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Describing Phenotypic Plasticity of *Pomacea canaliculata* From Irrigated Rice Fields of Diplahan, Zamboanga Sibugay Using Landmark-Based Geometric Morphometric Analysis

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ABSTRACT

The *Pomacea canaliculata* is one of the most successful invasive species that are capable of destroying a wide range of plants especially rice fields. The dynamic environment of rice fields and farmers' management practices to control pests may trigger these organisms to change their morphology and genetics. Thus, this study is conducted to observe the phenotypic variations of *P. canaliculata* in relation to rice varieties and geographic distance. This study used landmark-based geometric morphometric analysis to evaluate the morphology of golden apple snails. The samples of *P. canaliculata* were collected from three different rice varieties within three barangays of Diplahan, Philippines. The results show that sexual dimorphism and shell shape variations exist within, between, and among the populations of *P. canaliculata*. Phenotypic plasticity and genetic differentiation may have been the coping mechanisms for *P. canaliculata* to survive in the rice field environments and geographic isolation. It is recommended however that more studies be conducted about *P. canaliculata's* responses towards different environments not only in rice fields but in other fresh water ecosystems.

Keywords : *Pomacea canaliculata*, phenotypic plasticity, geometric morphometric analysis, sexual dimorphism, rice varieties, geographical distances

INTRODUCTION

PHENOTYPIC plasticity refers to an organism's ability to alter phenotypically in response to its surroundings (Madjos and Anies, 2016). Individual organisms that belong in different environments are able to detect environmental cues early in life and display phenotypes that are most suited to the environment they will experience later in life. Phenotypic plasticity can be adaptive or non-adaptive, but it always involves a change in gene expression, whether morphological, physiological, or behavioral, and some cases include all three (West-Eberhard, 2008). Gilbert (2010) emphasized that an individual's phenotypic plasticity in a community of organisms can be influenced by both genetic and environmental factors, particularly the interaction of biotic and abiotic traits.

P. canaliculata an invasive agricultural pest has always been a topic for having a disastrous characteristics in many agro-ecosystems making them as one of the 100 world worst invasive species. They can destroy wide range of plants such as azolla, duck weed, algae, water hyacinth, rice seedlings and other succulent leafy plants. This makes them an organism that has evolved suitable phenotypes as a form of adaptive mechanism and is potentially capable of causing ecological damage, particularly in agricultural fields, if not controlled (Guerrero, 1989; Cagauan and Joshi, 2003; Joshi, 2005; Joshi et al., 2005; Cowie, 2006; Kho, 2011; Mahilum and Demayo, 2014). Many farmers in the Philippines are battling the invasive organism golden apple snail, which poses a severe threat to rice production due to its plant-eating and reproductive abilities. *P.canaliculata* in the Philippines behaves differently than in other countries, such as Japan and Argentina, in that it takes longer to mature and lay eggs (Dong et al., 2011). According to Cabuga et al. (2017), geographical location is a crucial element in the species' spreading.

The recent study concerns on describing the phenotypic plasticity within, betweem, and among the populations *P. canaliculata* using geometric morphometric analysis. Geometric morphometric is the finest technique for characterizing shape and shape variations among biotic elements. Geometric morphometric analysis has advantages over standard measurements when it comes to studying the consequences of differences in organism orientation, location, and position

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(Bookatstien, 1991; Chiu et al., 2002; Rohlf, 2003; Zelditch et al., 2004; Cabuga, 2017). As a result, this study employs the geometric morphometrics method, particularly landmark-based geometric morphometric analysis, to examine the shell shape of *P. canaliculata* that was collected from various irrigated rice fields in Diplahan, Mindanao, Philippines. Because the irrigated rice fields were geographically isolated, this study will offer fresh information on how the population of the various organisms varies in each location.

METHODOLOGY

Collection and Preparation of Samples

Collection of golden apple snail samples in rice fields were handpicked by the researcher while wearing rubber gloves. Barangay Ditay, Barangay Pilar, and Barangay Tinongtongan were the three barangays of Diplahan, Mindanao, Philippines, that were chosen as golden apple snail sampling locations (Figure 1). Each location used the same rice varieties for the collection of samples (Table 1). A total of 270 individuals of golden apple snails were collected, with 30 samples (15 male and 15 female) of each rice variety in each barangay. The shells were boiled with water, then was cleaned with tap water after removing the meat with a pin (Mahilum & Demayo, 2014). Cracked shells and spires that had eroded were discarded. The presence of the penis sheath, which is indicative of males, are used classify snails according to sex (Torres et al., 2011) (Figure 2). To obtain the desired images of the shell, an iphone 7 was used. The camera was positioned on a tripod from the top of the shell to keep the distance consistent and to get good images of the samples (Mahilum & Demayo, 2014). The shells are photographed in 2D orientation with its aperture and ventral side pointing to the x axis.

