Macro-Geographic Variations of the Invasive Giant African Snail *Achatina fulica* Populations in Mindanao, Philippines

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ABSTRACT It is argued that widely distributed populations of the ecologically damaging land snail *Achatina fulica* are expected to harbor less genetic variation due to multiple introductions. Despite of this likely decreased genetic variation, they have successfully established cosmopolitan distribution and invasive status out from their native origin in East Africa. Successful invading species are found to exhibit increased plasticity which may indicate local adaptation to maximize its fitness. Thus, this study was conducted to examine plasticity in the phenotype of the species by employing the tools of both meristic and geometric morphometrics. Variations in the ventral, apertural view of *Achatina* were described and compared subjecting them to Correlation Analysis Based on Distances (CORIANDIS). This method visualizes congruence and disparity of multivariate traits. Results of the study show variability within, between and among populations of *A. fulica*. The plasticity observed was not based on distance and may indicate their inherent ability to adapt to local conditions characterized by the kind of host plants available and maybe due to the differences in the physico-chemical environment of the sampling areas.

KEYWORDS: macro-geographic, plasticity, meristic, geometric morphometrics, *Achatina*

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TRANSACTIONS ON SCIENCE AND TECHNOLOGY

INTRODUCTION

Evolutionary changes such as increased plasticity are found to be widespread in successful invading species such as *Achatina fulica*, an introduced land snail from East Africa. Such phenotypic plasticity is an indication of rapid evolution, a crucial mechanism for its invasion success since variability provides the raw material for sustained adaptability and long-term survivability as it invades novel environments with varying community structures (Dlugosh & Parker, 2008; Holt, 1987; Torres *et al.*, 2011). These phenotypic plastic responses could be a major contributing factor making it second among the Worlds' Worst 100 Invasive Species (IUCN 2014).

Phenotypic plasticity responses are those environmentally-induced phenotypic variations which could be attributed to the following ecological sources: geographical isolation, resource use and habitat selection, among others (Gilbert, 2000; Lacase *et al.*, 2009; Madjos *et al.*, 2015; Piglucci *et al.*, 2006; Scheiner, 1993). It is of interest to evaluate relationship between these environmental factors and phenotypic variation to further understand the biology of this invasive land snail (Sobrepena & Demayo, 2014) as variation in invasions has never been quantified (Dlugosh & Parker, 2008). Quantifying phenotypic variation provides a powerful alternative approach in invasion studies and information about how traits differ can reveal the evolutionary dynamics that shape populations Byers, 2008).

Phenotypic plasticity in morphometric traits may often be adaptive reflecting the effects of exogenous factors in a population (Stankowski & Johnson, 2014) and can be evolutionarily advantageous by contributing to a species success in invading new environments (Barret, 2005; Stearns, 1989). Evaluating the different forces documenting *A. fulica's* populations to diverge as they colonize a wide range of ecological habitat condition is essential if effective management strategies are to be developed in these invasive gastropod species. Thus, this study sought to determine macro-geographic variations from selected protected and suburban ecosystem types in Mindanao, Philippines through the use of meristic and geometric morphometrics tools.

METHODOLOGY

Study site

Thirty (30) adult individuals of *A. fulica* were purposively collected from each four geographically isolated protected montane areas and suburban places in Mindanao, Philippines (Fig. 1). Pasonanca Natural Park (PNP, Zamboanga City) and Initao-Libertad Protected Landscape and Seascape (ILPLS, Misamis Oriental) are Biodiversity Monitoring Areas of the DENR-Regions IX and X respectively, with Global Positioning System (GPS) readings of N 06° 58′ 28.8″, E 122° 04′ 02.5″ and N 08° 33′ 46″, E 124° 31′ 22″ and elevation of 62 meters asl (above sea level) and 10 meters asl respectively. The other two suburban locations are in Pagadian City (elevated at 60 meters asl) and Cagayan de Oro City (CDO), located at N 07° 50′, E 123° 26′ and N 08° 25.464′, E 124° 36.611′, with 60 meters asl and 63 meters asl elevations respectively. These sampling sites were further evaluated in terms of its host plant availability and abiotic parameters.

