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Annals of Biological Research, 2014, 5 (1):64-79
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0976-1233
CODEN (USA): ABRNBW

Banding pattern and shape morphology variations on shells of the invasive giant African land snail *Achatinafulica* (Bowdich 1822) from the Philippines

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ABSTRACT

One of the most notable variations observed in the morphology of the invasive giant African land snail Achatinafulica is the different banding patterns and shell shape morphology. This study aims to assess the correlation of shell shape and banding patterns among populations of A. fulica. Samples were collected from 15 different provinces in the Philippines. A total of 14 banding patterns were assigned based on the streaks observed on the body whorl. Generally, relative warp analysis showed variation in shell shape which could be slender shaped or round-looking shells. A varied spire-whorl length and aperture shape was also observed in the samples. Histograms and box-and-whiskers plots illustrated multimodal variationson the shell shapes of the banding patterns. Canonical variance analysis scatter plots presentedoverlapping of populations of the different banding patterns. Although there were no observable differences on the mean shapes of the different banding patterns, the MANOVA/CVA scores, Kruskal-Wallist test, and Cluster analysis showed significant morphology variations. The scatters distribution and short distance of variation suggest a wide intrapopulation variation. The findings of this study noted that although there were differences and similarities in the shell shapes or mean shapes of a banding pattern, it is not substantial to conclude that genetics or environmental factor alone caused the phenomena.

Key words: *A. fulica*, banding pattern, geometric morphometrics, invasive snail, morphology, shell shape

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INTRODUCTION

The giant African snail was reported as one of the most ecologically damaging land snails [1,2] and the Global Invasive Species Database ranked it as number two among the “100 Worst Alien Invasive Species” [3]. Their invasiveness connotes how they are able to adapt to different environmental conditions [4].This foraging species multiply rapidly. Additionally, host range of these snails includes 500 plants species [5]. This snail my reach high abundance and cause important economic loose under favorable environmental conditions [6].

Several studies on*A. fulica*have been focused on its dispersal, distribution and biology around the world [6-20]. However, information of the known factors affecting their biology is still lacking. Being an invasive species makes *A. fulica*an interesting species in studying evolution sincethis species exhibit wide morphological diversity both within and among populationsthus providing an excellent opportunity to study the evolution of phenotypic differences[21-25]. From its origin in East Africa to the different parts of the world including the Philippines, many variations of the giant African snail have been observed. Variations within and among populations, may indicate that this species may have diverged from its native population since its introduction to the country [26]. Morphological variationsin the species in terms of size, shape and colour has been largely attributed to environmental conditions [8]. It was therefore the objective of this study to determine the relationship between banding pattern and shell shape to be able to understand the nature of variations in the species. The variations among populations with different banding patternswere quantitatively described by applying the tools of geometric morphometrics (GM)specifically relative warp analysis.

MATERIALS AND METHODS

Sampling area

Snail samples were collected from 15 different provinces across the Philippine islands: Cagayan (Tuguegarao City), Pangasinan (Calasiao), Quezon (Lucena City), and Rizal (Antipolo City) in Luzon; Bohol (Agapi, Ondol, and Kinogitan) and Southern Leyte (Sogod) in Visayas; and Compostela Valley (Las Arenas), Davao del Norte (New Corella and Panabo City), Davao del Sur (Emily Holmes, Riverside, Nova Tierra Village, Mandug in Davao City and Padada), Lanao del Norte (MSU-IIT campus and Mimbalot Falls, Iligan City), Lanao del Sur (Marawi City), Misamis Occidental (Cagayan de Oro City), South Cotabato (General Santos City), and ZamboangaSibugay (Ipil). Sampling site locations were plotted in Figure 1.

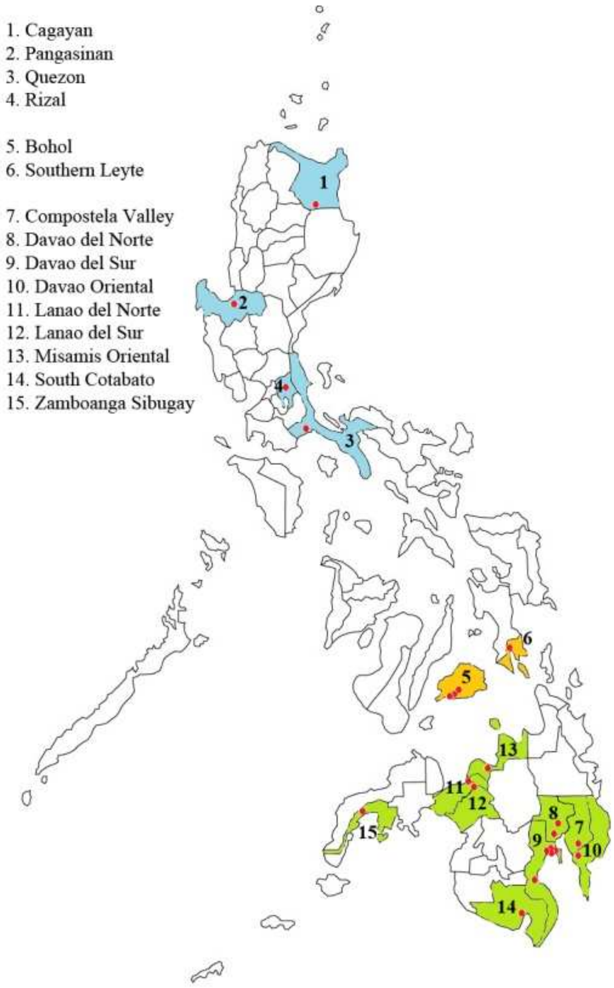

Figure 1. Map of the Philippines. Red dots correspond to locations of the different sampling sites