Table 1. Summary of the rice varieties from the different micro-geographical distances.

Ditay	Pilar	Tinongtongan
NSIC Rc 160 (Tubigan 14)	NSIC Rc 160 (Tubigan 14)	NSIC Rc 160 (Tubigan 14)
NSIC Rc 222 (Tubigan 18)	NSIC Rc 222 (Tubigan 18)	NSIC Rc 222 (Tubigan 18)
NSIC Rc 400 (Tubigan 35)	NSIC Rc 400 (Tubigan 35)	NSIC Rc 400 (Tubigan 35)

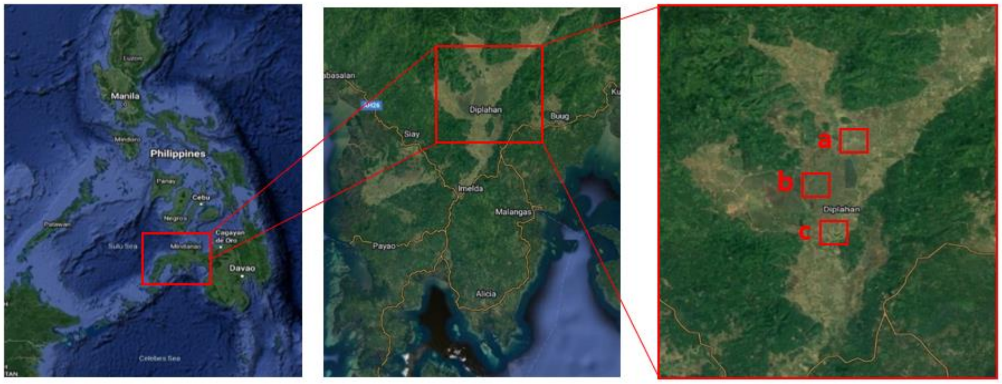

Figure 1. Geographical presentation of the different sampling sites, (a) Barangay Ditay (7.7324° N, 122.9635° E), (b) Barangay Pilar (7.6853° N, 122.9945° E) and, (c) Barangay Tinongtongan (7.8197° N, 122.9297° E).

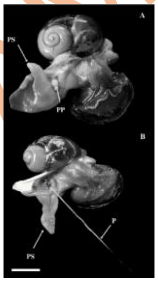

Figure 2. An image of the *Pomacea canaliculata's* penis sheath in front of the gills which is located inside of its mantle cavity (Lui et al. 2016)

Data Acquisition

Landmark Assignments

The number of landmarks on the shells were digitized in two-dimensional Cartesian coordinates using the tpsDig ver.2 program (Fig. 3) (Rohlf, 2004). All specimens were photographed with three replicates to minimize measurement error (Dvorak et al., 2005; Hampong O. B., 2018). The software obtained the landmark points' x and y coordinates, were used as raw data for further analysis. The GLS (General Least Squares) Procrustes superimposition method was used to scale, translate, and rotate the landmark configurations against the consensus configuration (Bookstein, 1991; Rohlf and Marcus, 1993; Dryden and Mardia, 1998; Hampong O. B., 2018).

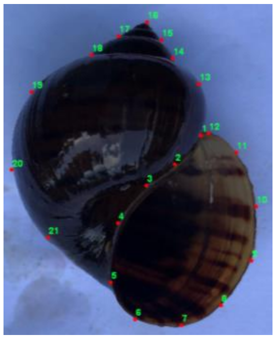

Figure 3. Designated landmarks of the ventral/apertural view of P. canaliculata's shell.

