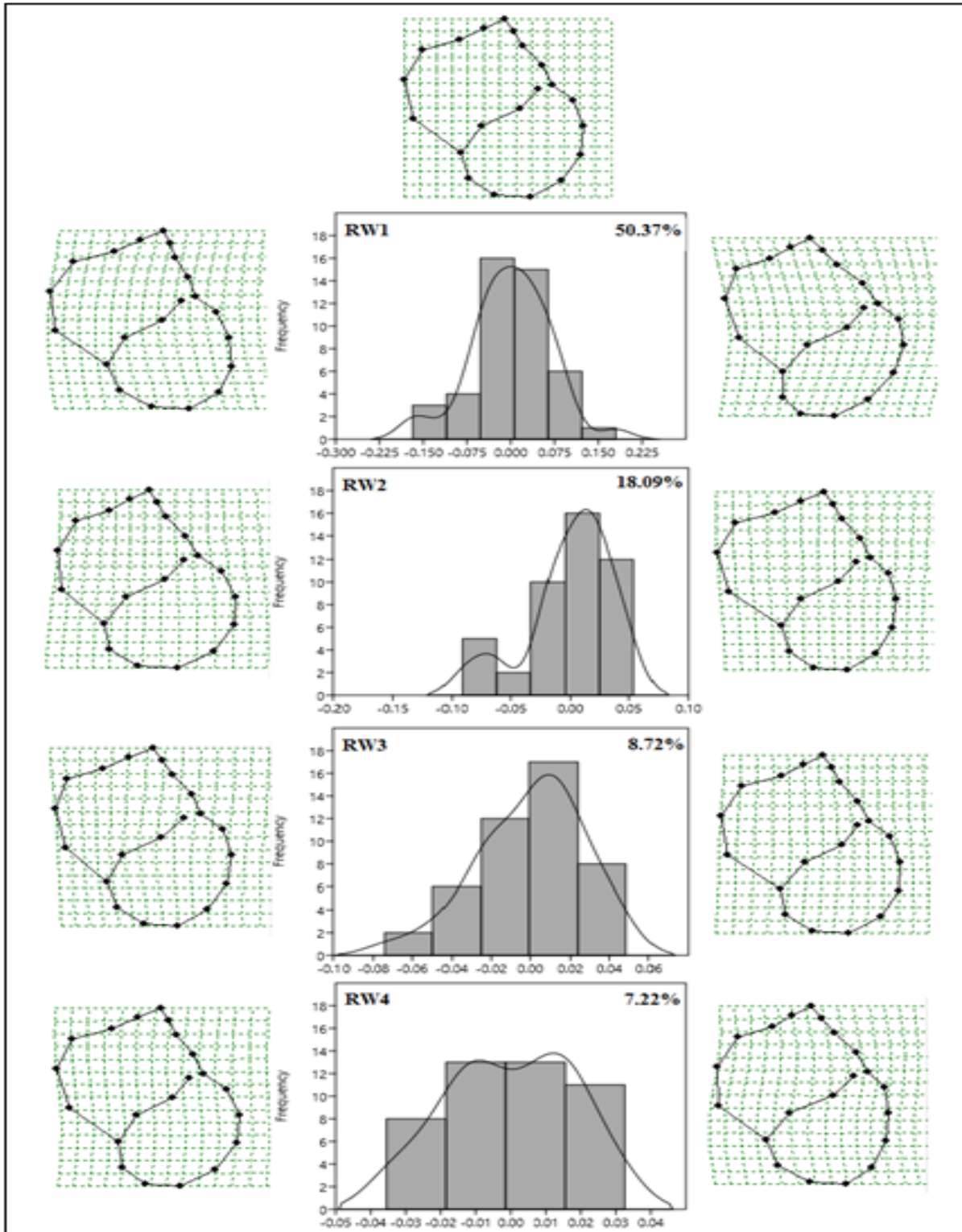


Fig. 4. Summary of the geometric morphometric analysis showing the mean shape and variation in ventral of male populations of *Goldenapple snail* generated by relative warps.