Banding pattern categorization

Banding patterns of the shells were based on the configuration of bands or streaks on the body whorl of the shell from all the samples of the different sampling locations. Categorization of the banding patterns was based on the band frequency, size (length and width), shape, and color. The patterns were then narrowed down to general configurations for the finally assignments of banding pattern categorization. The banding patterns were categorized into 14 major patterns (Figure 2). A total of 1309 shells were categorized under the 14 body whorl banding pattern described in this study.

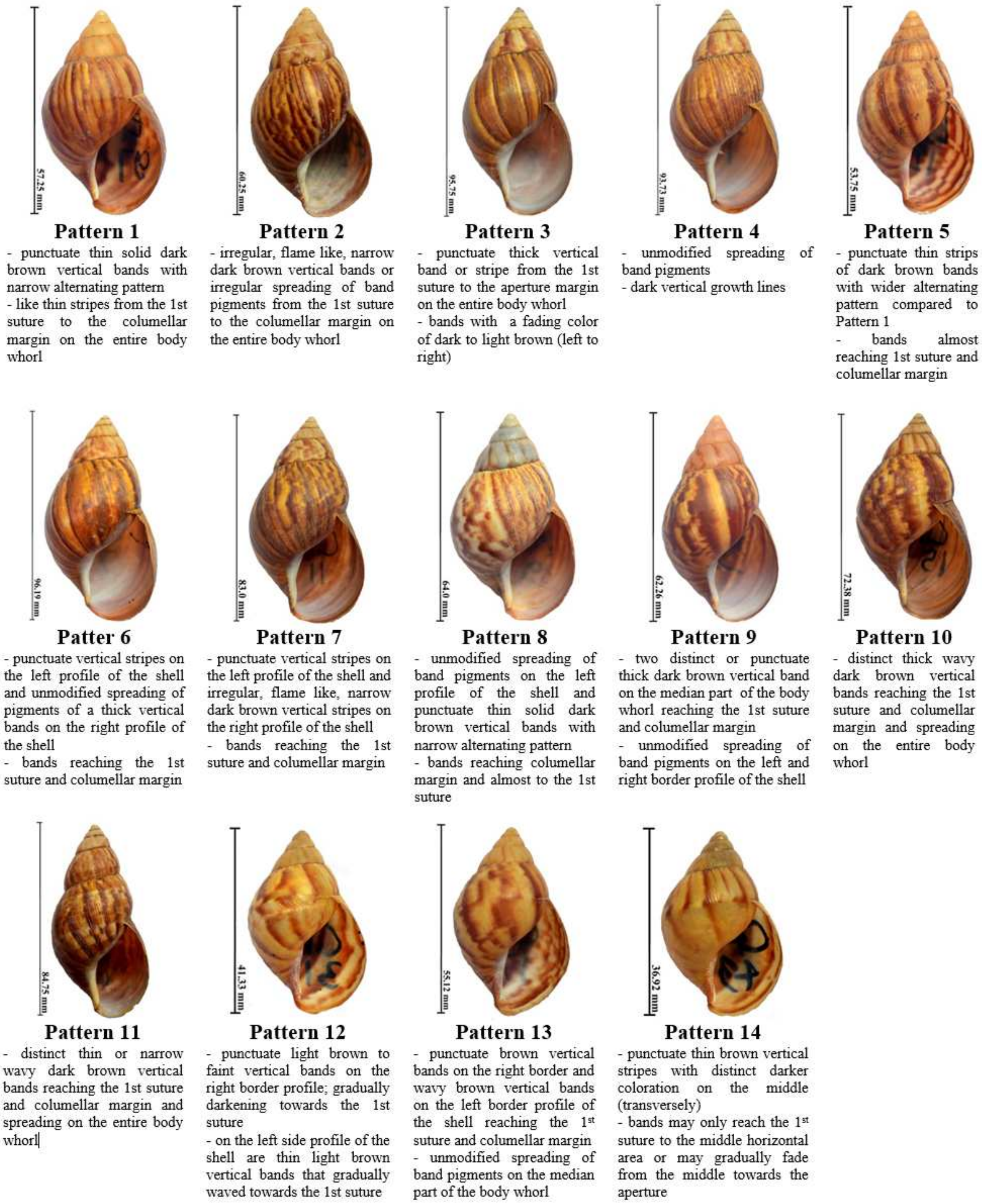

Figure 2. Images with descriptions of the different major body whorl banding patterns assigned to the shells of *A. fulica*

Imaging
Digital images of the ventral view of shells were then captured using a high resolution single-lens reflex (DSLR) camera mounted on a tripod to insure uniformity and minimize errors. Shells were positioned in such a way that the columellar is at 90° of the x-axis in the aperture view and in the orientation in which the apex is visible.