Analysis of Data

Landmarked-based Geometric Morphometric (GM) Analysis

Thin plate splines were used to graphically demonstrate patterns of shape variations based on landmarks that indicates the alteration of the reference to each specimen (Bookstein, 1991). The digitized landmark shells of male and female *P. canaliculata* was run individually. All specimens were digitized, including 15 male and 15 female specimens each rice variety. tpsDig ver.2 software was used in digitizing the two-dimensional Cartesian coordinates of the obtained number of landmarks on both the dorsal and ventral/apertural sides of the shell (Fig.3) (Rohlf, 2004). All specimens were digitized with three replicates to minimize measurement error (Dvarak et al., 2005). The software obtained x and y coordinates of the landmark points which is considered as the raw data was used for further analysis (Hampong, 2018). The TpsUtil program was used to build Tps file and make links file.

Relative Warps Analysis

The GLS (General Least Squares) Procrustes superimposition method were used to scale, translate, and rotate the landmark configurations against the consensus configuration (Bookstein, 1991; Rohlf and Marcus, 1993; Dryden and Mardia, 1998). The tpsRelw version 1.46 were used to do the relative warps analysis (Bookstein, 1991). (Rohlf, 2008). Relative warps analysis corresponds to a Principal Components analysis of the covariance matrix of the partial warp scores, which are different scales of a thin-plate spline transformation of landmarks (Fig. 4) (Frieß, 2003). The first and second relative warps, according to Hammer et al. (2001), are usually the most relevant.

Discriminant Function Analysis

The discriminant analysis was performed to see if the observed variance in shell shapes in golden apple snail populations between rice varieties is statistically significant. It was used to determine what percentage of right classifications are equal to or higher than the cut-off score of 75%, which is considered significantly different (Hammer et al., 2009). It was also utilized to determine population shape differences and equality of means of the compared groups using Hotelling's t-squared, which yielded a p value (p0.05). PAST version 3.0 software was used to conduct the statistical analysis.

Multivariate Analysis of Variance

Multivariate Analysis of Variances (MANOVA) was used for the analysis of the relative warp scores for the shape of male and female shell per variety and per sites populations of golden apple snail. MANOVA is a type of multivariate analysis that determines whether or not numerous samples have the same shape. Statistical values such as Hotelling's p(same), Wilk's lambda, and Pillai trace are available. The p(same) of Hotelling denotes a pairwise comparison of the mean shape. A comparison of the error variance/covariance matrix and the effect variance/covariance matrix yields Wilk's lambda. It would establish the link between a numbers of factors. Wilk's lambda has a small (near to 0) value when the groups are well divided, and a large (close to 1) value when the groups are poorly separated (Torres et al., 2010). The Pillai trace is used to determine whether two sets of variables are independent. The greater the value of the Pillai trace, the greater the contribution of the given effect to the variance. PAST version 3.0 will be used to perform MANOVA.

Canonical Variate Analysis

The male and female datasets was combined and analyzed using Canonical Variate analysis to detect variance between groups as compared to the pooled within-group variation. To compare patterns of inter-population variance, CVA was used. The canonical variate were displayed in the form of an ordination and will be dispersed throughout the groups. PAST 3.0 were used to perform CVA.

RESULTS AND DISCUSSION

Sexual Dimorphism

Within the populations of *P. canaliculata* from the barangay of Ditay, Pilar, and Tinontongan the difference in the shell shape mean is illustrated in Table 2. It indicates a considerable disparity in the shell shape of male and female populations within the different micro-geographic distances.

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Table 2. Confirmatory MANOVA results on shell shape variance in pooled male and female populations collected from different micro-geographic locations utilizing landmark-based geometric morphometric analysis.

MALE AND FEMALE	WITHIN	Wilk's lambda	Pillai Trace	p(same)	Remarks
	Ditay	0.6366	0.3634	3.145E-09	Significant
	Pilar	0.4666	0.5334	7.042E-22	Significant
	Tinongtongan	0.6381	0.3619	3.861E-09	Significant