Moreover, in this analysis, the peaks of the histogram (Figs 2-5) can possibly explain the mode of speciation of *P. canaliculata*. Considering the higher-order relative warp (RW1), the males exhibit two or more peaks while the female has one peak. This can be interpreted that male *P. canaliculata* may have undergone disruptive selection while females have stabilizing selection. Disruptive selection would mean that there could be possible divergence among male *P. canaliculata* because of selection pressures, as supported in the study of Bos and Scharloo (1973), that disruptive selection can cause a large increase of phenotypic variance while stabilizing selection did not increase phenotypic variance. The wider single peak in the case of females would mean that though it is more generalist than the male, it still maintains the degree of variability, or it could still undergo divergence, as shown in RW2 and RW3 (Figs 3 and 4). The possible earlier divergence of male *P. canaliculata* than female could be attributed to its size by which female is usually larger than male (Riascos and Guzman, 2010). So, the males having smaller sizes are possibly more susceptible to selection pressures such as competition with other species or gaps in the underlying resource distribution (Hendry *et al.*, 2009).

Variability is one of the characteristics of life. The minor differences in this study would possibly imply that there is a divergence of two different species based on the histogram that probably can affect the genotypes in the future. Different shell shapes could arise as a collateral outcome of genetically different reproductive behaviours (Estenebet and Martin, 2003). Since Darwin's time, the question "what is a species" has provoked fierce disputes and a tremendous number of publications, from short opinion papers to thick volumes (Zinner and Roos, 2014). The need to use more than one species concept in order to be applicable raises the idea of a pluralistic species concept (Aldhebiani, 2018). In this study, different species concepts could possibly be applied. The typological species concept indicating morphological differences should not be ignored because, even if it is minor, it can eventually lead to

genetic changes (Gregory, 2009). In evolutionary biology, even one percent variation is significant, and may affect the overall variation. This may have high fitness value than the rest and may dominate other traits in the future. In the curve, this small percentage of variation is found in the intermittent part, which could have more selection pressures than in the extremes. The question now is, "What are the possible traits in this middle phenotype?" Sometimes morphological characteristics are subjective and depend on 'expert' opinion for key traits and in some cases, the species are sympatric (morphologically indistinguishable) but are clearly different lineages (Aldhebiani, 2018). This possible divergence can also be supported by the 'cohesion species concept' which indicates that the most inclusive population with potential for phenotypic cohesion through intrinsic cohesion mechanisms can have the potential for genetic and/or demographic exchangeability (Wilkins, 2002). The cohesion concept is similar to the evolutionary species concept in the way that a population's genetic stress on the origins of phenotypic similarity within species (Aldhebiani, 2018). Furthermore, this possible speciation that may continue in the future from morphological to molecular level can lead us to this question, "What is the effective evolutionary significant unit (ESU) to be considered for the management of this pest?" that can be further investigated. ESU is synonymous with biospecies and evolutionary species. ESU specifies a population (or group of populations) that is substantially reproductively isolated from other conspecific population units and represents an important component in the evolutionary legacy of the species (Wilkins, 2002).

The ecological species concept could also be applied. Two organisms are similar to each other, and their needs are more likely to overlap. Therefore, they are expected to contest, and consequently, the more likely that they are of the same species (Castellanos-Morales *et al.*, 2016). It also has a problem similar to the morphological species concept which is: "at what point does one stop the process of splitting divergent forms into new species?" (Aldhebiani, 2018).