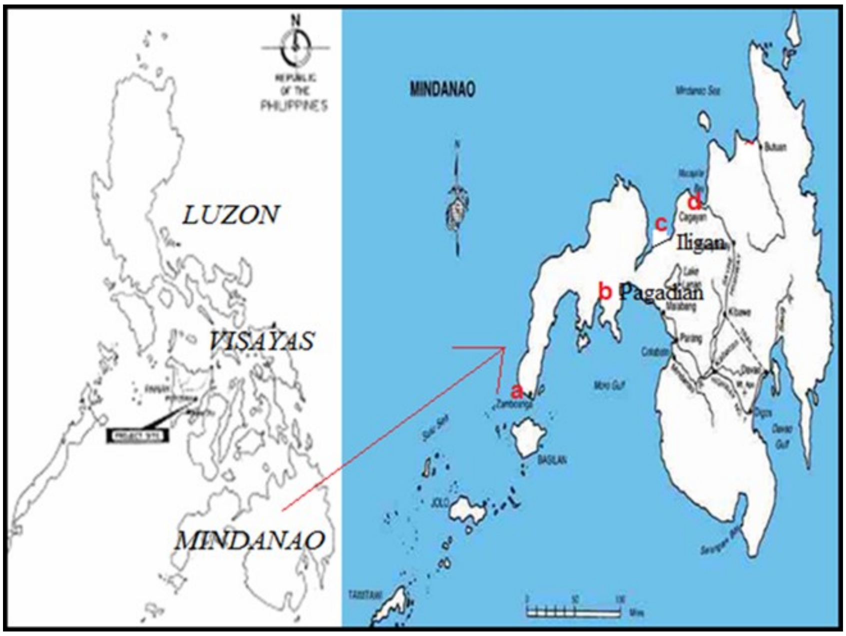


Figure 1. Location map of the four macro-geographically isolated study sites

Morphometrics Analysis

In quantifying morphometric traits, both meristic and geometric morphometrics were used as tools and subjected to Correlation Analysis Based on Distances (CORIANDIS) (Marquez & Knowles, 2007). These morphometric analyses can be used to quantify a trait of ecological significance by detecting phenotypic plasticity responses (Madjos and Anies, 2016).

For meristic measurements (Addis *et al.*, 2010), Vernier caliper was used to obtain the conchological character measurements (shell length, shell width, spire height, number of whorls, aperture height and aperture width). The shell length was taken from the apex of the shell to the base of the aperture. The shell width was measured at the widest part of the shell when the shell is oriented so that aperture faces the observer; specifically measured from the side of the body whorl to the outermost side of the aperture. Meanwhile, the spire height corresponds to the apical whorl, excluding the main body whorl. In counting the number of whorls, a complete turn indicates a whorl (Galan *et al.,* 2018). The aperture length and width were measured on the widest part, being oriented at 90°. Figure 2 shows the conchological characters taken through meristic tools.

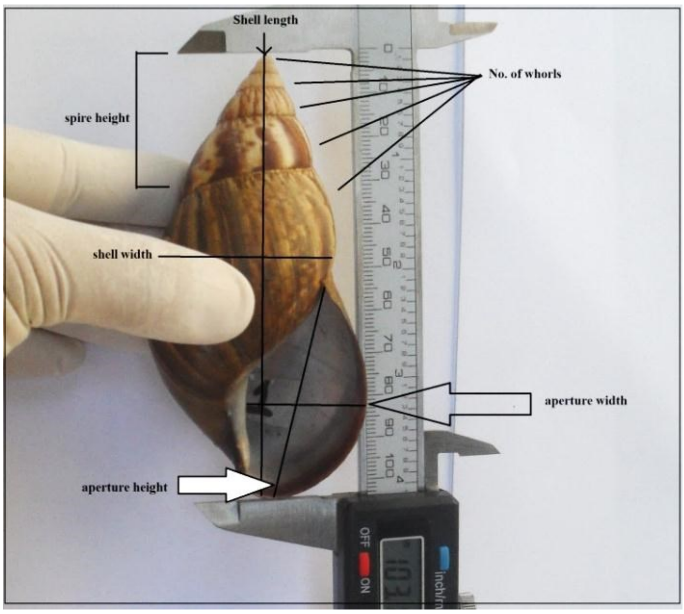


Figure 2. Conchological characters taken through meristic tools.

To test the hypothesis that conchological, meristic characters vary among the macro-geographically isolated areas, Multi-Variate Analysis of Variance (MANOVA) was used. On the other hand, geometric morphometrics is equally significant in demonstrating phenotypic plasticity in morphometric traits (Addis *et al.,* 2010), thus quantitative representation and analysis of morphological shape analysis of the shell made use of geometric coordinates. In coordinate acquisition, individuals of *A. fulica* from each population were photographed using WG1 Pentax camera (14 megapixel, optical zoom 10x) on its aperture, a shell part thought to have eco-evolutionary significance during locomotion, tumbling, mating and feeding.