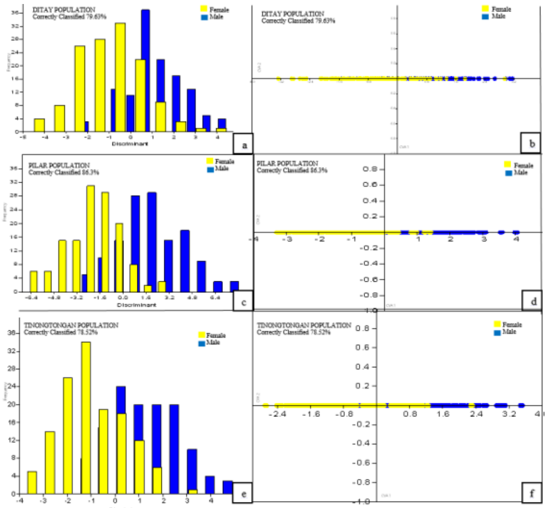

Figure 4. Discriminant Function Analysis (DFA) [a,c,e] and Cayhuuuuuunonical Variate Analysis (CVA) [b,d,f] plots showing distribution of relative warp scores of ventral shell shape variability of the pooled male and female individuals of P. canalculata populations of Brgy. Ditay, Brgy. Pilar, and Brgy. Tinongtongan, Diplahan, Zamboanga Sibugay areas using landmark-based GM analysis.

Figure 4 depicts the distribution of relative warp scores on ventral shell shape variability of male and female populations among the different areas using DFA and CVA plots. Barangay Ditay, Pilar, and Tinongtongan have percentage values of 79.63%, 86.3%, and 78.52% respectively. According to Hammer et al. (2009), correct categorization equal to or higher than the cut-off score of 75% is significant. As a result, these values imply that male and female populations have distinct shell shapes, which shows the prevalence of sexual dimorphism in the populations of *P. canaliculata* from different geographic locations. The graph, on the other hand, reveals a lot of overlap between male and female populations. This means that the shell morphologies of the various populations are still comparable (Hampong 2018).

3.2. Shell Shapes Variability Based on Rice Types

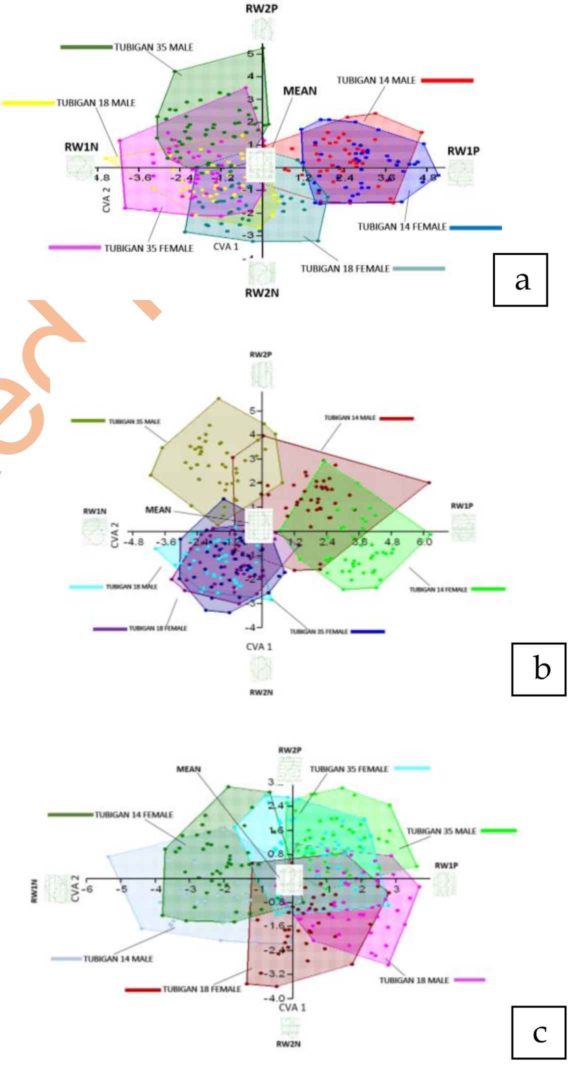

Figure 5. CVA scatter plot showing ventral shell shape variability among populations of *P. canaliculata* from different rice types collected from (a) Brgy. Ditay, (b) Brgy. Pilar, (c)

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Brgy. Tinongtongan, Diplahan, Philippines using landmark-based GM analysis.