Organizing and Digitalization
Shell samples were grouped according to their banding patterns under different provinces. Triplicate of an image was made to insure consistency. The images of the grouped samples were digitized using tpsUtil[27] and saved as thin-plate splines (TPS) files.

Landmark selection
Landmarks as well as pseudolandmarks were assigned to the prominent features in the shells of *A. fulica*. Landmarks were designated to homologous structures found in the shell to ensure consistency in number from shell to shell. A total of 50 landmarks were used to summarize the shape of the shell (Figure 3).The chosen landmarks were described in Table 1.

Landmarking
Digitalized images were then subjected to landmark acquisition using tpsDig2.12 [28] program to facilitate the establishment of "x" and "y" coordinated of the landmarks. Also with tpsDig2.12, data for the sliders and links were also generated to enhance the image for the specimens.

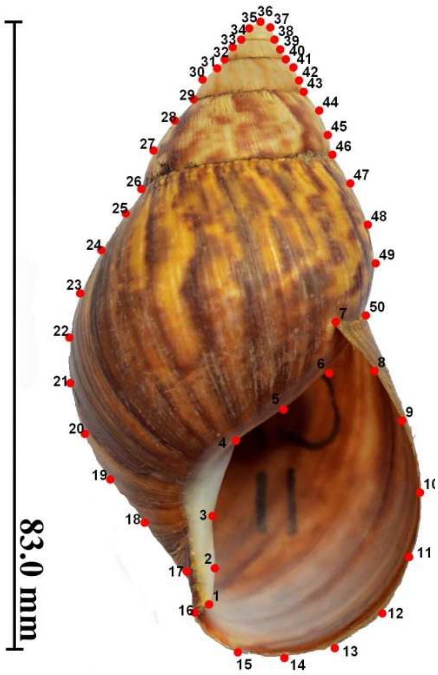

Figure 3. Designated landmarks and pseudolandmarks on the shell of *A. fulica*

Landmark-based and Statistical Analysis
Relative warp analysis using tpsRelw [29]was used to yield information on the variation in local shape, this involved fitting and interpolation function to homologous landmarks for each specimen in a sample. From the result of the relative warps of the shell shape, histogram and box and whiskers plots were generated using the Paleontological Statistics (PAST) software [30]. The most informative warp scores, first and second relative warps) were then subjected to Canonical Variance Analysis (CVA). Histogram, box plot, and CVA plot were used to visualize where data are centered and distributed over range of variables.

To determine the degree of variation between shell shapes of each banding patterns, the centroid size of the pooled pattern data was used for cluster analysis – helps separate multivariate data into a series of hierarchically related sets [31] – and the first and second relative warps scores of the pooled pattern data was subjected to Krustal-Wallis test, a nonparametric test used to compare independent groups of sample data and in this case determine the significance of difference (at 0.05 level of significance) in the shape variation of shells [32]. All statistical analyses were performed in PAST software.

Table 1. Description of the anatomical landmark points on the ventral view of *A. fulica* shell

LANDMARK NO.	DESCRIPTIONS OF LANDMARK POINTS
1	First LM in the end of the umbilicus perpendicular to LM 16
2	LM in the columellar margin between LM 1 and LM 3
3	LM in the columellar margin edge between LM 2 and LM 4
4	LM in the columellar margin edge between LM 3 and LM 5
5	LM in the columellar margin between LM 4 and LM 6
6	LM in the columellar margin between LM 5 and LM 7
7	First LM in the aperture margin
8	LM in the aperture margin perpendicular to LM 6
9	LM in the aperture margin perpendicular to LM 5
10	LM in the aperture margin between LM 9 and LM 11
11	LM in the aperture margin perpendicular to LM 2
12	LM in the aperture margin perpendicular to LM 1
13	LM in the aperture margin between LM 12 and LM 14
14	LM in the aperture margin between LM 13 and LM 15
15	LM in the aperture margin between LM 14 and LM 16
16	LM in the end of the aperture margin and umbilicus perpendicular to LM 1
17	First LM in the body whorl of the left border profile of the shell perpendicular to LM 2
18	LM in the body whorl of the left border profile of the shell perpendicular to LM 3
19	LM in the body whorl of the left border profile of the shell between LM 18 and LM 20
20	LM in the body whorl of the left border profile of the shell perpendicular to LM 4
21	LM in the body whorl of the left border profile of the shell perpendicular to LM 6
22	LM in the body whorl of the left border profile of the shell perpendicular to LM 7
23	LM in the body whorl of the left border profile of the shell perpendicular to LM 49
24	LM in the body whorl of the left border profile of the shell perpendicular to LM 48
25	Last LM in the body whorl of the left border profile of the shell perpendicular to LM 47
26	End of the first suture of the left border profile of the shell
27	LM on the first whorl of the left border profile of the shell perpendicular to LM 45
28	LM on the first whorl of the left border profile of the shell perpendicular to LM 44
29	End of the second suture of the left border profile of the shell
30	LM in the second whorl of the left border profile of the shell perpendicular to LM 42
31	LM in the second whorl of the left border profile of the shell perpendicular to LM 41
32	End of the third suture of the left border profile of the shell
33	LM in the third whorl of the left border profile of the shell perpendicular to LM 39
34	End of the forth suture of the left border profile of the shell
35	End of the fifth suture of the left border profile of the shell
36	Apex of the shell
37	End of the first suture of the right border profile of the shell
38	End of the forth suture of the right border profile of the shell
39	LM in the third whorl of the right border profile of the shell align to LM 33
40	End of the third suture of the right border profile of the shell
41	LM in the second whorl of the right border profile of the shell align to LM 31
42	LM in the second whorl of the right border profile of the shell align to LM 30
43	End of the second suture of the right border profile of the shell
44	LM in the first whorl of the right border profile of the shell align to LM 28
45	LM in the first whorl of the right border profile of the shell align to LM 27
46	End of the first suture of the right border profile of the shell
47	LM in the body whorl of the right border profile of the shell align to LM 25
48	LM in the body whorl of the right border profile of the shell align to LM 24
49	LM in the body whorl of the right border profile of the shell align to LM 23
50	End of the spire perpendicular to LM 7