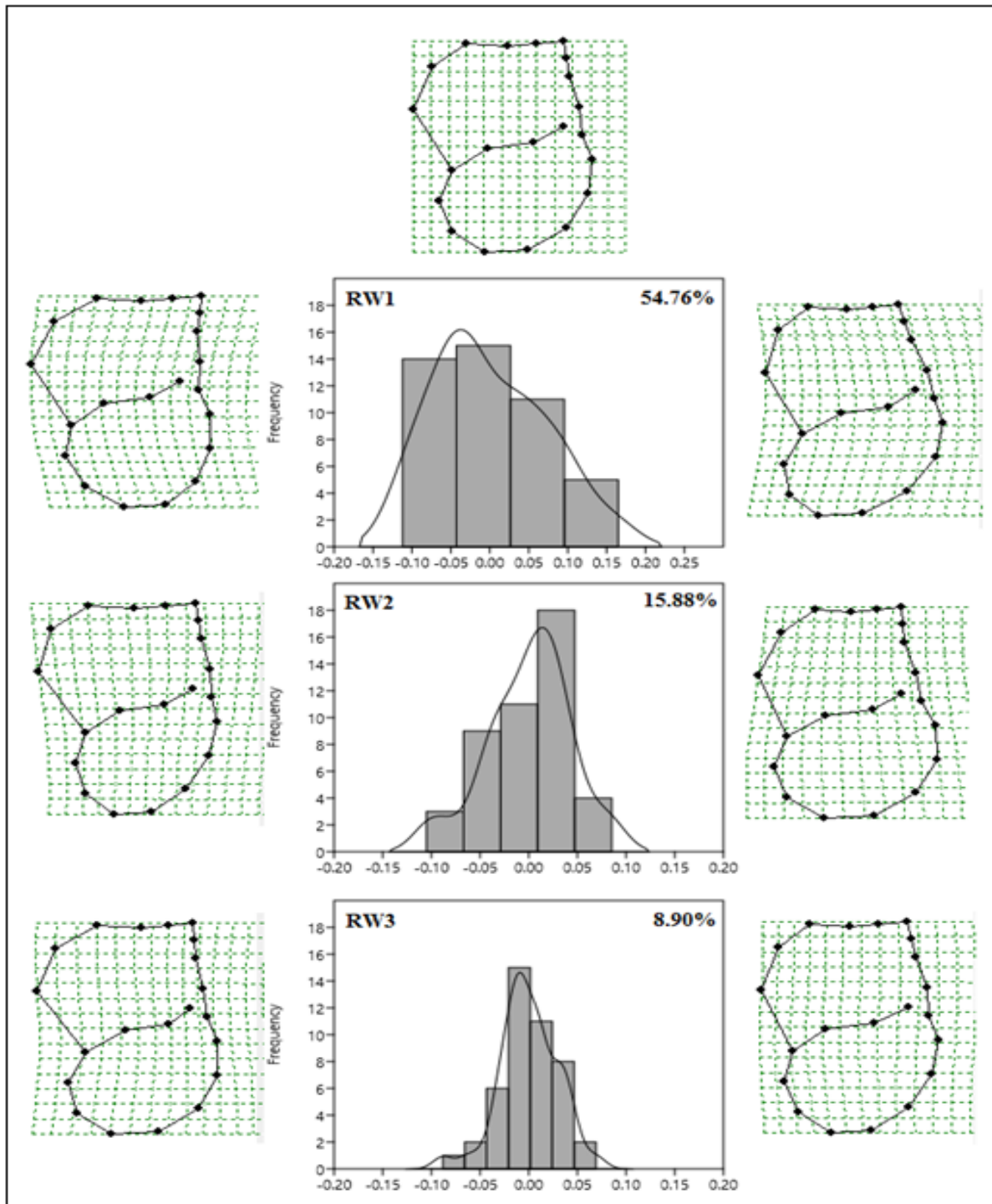


Fig. 5. Summary of the geometric morphometric analysis showing the mean shape and variation in ventral of female populations of *Goldenapple snail* generated by relative warps.

It could also be noted that the molecular approach could help in answering if this species could really undergo phenotypic plasticity. However, there is always a dilemma, "Is the genetic species concept applicable in this?" In genetic species concept, it specifies that a group of organisms may inherit characteristics from each other, a common gene pool,

and a reproductive community that forms a genetic unit (Wilkins 2002). The challenge is that it will only give us a "gene tree" but not a "species tree". Therefore, it can lead us to a bigger challenge in using the genetic species concept using DNA sequencing which is to include as many genes as possible to possibly obtain a species tree.

Conclusion

The results showed that relative warp analysis could be used for phenotypic variation assessment in shell shape morphology of golden apple snail. The variations could be due to selection pressures in the environment that may possibly lead to genetic changes and consequently can undergo phenotypic plasticity. Moreover, it can also help in the preliminary understanding of some of the species concepts and the concept of evolution as a whole which is all about the changes in the genetic composition of populations of organisms over time.

Acknowledgement

The researchers would like to recognize the authorities and locals of Steeltown, Brgy. Sta Elena, Iligan City for permitting us to conduct this study.