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. Figure 3 shows the landmarks acquired in the apertural view patterned after the works of Sobrepena & Demayo (2014 a,b).

Figure 3. Landmarks acquired in the apertural view of *A. fulica*

Fifty (50) landmarks were identified in the apertural view of *A. fulica.* Each set of co-ordinates were then submitted separately to a Generalized Procrustes Analysis (GPA) available in the tpsRelw software (Rohlf, 2007) to eliminate any morphological variations resulting from size, position or orientation of specimens. 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. 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) to provide a graphical representation of shape and to compare the sets of data. PCAs can identify any regularity within the sample (Addis *et al.,* 2010).

In a morphometrics analysis, regularities corresponding to simultaneous displacements of anatomical points are often observed in the specimens thus, 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). Differences in the centroid sizes among populations were then tested using Multivariate Analysis of Variance (MANOVA).

Both meristic and geometric data were then integrated from all the seven characters (non-landmarked length, width, spire height, number of whorls, aperture length and aperture width and the landmarked ventral, apertural view) in order to observe underlying differences and sources of variability among groups in terms of congruence among characters (Tabugo *et al.,* 2014; Anies *et al.,* 2015). CORIANDIS has been used to determine associations among multivariate datasets, trait variance or disparity, congruence and multivariate covariance measure on how similar the interspecific locations of characters of the species or populations. The option "Projections on compromise space" will plot all the specimens/groups and traits in the same space. The squared distances of each group to the origin are then computed for each of the shape data sets, and plotted in a stacked bar graph to give an overall impression of the differences between the populations of *A. fulica*.

RESULT AND DISCUSSION

Variations in meristic characters where the snails were collected were observed based on the availability of food plants and abiotic parameters (Table 1 and 2).

Table 1. Food plants available in the four sampling areas where *A. fulica* individuals were collected.

Native & Horticultural Flora	Sampling Sites			
	PNP, Zambo	Pagadian	ILPLS	CDO City
Livistona rotundifolia	■			
Abelmoschus manihot	■			
Ipomea batatas	■			
Oreganum vulgare	■	■		
Carica papaya	■	■		■
Musa paradisiaca	■	■		■
Anona muricata	■	■		■
Colocasia esculenta	■	■		■
Moringa oleifera	■	■		■
Plant Litters	■	■	■	■
Ipomea alba		■		■
Swetenia macrophylla			■	
Broussonetia luzonica			■	
Terminalia margarapali			■	
Steganotaenia asaliacea			■	
Psidium guajava			■	
Couroupita guianensis			■	
Strilitzia sp.			■	
Twigs				■
Cynodon sp.				■
Manihot esculenta				■
Operculina turphetum				■
Saccharum oficinarum				■
Citrus lima				■
Eugenia sp.				■

Monte Carlo: Mean – 0.9159; Std. Dev. – 0.0226; Z score - 2.052; p - 0.0402

Table 2. Mean temperature, humidity and precipitation in the different sampling areas at diiferent periods.

Sampling Areas	Mean Temperature (°C)				Mean Humidity (%)				Mean Precipitation (mm)*			
	Periods				Periods				Periods			
	1	2	3	4	1	2	3	4	1	2	3	4
PNP, Zbo. city	30.9	36.5	33.4	27.5	85 (Wet)	40 (Warm)	78.7 (Wet)	76.3 (Wet)	226.35	005	195	66.4
Pagadian City	29.1	33.7	33.4	29.6	59.3 (Warm)	35.3% (Warm)	38.7 (Warm)	63.3 (Warm)				
ILPLS, Mis. Or.	29	33.4	33.3	30.5	80 (Wet)	40% (Warm)	78% (Wet)	80.7% (Wet)	192	0.0	145	37.5
Cagayan de Oro City	30.4	33.7	33.4	27.5	35.7 (Warm)	34% (Warm)	40% (Warm)	76.7 (Wet)				

Legend: ** - source: Nearest PAGASA, DOST IX & X of the four sites*
1 – August-September 2015 (rainy season), 2 – March 2016 (El Niño phenomenon),
3 – June-July 2016 (end of summer season), 4 – November 2016 (onset of La Nina watch)

Seriation analysis inferred that there is a significant difference among the host plant community structures in the four sampling areas (PNP, Zamboanga City; Pagadian de Oro City; ILPLS, Mis. Oriental and Cagayan de Oro City) (Table 1). Variations in terms of abiotic parameters in varying periods were also documented in each sampling sites (Table 2). Tables 3 and 4 show the MANOVA results in terms of meristically evaluated conchological characters (length, width, spire height, number of whorls, aperture length and aperture width).