RESULTS AND DISCUSSION

Results of the relative warp (RW) analysis showed four general descriptions of shell shape morphology which are consistent with the results of a related study[26]: 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 (Figure 4). The descriptions of the shapes of the shells are presented in Table 2. The first relative warp (RW1) explains the majority of the variations in *A. fulica* shell morphology. The shells were either slender-shaped or round-looking shaped. RW1 also described the differences in shell width. Both RW1 and RW2 described the variation in spire-whorl length, where slender-shaped shells have elongated spire with narrow body whorl and round-shaped shells have short spire with wide body whorl. The second relative warp (RW 2) show the difference in aperture outer margin which varied from having a wider lower portion to a more concave shaped upper margin thus giving a different shape to the aperture margin of the shells. The third relative warp (RW 3) described the variations in shell orientation where the sutures are slanted creating a one side view of the spire border profile wider than the other side making the shell appear to be leaning towards the right or left direction. The fourth relative warp (RW4) described the variation in aperture shape and shell orientation. However, aperture size and shape, shell or spire orientation is not always similar in certain shell shapes.A closer look at the histograms and box-and-whiskers plots of the significant relative warps displayed a multimodal variation in the mode of distributions of the shellsfora defined banding pattern although the mean values of all the populations described are close to the mean value of the pooled pattern indicating a high degree of intrapopulational variation in the snail.