	T14M	T14F	T18M	T18F	T35M	T35F
T14M		3.23569E-10	5.00609E-10	0.00581439	1.20603E-07	7.71881E-10
T14F			2.83048E-05	1.51183E-10	0.000164316	0.00081728
T18M				1.07101E-11	1.79548E-06	0.000755536
T18F					9.53859E-09	1.53696E-12
T35M						0.000596681
T35F						

(a) *Wilk’s lambda:* 0.03032 F: 6.091 p(same): 1.208E-85 *Pillai trace:* 2.314 F: 5.238 p(same): 3.271E-71

	T14M	T14F	T18M	T18F	T35M	T35F
T14M		8.37602E-11	6.00324E-10	2.614561E-09	4.70561E-09	8.9692E-11
T14F			9.16283E-10	1.09484E-13	0.0142274	0.000127168
T18M				8.30278E-16	2.51064E-10	7.66178E-10
T18F					1.89244E-12	7.94058E-12
T35M						0.000151588
T35F						

(b) *Wilk’s lambda:* 0.0134 F: 8.245 p(same): 1.862E-120 *Pillai trace:* 2.656 F: 6.89 p(same): 8.841E-100

	T14M	T14F	T18M	T18F	T35M	T35F
T14M		5.67607E-09	9.2361E-08	0.00597969	8.18874E-05	5.61772E-07
T14F			0.000100471	3.13143E-09	0.0101495	0.000429034
T18M				3.4352E-08	5.55825E-05	0.000143825
T18F					3.52909E-06	2.33337E-05
T35M						0.000332923
T35F						

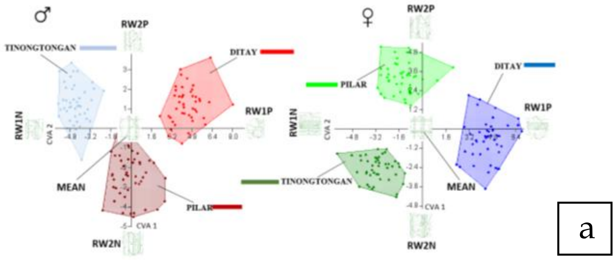
(c) *Wilk’s lambda:* 0.04896 F: 4.982 p(same): 3.714E-66 *Pillai trace:* 2.136 F: 4.535 p(same): 2.2E-58

Legend: Tubigan 14 Male (T14M), Tubigan 14 Female (T14F), Tubigan 18 Male (T18M), Tubigan 18 Female (T18F), Tubigan 35 Male (T35M). Tubigan 35 Female (T35F)

Table 3. Hotelling’s pairwise comparison in the ventral shell shape variances between pooled individuals of *P. canaliculata* of the different rice varieties (Tubigan 14, Tubigan 18, and Tubigan 35) from (a) Brgy. Ditay, (b) Brgy. Pilar, (c) Brgy. Tinongtongan, Diplahan, Philippines using landmark-based GM analysis.

Figure 5 shows a scatter plot of populations from several rice varieties, both male and female, with a low degree of overlapping, indicating a meaningful pairwise comparison result. The figure also shows how variants are split apart. The relative warps (RW1 and RW2) in the figure also indicate the variability in shell shape between populations. This results is supported by the the Hotelling pairwise comparison of shell shape variability between GAS populations that is shown in table 3. It demonstrates that the pairwise comparison of male and female, as well as the population of different rice types, has significant p(same) values. It confirms that the p(same) comparison of sex and rice types groups has significant values, indicating that there is sexual dimorphism and differentiation because of rice types in the population. The Wilk's Lambda is also near to zero, whereas the Pillai trace has a large value, indicating that the groups are highly divided (Torres et al 2010).

Shell Shapes Variability Based on Micro-Geographic Distances



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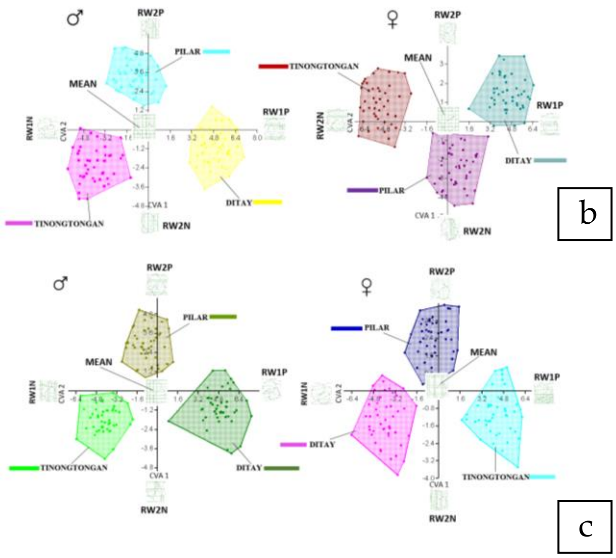

Figure 6. CVA scatter plots showing shell shape variability among populations of GAS within sexes and between micro-geographic locations collected from (a) Tubigan 14, (b) Tubigan 18, (c)Tubigan 35 rice varieties using landmarked-based GM analysis.