Table 3. MANOVA results of the four populations in terms of meristically evaluated conchological characters.

Wilks lambda	df1	df2	F	p(same)
0.3115	18	993.3	26.81	5.135E-73*
Pillai trace	**df1**	**df2**	**F**	**p(same)**
0.8473	18	1059	22.32	6.009E-62*

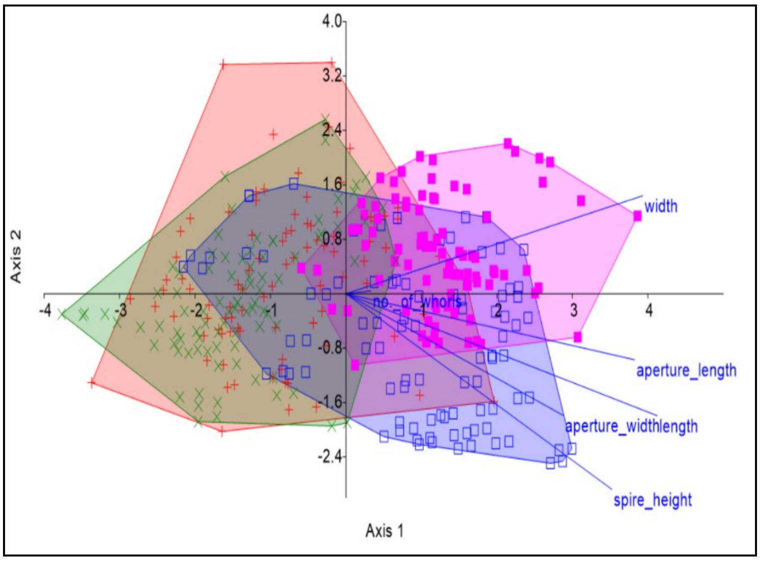
* significant at α – 0.0001

Table 4. Multivariate Analysis of Variance (MANOVA) between groups.

	CDO	ILPLS	Pagadian	PNP, Z.C.
CDO	-	1.38845E-24*	1.14753E-27*	3.42051E-07*
ILPLS		-	4.87279E-12*	6.90765E-33*
Pagadian			-	2.96069E-38*
PNP, Z.C.				-

* significant at α – 0.0001

The distribution of individuals among the four populations of *A. fulica* in the CVA plot show how each population vary based on the evaluated conchological characters (length, width, spire height, no. of whorls, aperture length and aperture width) (Fig. 4). It can be inferred from the results that the differences in conchological characters between the groups can be attributed to variations between individuals within the groups. Discriminant analysis shows the frequencies of individuals that are similar to other groups (Table 5). The variations between individuals and the observed differences between populations could be attributed to phenotypic plasticity (Vinic *et al.*, 2006).



Legend: red - CDO; green –ILPLS; blue - Pagadian City; pink-Zambo. City

Figure 4. CVA scatterplot showing variations among the four populations in terms of the six meristic characters (length, width, spire height, number of whorls, aperture length and aperture width).

Table 5. Confusion matrix (in percent) between populations of *A. fulica* based on meristic characters.

	CDO	**ILPLS**	**Pagadian**	**PNP, Z.C.**
CDO	46.68(42)	7.78(7)	12.22(11)	33.33(30)
ILPLS	16.67 (15)	54.44(49)	25.55(23)	3.33(3)
Pagadian	0	20(18)	75.56(68)	4.44(4)
PNP, Z.C.	20(18)	2.22(2)	10(9)	67.78(61)

Legend: *upper values (%); lower values (actual number)*

For shape analysis, results of the relative warp (RW) analysis showed three among the four general descriptions of shell shape morphology were observed based on the works of Sobrepena & Demayo (2014a). These are as follows: elongated spire with narrow body whorl and narrow aperture, elongated spire with narrow body whorl and rounded aperture, short spire with wide body whorl and narrow aperture, and short spire with wide body whorl and rounded aperture. Thin-Plate Spline (TPS) provides the visual presentation of the ventral shapes of *A. fulica* shells in four geographic locations (Fig. 5). It can be seen from the figure that a narrow body whorl and narrow aperture characteristics of the shell were commonly observed among shells of the population from Pagadian City (C) while an elongated spire with narrow body whorl and rounded aperture were commonly observed in both CDO and ILPLS populations. Most individuals of the Zamboanga City population showed shell shapes with short spire with wide body whorl and narrow aperture. While these variations were significant from the results of the MANOVA analysis of the shapes of individuals in the four populations (Tables 6), only the Cagayan de Oro suburban populations significantly differs in shapes when compared to other populations (Table 7). The population differences like the meristic characters are attributed to variations in individual shapes within populations (Table 8, Fig. 6).