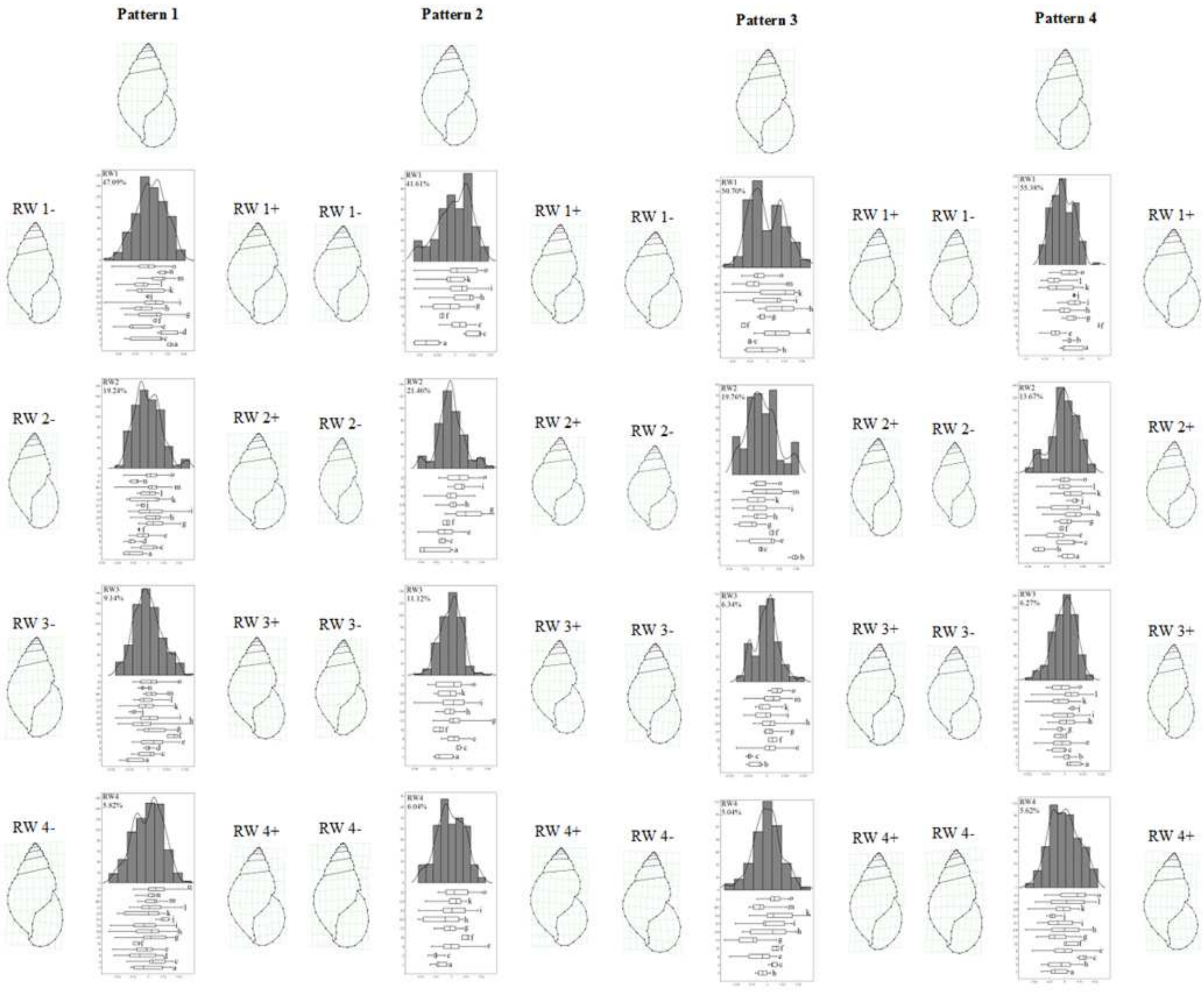

Figure 4. Summary of the landmark based geometric morphometric analysis showing the consensus morphology (uppermost pannel) and the variation in shape of the shells of *A. fulica* explained by each of the significant relative warps
Pattern 1 (a), Pattern 2 (b), Pattern 3 (c), Pattern 4 (d), Pattern 5 (e), Pattern 6 (f), Pattern g), Pattern 8 (h), Pattern 9 (i), Pattern 10 (i), Pattern 11 (k), Pattern 12 (l), Pattern 13 (m), and Pattern 14 (n)