References

- Aldhebiani A.** 2018. Species concept and speciation. Saudi Journal of Biological Sciences **25**, 437–440.
- Avaca MS, Narvarte M, Martín P.** 2013. Shell shape variation in the Nassariid *Buccinanops globulosus* in northern Patagonia. Helgoland Marine Research **67**, 567–577.
- Bae MJ, Kim EJ, Park YS.** 2021. Comparison of Invasive Apple Snail (*Pomacea canaliculata*) Behaviors in Different Water Temperature Gradients. Water **13(9)**, 1149.
- Balbosa KKH, Torres MAJ, Demayo CG.** 2012. Microgeographic Differentiation in Populations of the Golden Apple Snail, *Pomacea canaliculata* Lamarck. The Second International Congress on Interdisciplinary Research and Development, Thailand **811(1)**.
- Bos M, Scharloo W.** 1973. The effects of disruptive and stabilizing selection on body size in *Drosophila melanogaster*. II. Analysis of responses in the thorax selection lines. Genetics **75(4)**, 695–708. <http://dx.doi.org/10.1093/genetics/75.4.695>.
- Buddie AG, Rwomushana I, Offord LC, Kibet S, Makale F, Djeddour D, Cafa G, Vincent KK, Muvea A, Chacha D, Day RK.** 2021. First report of the invasive snail *Pomacea canaliculata* in Kenya. CABI Agriculture and Bioscience **2(1)**. <http://dx.doi.org/10.1186/s43170-021-00032-z>
- Cabuga C, Sularte R, Acob M, Eleazar M, Tiempo C, Joseph C, Angco M, Calub A, Havana H, Dominguito J, Budlayan M, Colon R, Guilleno J, Arriza S.** 2017. Describing shell shape variations and sexual dimorphism of golden apple snail, *Pomacea canaliculata* (Lamarck, 1822) using geometric morphometric analysis. Computational Ecology and Softwares **7(3)**, 123–139
- Castellanos-Morales G, Gámez N, Castillo-Gámez RA, Eguiarte LE.** 2016. Peripatric speciation of an endemic species driven by Pleistocene climate change: The case of the Mexican prairie dog (*Cynomys mexicanus*). Molecular Phylogenetics and Evolution **94**, 171–181. <http://dx.doi.org/10.1016/j.ympev.2015.08.027>
- Estebenet AL, Martín PR.** 2002. *Pomacea canaliculata* (Gastropoda: Ampullariidae): Life-history traits and their plasticity. Biocell **26(1)**, 83–89.
- Estebenet A, Martín P.** 2003. Shell interpopulation variation and its origin in *Pomacea canaliculata* (Gastropoda: Ampullariidae) from Southern Pampas, Argentina. Journal of Molluscan Studies **69**, 301–310.
- Gregory TR.** 2009. Understanding Natural Selection: Essential Concepts and Common Misconceptions. Evo Edu Outreach **2**, 156–175. <http://dx.doi.org/10.1007/s12052-009-0128-1>
- Grenier S, Barre P, Litrico I.** 2016. Phenotypic Plasticity and Selection: Nonexclusive Mechanisms of Adaptation", Scientifica, Article ID 7021701, 9 pages. <https://doi.org/10.1155/2016/7021701>