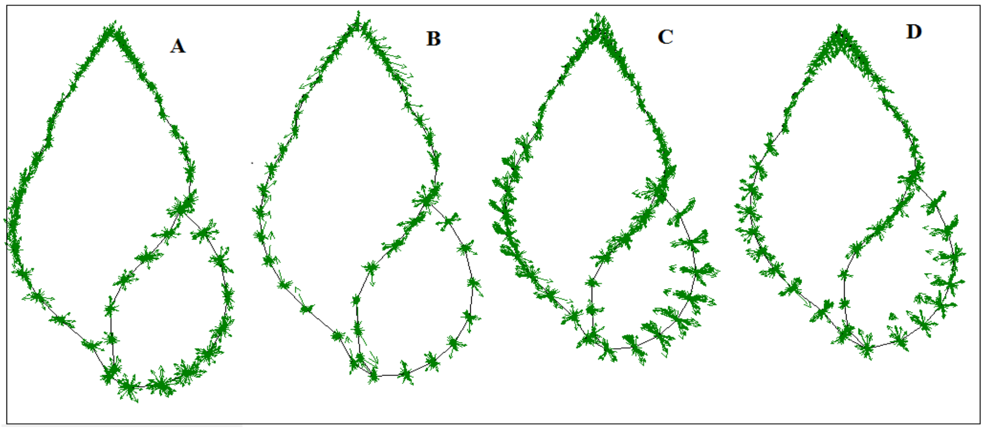


Figure 5. Conchological mean shape variations in (A) Cagayan de Oro; (B) Initao; (C) Pagadian and (D) PNP, Zamboanga City.

Table 6. MANOVA between the four populations of *A. fulica* based on significant RW.

Wilk's lambda	df1	df2	F	p(same)
0.9374	9	857	2.561	0.006571*
Pillai trace	df1	df2	F	p(same)
0.062779	10	62	2.522	0.007344*

* significant at $\alpha - 0.01$

Table 7. Comparison of populations based on significant RW of shape data in *A. fulica*.

	ILPS	Pagadian	PNP,Z.C.
CDO	0.0477976*	0.00297918*	0.000458792*
ILPS		0.710422	0.511036
Pagadian			0.694401

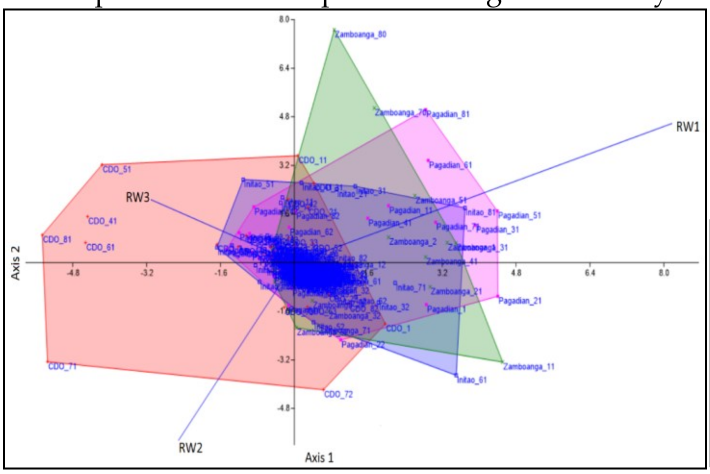
* significant at $\alpha - 0.01$

Table 8. Confusion matrix (in percent) between populations of *A. fulica* based on significant RW.

	CDO	**ILPLS**	**Pagadian**	**PNP, Z.C.**
CDO	36.67(33)	46.67(42)	7.78(7)	8.89(8)
ILPLS	26.97(24)	48.31(43)	6.74(6)	17.98(16)
Pagadian	22.22(20)	48.89((44)	11.11(10)	17.78(16)
PNP, Z.C.	16.85(15)	48.331(43)	7.54(27)	18.72(67)

Legend: *upper values (%); lower values (actual number)*

These variations in shapes are further explored through CVA analysis (Fig. 7)



Legend:
red – Cagayan de Oro populations;
green – Pagadian City;
blue – ILPLS, Misamis Oriental;
pink – PNP, Zamboanga City

Figure 6. CVA scatter plot showing variability among the four populations based on significant RW.