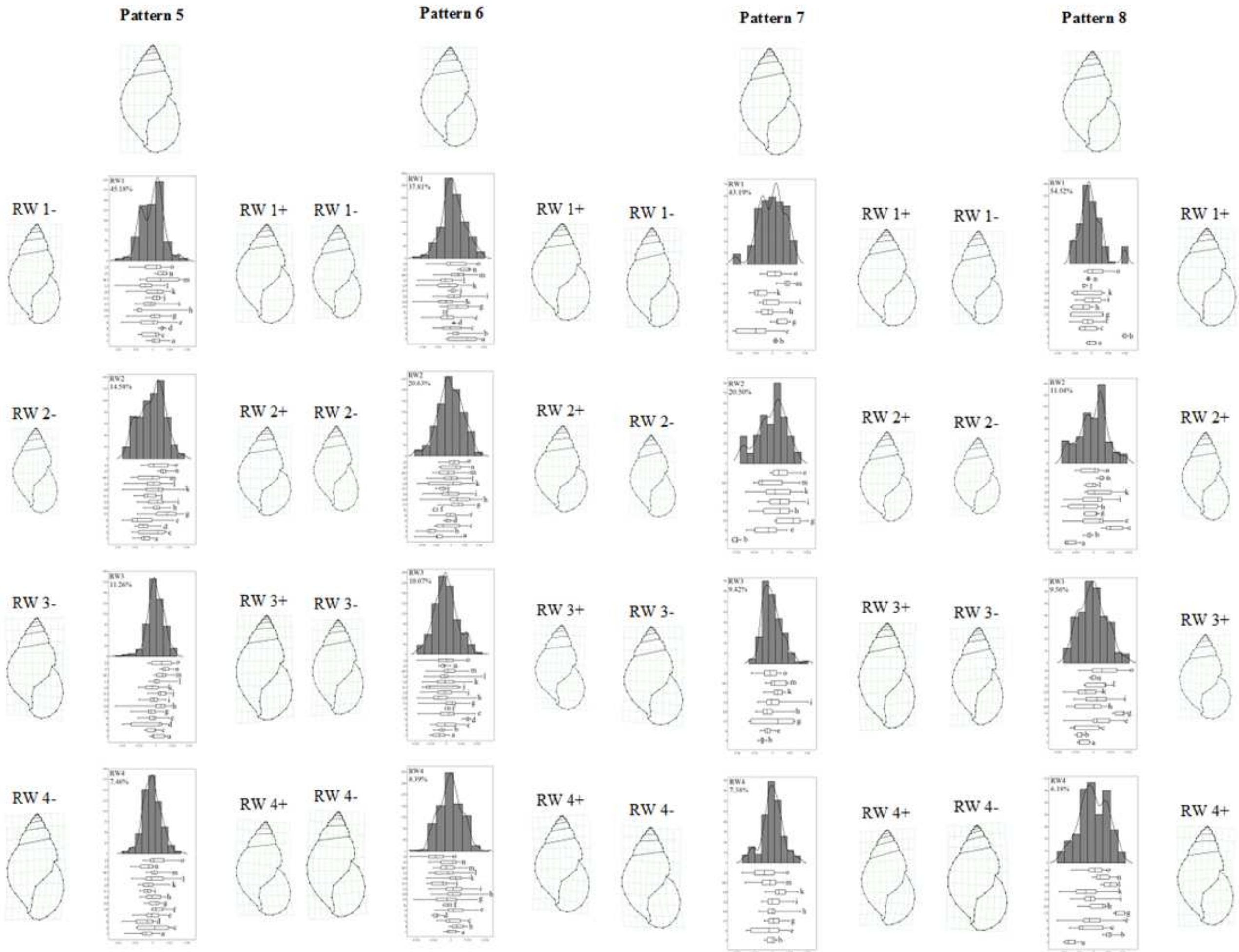

Figure 4 (cont.). Summary of the landmark based geometric morphometric analysis showing the consensus morphology (uppermost pannel) and the variation in shape of the shells of *A. fulica* explained by each of the significant relative warps
Legend: Pattern 1 (a), Pattern 2 (b), Pattern 3 (c), Pattern 4 (d), Pattern 5 (e), Pattern 6 (f), Pattern g), Pattern 8 (h), Pattern 9 (i), Pattern 10 (i), Pattern 11 (k), Pattern 12 (l), Pattern 13 (m), and Pattern 14 (n)

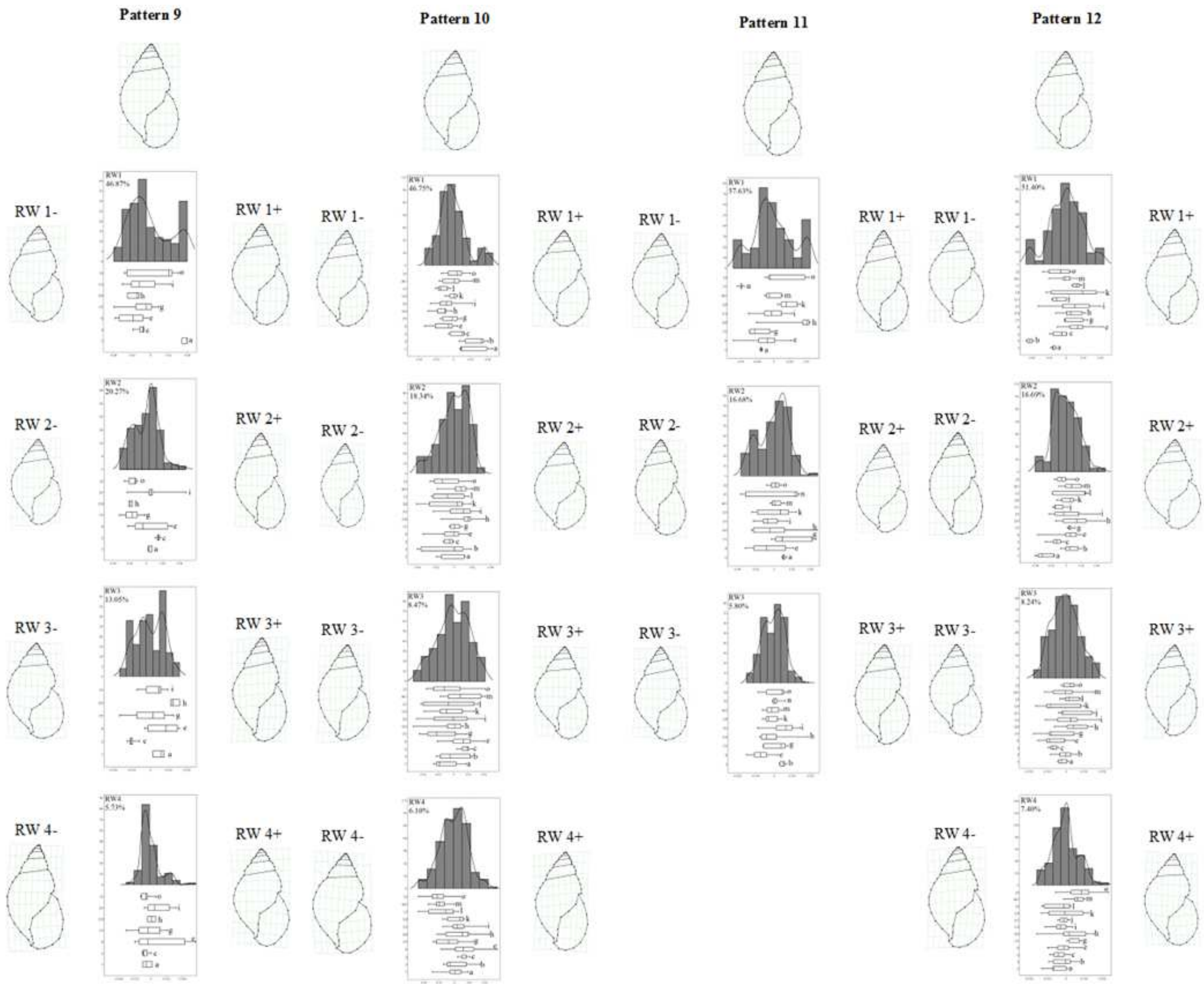

Figure 4 (cont.) Summary of the landmark based geometric morphometric analysis showing the consensus morphology (uppermost pannel) and the variation in shape of the shells of *A. fulica* explained by each of the significant relative warps