- Hayes KA, Joshi RC, Thiengo SC, Cowie RH.** 2008. Out of South America: Multiple origins of non-native apple snails in Asia. *Diversity and Distributions* **14(4)**, 701–712.
- Hendry A, Huber S, De Leo'n L, Herrel A, Podos J.** 2009. Disruptive selection in a bimodal population of Darwin's finches. *Proceedings of the Royal Society of London B*, **276**, 753–759.
<http://dx.doi.org/10.1098/rspb.2008.1321>
- Johnsen A, Delhey K, Andersson S, Kempnaers B.** 2003. Plumage colour in nestling blue tits: Sexual dichromatism, condition dependence and genetic effects. *Proceedings of the Royal Society of London B*, **270(1521)**, 1263–1270.
- Madjos GG, Aniez OS.** 2016. Morphometrics approaches to studying phenotypic plasticity in *Pomacea canaliculata* (Golden apple snail). *International Journal of Advanced and Applied Sciences* **3(4)**, 50–56.
- Mahilum JJM, Demayo CG.** 2014a. Describing Lake Populations of the golden apple snail, *Pomacea canaliculata* using landmark-based geometric morphometric analysis. *Journal of Entomology and Zoology Studies* **2(4)**, 139–144.
- Mahilum JJM, Demayo CG.** 2014b. Sexual Dimorphism on Shell Shape of *Pomacea canaliculata* Lamarck Thriving in Lakes Using the Geometric Morphometric Approach. *International Journal of Bioscience, Biochemistry and Bioinformatics* **4(4)**, 284–289.
- Mahilum JJM, Demayo CG.** 2014c. Comparing Compartmentalization in the Shell of Two Populations of Golden Apple Snails, *Pomacea canaliculata* Lamarck (Gastropoda: Ampullariidae). *International Journal of Fisheries and Aquatic Sciences* **3(2)**, 26–31.
- Maps of Mindanao Island, Philippines.** (2022, March 23) Retrieved from <http://printable-maps.blogspot.com/2010/07/maps-of-mindanao-island-philippines.html>
- Minton RL, Wang LL.** 2011. Evidence of Sexual Shape Dimorphism in Viviparus (Gastropoda: Viviparidae). *Journal of Molluscan Studies*, 1–3.
- Ordynets A, Keßler S, Langer E.** 2021. Geometric morphometric analysis of spore shapes improves identification of fungi. *PLOS ONE* **16(8)**, e0250477.
<https://doi.org/10.1371/journal.pone.0250477>
- Philippines Physical Map.** (2022, March 23) Retrieved from <http://www.freeworldmaps.net/asia/philippines/map.html>
- Ponder WF, Lindberg DR, Ponder JM.** 2019. *Shell, Body, and Muscles from: Biology and Evolution of the Mollusca* CRC Press. [Routledgehandbooks.com http://dx.doi.org/10.1201/9781351115667-3](http://dx.doi.org/10.1201/9781351115667-3)
- Riascos JM, Guzman PA.** 2010. The ecological significance of growth rate, sexual dimorphism and size at maturity of *Littoraria zebra* and *L. variegata* (Gastropoda: Littorinidae). *Journal of Molluscan Studies* **76(3)**, 289–295.
- Sta. Elena Iligan City.** (2022, March 23) Retrieved from <https://www.google.com/search?q=sta+elena+iligan+city&npic=0&rflfq=1&rlha=0&rlag=8211721,124230843,1913&tbm=lcl&ved=0ahUKEWjwku3YsqfbAhWBW5QKHUiBCBYQtgMIKA&tbs=lrf:!3sIAE,lf:1,lf ui:1&rlidoc=1>
- Tamburi NE, Martin PR.** 2009. Reaction norms of size and age at maturity of *Pomacea canaliculata* (Gastropoda: Ampullariidae) under a gradient of food deprivation. *Journal of Molluscan Studies*, **75(1)**, 19–26.
- Thaewnon-ngiw B, Klinbunga S, Phanwichien K, Sangduen N, Lauhachinda N, Menasveta P.** 2004. Genetic diversity and molecular markers in

introduced and Thai native apple snails (*Pomacea* and *Pila*). Journal of Biochemistry and Molecular Biology **3(4)**, 493-502.

Thelwell M, Bullas A, Kühnapfel A, Hart J, Ahnert P, Wheat J, Loeffler M, Scholz M, Choppin S. 2022. Modelling of human torso shape variation inferred by geometric morphometrics. PLOS ONE **17(3)**, e0265255.

<https://doi.org/10.1371/journal.pone.0265255>

Torres MAJ, Cabahug TEO, Joshi RC, Baoanan ZG, Demayo CG. 2013. Variability in Populations of Golden Apple Snail, *Pomacea canaliculata*, (Lamarck, 1822) in selected locations from The Philippines. Research Journal of Recent Sciences **2(8)**, 12-19.

Torres MAJ, Joshi RC, Sebastian LS, Demayo CG. 2011. Geographic phenetic variation in the

golden apple snail, *Pomacea canaliculata* (Ampullariidae) based on geometric approaches to morphometrics. Advances in Environmental Science-International Journal of the Bioflux Society **3(3)**, 243-258.

Tripoli FFD, Genecera J, Matela MNV, Fanuga KJD, Velasco DGM, Landero RS, Cataluña RB, Torres MAJ, Requieron EA, Bigsang RT. 2015. Sexual dimorphism in the shell shape of the golden apple snail, *Pomacea canaliculata*. AACL Bioflux **8(6)**, 910-923.

Wilkins J. 2002. Summary of 26 species concepts. 2002.

john.wilkins@bigpond.com

Zinner D, Roos C. 2014. So what is a Species Anyway? A Primatological Perspective. Evolutionary Anthropology **23**, 21-23.