Further analysis in understanding variations between populations of the snails made use of multiple data sets of characters from meristic measurements and the shapes of shells based on relative warps. These data were analyzed using the CORIANDIS software for the demonstration of phenotypic plasticity in the snails (Márquez & Knowles, 2007). Figure 7 shows the plot of the principal components of "compromise" space axis accounting for 79.83%, 11.25% and 8.197% of the total compromise variance. Quality of the compromise was 86.94%. Based on the plot, the congruence and multivariate measure on how related the interspecific locations of conchological characteristics (represented as colored points) are shown. Between pairs of the four snail populations, they are said to be positively congruent and tend to cluster together based on whether two traits tend to be consistently similar (Marquez and Knowles 2007). In the result, Cagayan de Oro and Initao National Park populations cluster together and primarily differ with Pagadian and Zamboanga populations based on the seven shell shape datasets.

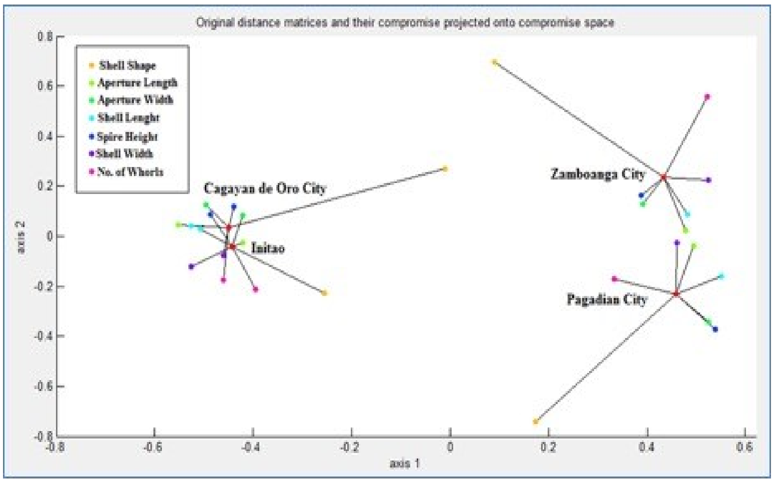


Figure 7. Plot of the principal components of "compromise" space axis of the *A. fulica* populations.

Codisparities to determine correlation between populations are shown in the disparity plots indicating the relative contribution of different characters of *A. fulica* to the divergence of the four populations (Fig. 8).

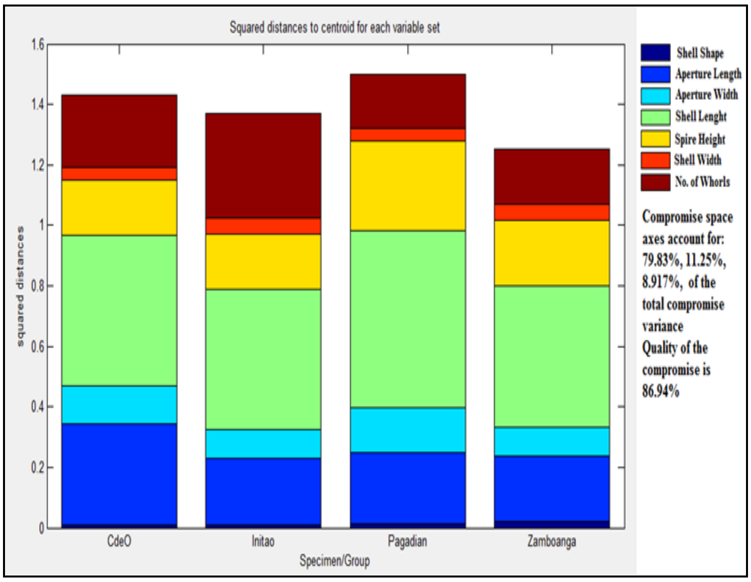


Figure 8. Stacked bar graphs showing disparity among the four populations of *A. fulica* with regards to 7 conchological characters (landmarked ventral, apertural view and non-landmarked length, width, spire height, number of whorls, aperture length and aperture width).

The total height of the stacked bar chart results from the addition of a squared distances of each trait separately (a measure of trait disparity). Based on the heights of the stacked bar graphs, the results show how populations differ in selected morphological traits. Pagadian City *Achatina* populations differ from the other populations based on shell length. The narrowest aperture width but more protruding whorls was a characteristic which differentiate the ILPLS populations from the rest. This smaller aperture and more protruding whorls may be for the adaptive responses of *A. fulica*'s burrowing lifestyle (Chiba, 2009), or an adaptation minimizing the area of exposed surface, thus having the advantage by minimizing the loss of humidity under stressful conditions (Pfenninger & Magnin, 2001). Spire height is observed to be defining the population of the population of the snail from Pagadian. This trait is considered added advantage to deter predation.