Pattern 1 (a), Pattern 2 (b), Pattern 3 (c), Pattern 4 (d), Pattern 5 (e), Pattern 6 (f), Pattern g), Pattern 8 (h), Pattern 9 (i), Pattern 10 (i), Pattern 11 (k), Pattern 12 (l), Pattern 13 (m), and Pattern 14 (n)

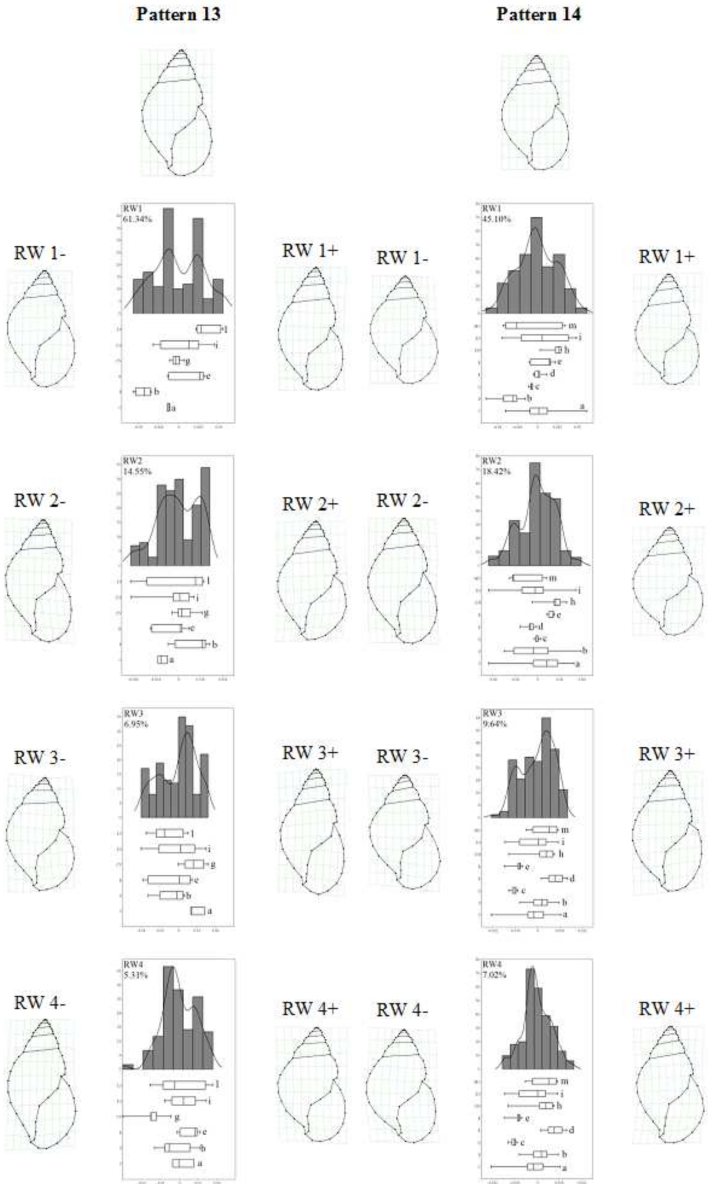

Figure 4 (cont.). Summary of the landmark based geometric morphometric analysis showing the consensus morphology (uppermost pannel) and the variation in shape of the shells of *A. fulica* explained by each of the significant relative warps
Pattern 1 (a), Pattern 2 (b), Pattern 3 (c), Pattern 4 (d), Pattern 5 (e), Pattern 6 (f), Pattern g), Pattern 8 (h), Pattern 9 (i), Pattern 10 (i), Pattern 11 (k), Pattern 12 (l), Pattern 13 (m), and Pattern 14 (n)

Table 2. Variability in the shells of *A. fulica* shell as explained by the significant relative warp

RW	Pattern 1	Pattern 2
1	- Variation in body size; wider body width in (+) than in (-). - Variation in spire-body whorl length aspect; higher spire and length and shorter body whorl length (-), shorter spire length and longer body whorl length (+). - Variation in the aperture size; wider and longer aperture in (+) than (-).	- Variation in the shell shape; slender or more elongated shape with narrow body width (-) and rounder shape with wider body whorl size (+). - Difference in spire-body whorl length aspect; slender shell shape (-) have higher spire length with shorter body whorl length, while the rounder shell shape have the opposite (+). - Difference in aperture length; longer length in (-) than (+) warp.
2	- Variation in shell width; wider body whorl and spire in (+) than (-) - Difference in aperture size; longer aperture length and wider width (-)	- Variation in shell width; wider body whorl and spire in (+) - Difference in aperture width; wider opening in (+).
3	- Difference in shell orientation; leaning to the right (-) and towards the left (+) direction	- Variation in aperture shape; wider lower aperture outer margin in (+) giving a wider opening than (-). - Difference in LM 18 and 19 giving shell in (+) warp a more pronounce or rounder looking body whorl shape.
4	- Difference in shell orientation; shell in (-) warps tends to lean towards the left direction while shell in (+) is more upright orientation.	- Distinct variation in body size; narrow body whorl and spire (-) and wider shell size (+). - Slight difference in spire-body whorl length aspect; shorter spire height with longer body whorl length (+) and longer spire with shorter body whorl (-). - Difference in aperture size and shape; longer length with elongated lower outer margin (-) and shorter but wider opening (+).