(a)

MALE	Ditay	Pilar	Tinongtongan
Ditay		1.2943E-14	1.04271E-22
Pilar			2.20831E-14
Tinongtongan			

Wilk's lambda: 0.01725 F: 16.54 p(same): 7.993E-54 *Pillai trace:* 1.686 F: 13.58 p(same): 1.263E-47

FEMALE	Ditay	Pilar	Tinongtongan
Ditay		2.58049E-18	3.78123E-20
Pilar			6.72467E-15
Tinongtongan			

Wilk's lambda: 0.01294 F: 19.47 p(same): 1.991E-59 *Pillai trace:* 1.764 F: 18.84 p(same): 1.04E-58

(b)

MALE	Ditay	Pilar	Tinongtongan
Ditay		1.86543E-19	3.87048E-23
Pilar			2.70125E-16
Tinongtongan			

Wilk's lambda: 0.009578 F: 23.04 p(same): 2.346E-65 *Pillai trace:* 1.79 F: 21.53 p(same): 2.062E-63

FEMALE	Ditay	Pilar	Tinongtongan
Ditay		6.65345E-14	6.79201E-25
Pilar			1.421E-17
Tinongtongan			

Wilk's lambda: 0.01394 F: 18.67 p(same): 5.709E-58 *Pillai trace:* 1.701 F: 14.35 p(same): 1.946E-49

(c)

MALE	Ditay	Pilar	Tinongtongan
Ditay		5.53589E-18	1.76639E-23
Pilar			1.85806E-14
Tinongtongan			

Wilk's lambda: 0.0125 F: 19.86 p(same): 4.191E-60 *Pillai trace:* 1.743 F: 17.15 p(same): 1.893E-55

FEMALE	Ditay	Pilar	Tinongtongan
Ditay		1.09961E-12	2.55013-21
Pilar			2.02545E-14
Tinongtongan			

Wilk's lambda: 0.02051 F: 14.96 p(same): 1.822E-50 *Pillai trace:* 1.665 F: 12.54 p(same): 4.812E-45

Table **4.** Hotelling's pairwise comparison in the same rice variety's [(a) Tubigan 14, (b) Tubigan 18, (c) Tubigan 35] ventral shell shape variances between pooled individuals of *P. canaliculata* among different areas using landmark-based GM analysis.

Table 4 shows the mean shell shape variability by sex and location as described by Hotelling's pairwise comparison. Within sexes and between micro-geographic distances, significant p(same) values are found, implying that sexual dimorphism and differentiation because of geographic distances exists. The CVA scatter plots in Figure 6, which show the general split-ups of the variants, confirm this results. The image depicts how male and female populations from various micro-geographic locations are well segregated. This is supported by Table 4, which shows that Wilk's lambda is near to zero in male and female populations from different locales. This indicates that the geographic distances have a significant impact on *P. canaliculata* population differentiation.

The results showed that morphological differences between sexes, rice types, and geographical distances have significant values. This tells us three things: first, the large variation between sexes indicates that there is sexual dimorphism in terms of the shell. Second, the large differences between rice varieties suggest that *P. canaliculata* phenotypic characteristics are linked to the hosts they utilize. Third, the considerable variance between *P. canaliculata* populations over short geographical distances indicates that environmental factors and their low mobility influence the phenotypes of population members.

CONCLUSION

The findings of this study show that sexual dimorphism and shell shape variations exists between the populations of *P. canaliculata* collected from different rice types and short geographic distances. In addition, geometric morphometric, particularly relative warp analysis and superimposition, can be utilized to assess phenotypic changes in *P. canaliculata* shell shape morphology. The observed shell shape differences in this study could be caused by various environmental and genetic factors that lead to phenotypic plasticity of this invasive species in response to various rice varieties and geographic distances. Moreover, the causes of sexual dimorphism on organisms are not yet clear, more genetic and ecological research is needed to better understand the nature of the organism's variations. In addition, the conclusions and findings of this study in relation to the rising problem of invasive species may lead to the reconsideration of an effective management strategies and proper control of *P. canaliculata*.

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