There could be many ecological factors that may have shaped phenotypic trait variations within and between populations of this invasive species. Habitat conditions are heterogeneous and have many variables such as food availability, predator occurrence, ease of defense, likelihood of offspring survival, microclimate changes, distance to human settlements, and many others (Holt, 2007; Jones, 2001). These may have contributed to variations in morphometry in the snail's shell. Due to multiple introductions in many types of environment, the introduced *A. fulica* may have encountered also novel selection pressures and challenges that permit population differentiation expressed as variability in morphologic and genetic characteristics (Fontanilla *et al.*, 2014). Though genetic variability was not examined in this study, the observed phenotypic variations can be reflected in the ability of the snail to alter growth form or develop alternative phenotypes to suit current ecological conditions (Goodfriend, 1986; Miner *et al.*, 2005; Wagele, 2004). This is an indication of their adaptive strategy to minimize loss of fitness in a harsher environment or to maximize fitness in a favorable environment (Madjos *et al.*, 2015).

CONCLUSION

Results in this study show how the different populations of *A. fulica* vary. Variability between the populations was observed to be due to variations observed in meristic measurements and analysis of shell shapes within each of the populations. While similarities were observed, the differences between the populations were attributed to the existence of individuals that are distinct for the populations where they were collected. Population differences cannot be attributed to geographical distance alone but could also be due to differences in habitats. Variations in the availability of food plants and other ecological conditions in the collection areas plus genetic predisposition to variable selection pressures may have contributed to the great potential of survival and adaptation of this species thus its invasiveness.

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REFERENCES

[1] Addis, P., Melis, P., Cannas, R., Secci, M., Tinti, F., Piccinetti, C. and Cau, A. (2010). A morphometric approach for the analysis of body shape in bluefin tuna: preliminary results. *Collect Vol Sci Pap ICCAT*, **65**(3), 982-987.

[2] Anies, O., Demayo, C. and Manting, M. M. (2015). Correlation Analysis Based on Distances of Facial Shapes of the Four Moro Gropus in Taluksangay, Zamboanga City, Philippines. *Aust. J. Basic and Applied Science*, **9**(28), 64-71.

[3] Barrett, R. D. H. (2005). Experimental Evolution of Pseudomonas flourescens in simple and complex environments. *American Naturalists*, **166**:470-480.

[4] Burela S. and Martin, P. (2011). Evolution and functional significance of lengthy copulations in a promiscuous apple snail, *Pomacea canaliculata* (Caenogastropoda: Ampullariidae). *Journal of Molluscan Studies*, **77**:54-64.

[5] Byers, D. L. (2008). Adaptation and Phenotypic Variance. *Nature Education*, 1(1):161.

[6] Chiba, S. (2009). Morphological divergence as a result of common adaptation to a shared environment in land snails of the genus *Hirasea.* Journal of Molluscan Studies, 75: 253–259.

[7] Dlugosh, K. M. & Parker, I. M. (2008). Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, **17**, 431-449.

[8] Fontanilla, I. K. C., Sta. Maria, I. M. P., Garcia, J. R. M., Ghate, H., Naggs, F. and Wade, C. M. (2014). Restricted Genetic Variation in Populations of *Achatina* (*Lissachatina*) *fulica* outside of East Africa and the Indian Ocean Islands Points to the Indian Ocean Islands as the Earliest Known Common Source. *PLoS ONE*, **9**(9): e105151. doi:10. 1371/journal.pone.0105151

[9] Galan, G. L, Porquis, H. C. and Bulasa, M. A. R. (2015). Shell band pattern of golden apple snail (*Pomacea canaliculata,* Lamarck) in selected aquatic habitats. *International Journal of Environmental Science and Development*, **6**(8):625-628.

[10] Gilbert, S. F. (2000). Environmental regulation of normal development. In: Developmental Biology. 6th edition, Sunderland (MA), Sinauer Associates, 580-581.

[11] Goodfriend, G. A. (1986). Variation in land-snail shell form and size and its causes: A review. *Systematic Biology*.

[12] Holt, R. (1987). Population dynamics and evolutionary process: the manifold roles of habitat selection. *Evolutionary Ecology*, **1**:331-347.

[13] UCN Website (2014). Invasive alien species: a selection from the Global Invasive Species Database. The Invasive Species Specialist Group (ISSG)-International Union for the Conservation of Nature (IUCN), p. 12.

[14] Jones, J. (2001). Habitat Selection Studies in Avian Ecology: A Critical Review. *The Auk*, **118**, 556-562.

[15] Lacaze, X., Hayes, P. M. and Korol, A. (2009). Genetics of phenotypic plasticity: QTL analysis

[16] in barley, *Hordeum vulgare. Heredity*, **102,** 163–173.

[17] Madjos, G. G. and Anies, O. S. (2016). Morphometrics approaches to studying phenotypic plasticity in *Pomacea canaliculata* (Golden apple snail). *International Journal of Advanced and Applied Sciences*, **3**(4), 50-56.

[18] Madjos, G., Demetillo, M., Baguio, M. and Torres, M. A. (2015). Phenotypic variations in populations of *Pomacea canaliculata* (golden apple snail): a case of agroecotypes? *Advances in Environmental Studies-International Journal of the Bioflux Society*, **7**(3), 432-441.

[19] Márquez, E. J. and Knowles, L. L. (2007). Correlated evolution of multivariate traits: detecting codivergence across multiple dimensions. *Journal of Evolutionary Biology*, **20**(6), 2334-2348.

[20] Miner, B. G., Sultan, S. E., Morgan, S. G., Padilla, D. K. and Relyea, R. A. (2005). Ecological consequences of phenotypic plasticity. *Trends in Ecology and Evolution*, **20**(12), 685-692.

[21] Pfenninger, M. and Magnin, F. (2001). Phenotypic evolution and hidden speciation in *Candidula unifasciata* ssp. (Helicellinae, Gastropoda) inferred by 16s variation and quantitative shell traits. *Molecular Ecology*, **10**(10), 2541-2554.

[22] Piglucci, M., Murren, C. and Schlichting, C. (2006). Phenotypic plasticity and evolution by genetic assimilation. *Journal of Experimental Biology*.

[23] Rohlf, F. J. (2006). TpsDig Version 2.10 – Department of Ecology and Evolution, State University of New York at Stony Brook, New York.

[24] Rohlf, F. J. (2007). TpsRelw Version 1.41 - Department of Ecology and Evolution, State University of New York at Stony Brook, New York.

[25] Scheiner, S. M. (1993). Genetics and Evolution of Phenotypic Plasticity. Annual Review of *Ecology and Systematics*, **24,** 35-68.

[26] Sobrepeña, J. M. and Demayo, C. G. (2014a). Outline-based geometric morphometric analysis of shell shapes in geographically isolated populations of Achatina fulica from the Philippines *Journal of Entomology and Zoology Studies*, **2**(4), 237-243.

[27] Sobrepeña, J. M. and Demayo, C. G. (2014b). Banding pattern and shape morphology variations on shells of *Achatina fulica* (Bowdich 1822) from the Philippines. *Annals of Biological Research*, **5**(1), 64-79.

[28] Sobrepeña, J. M. and Demayo C. G. (2014c). Assessing Geographic Conchological Variations of The Different Banding Patterns in the Invasive Giant African Land Snail Achatina fulica From the Philippines. *International Journal of Bioscience, Biochemistry and Bioinformatics,* **4**(4), 290-298.

[29] Stankowski, S. and Johnson, M. S. (2014). Biogeographic discordance of molecular phylogenetic and phenotypic variation in a continental archipelago radiation of land snails. *BioMed Central Evolutionary Biology*, **14,** 2.

[30] Stearns S. (1989). The evolutionary significance of phenotypic plasticity: phenotypic sources of variation among organisms can be described by developmental switches and reaction norms. *BioScience*, **39**(7), 436.

[31] Tabugo, S. R., Torres, M. A. and Demayo, C. G. (2014). Elliptic Fourier Analysis in describing shape of male appendages in Neurothemis species found in Iligan City, Philippines. *Natura Journal* **18**(1), 1-18.

[32] Torres, M. A., Joshi, R. C., Sebastian, L. S. and Demayo, C. G. (2011). Geographic phenetic variation in the golden apple snail, *Pomacea canaliculata* (Ampullariidae) based on geometric approaches to morphometrics. *Advances in Environmental Sciences -International Journal of the Bioflux Society*, **3**(3), 243-258.

[33] Vinic, G. K., Unnithan, V. K. and Sugunan, V. V. (1998). Farming of the Giant African Snail, *A. fulica*. India: Central Inland Capture Fisheries Research Institute no. 56, p. 1-24.

[34] Wagele, H. (2004). Potential key characters in Opisthobranchia (Gastropoda, Mollusca) Enhancing Adaptive Radiation. *Organisms, Diversity & Evolution*, **4,** 175–188.

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