RW	Pattern 3	Pattern 4
1	- Variation in the shell shape; slender or more elongated (+) and rounder (-) shaped. - Difference in spire-body whorl length aspect; slender shell shape (+) have higher spire length with shorter body whorl length, while the rounder shell shape have the opposite (-). - Difference in shell width; wider width in rounder shell (-) and narrower body width in slender shell (+). - Difference in aperture margin length; longer in (-+) and shorter in (+).	- Variation in shell shape; slender or more elongated (-) and rounder (+) shaped. - - Difference in spire-body whorl length aspect; slender shell shape (-) have higher spire length with shorter body whorl length, while the rounder shell shape have the opposite (+). - Difference in aperture length; longer in (-) and shorter in (+).
2	- Variation in aperture size; longer length with more elongated lower outer margin (+) and wide shorter opening with round-like shape (-). - Variation in the shell shape; slender or more elongated (+) and rounder (-) shaped. - Difference in spire-body whorl length aspect; slender shell shape (+) have higher spire length with shorter body whorl length, while the rounder shell shape have the opposite (-). - Difference in shell width; wider width in rounder shell (-) and narrower body width in slender shell (+).	- Variation in spire compartmentalization; sutures in (+) warp are farther apart than in (-) warp giving wider spires. - Variation in aperture outer margin shape; wider or a more elongated lower portion (-) and a wider upper part (+).
3	- Variation in shell orientation; leaning to the left (-) and towards the right (+) direction. - Difference in aperture outer margin shape; elongated in the lower part (-) and wider in the upper part (+).	- Slight difference in spire-body whorl length aspect; longer spire shorter body whorl (+) and shorter spire longer body whorl length (-). - Variation in aperture size; longer but narrow aperture (+) and shorter but wider aperture (-).
4	- Variation in aperture margin; difference in LM 2 and 3 give shell in (-) warp pronounce inner margin, and difference in the lower outer margin giving shell in (-) warp a wider aperture while narrow opening in (+) warp. - Difference in aperture size; shorter and wider aperture in (-), and longer and narrow aperture opening in (+).	- Variation in shell orientation; leaning to the left (-) and towards the right (+) direction.

Table 2 (cont.) Variability in the shells of *A. fulica* shell as explained by the significant relative warp

RW	Pattern 5	Pattern 6
1	- Variation in the shell shape; slender or more elongated (-) and rounder (+) shaped with wider shell width. - Difference in spire-body whorl length aspect; slender shell shape (-) have higher spire length with shorter body whorl length, while the rounder shell shape (+) have the opposite. - Difference in aperture size; higher length and width in (+) than in (-).	- Variation in the shell shape; slender or more elongated (-) and rounder (+) shaped with wider shell width. - Variation in spire-body whorl length aspect; longer spire with shorter body whorl length (+) and shorter spire with loner body whorl length (-). - Difference in body or shell width, wider shell size in (-) than (+). - Difference in aperture size; longer and wider aperture in shell on the (+) warp.
2	- Variation in shell width; wider body whorl, spire, and aperture width in shell of (+) warp. - Difference in aperture outer margin shape; wider lower part of the aperture giving off a longer length (-) and wider upper portion making a wider aperture (+).	- Variation shell size; wider shell in the (+) warp than in (-) warp. - Variation in aperture outer margin shape; elongated or wider in the lower part of the shell in the (-) warp and wider upper part on shell of the (+) warp.
3	- Variation in aperture length; longer aperture in (+) warp. - Difference in LM 2 making a more pronounce inner aperture margin shape in shell of (-) warp. Difference in LM 18 and 19 making a rounder looking body whorl shape in shell of (-) warp.	- Variation in shell orientation; leaning to the left (-) and upright direction (+). - Variation in aperture outer margin shape; a longer aperture length (-) and a more elongated lower part of the margin (+). - Difference LM 18 and 19; pronounce body whorl and umbilicus shape of shell in (+) warp.
4	- Variation in shell orientation; leaning to the left (-) and to the right (+) direction. - Difference in aperture size; wide shorter aperture (+) and narrow longer aperture (-).	- Variation in shell orientation; leaning to the right (+) and leaning to the left (-).
5		- Variation in shell width and aperture size aspect; wider shell with wide shorter aperture (+) and smaller shell width with narrow longer aperture size (-).

RW	Pattern 7	Pattern 8
1	- Variation in the shell shape; slender or more elongated (-) and rounder (+) shaped with wider shell width. - Difference in spire-body whorl length aspect; slender shell shape (-) have higher spire length with shorter body whorl length, while the rounder shell shape (+) have the opposite. - Difference in aperture size; higher length and width in (+) than in (-).	- Variation in the shell shape; slender or more elongated (-) and rounder (+) shaped with wider shell width. - Difference in spire-body whorl length aspect; slender shell shape (-) have higher spire length with shorter body whorl length, while the rounder shell shape (+) have the opposite. - Difference in aperture size; higher length and width in (+) than in (-).
2	- Variation in shell width; wider body whorl, spire, and aperture width in shell of (+) warp. - Difference in aperture outer margin shape; wider lower part of the aperture giving off a longer length (-) and wider upper portion making a wider aperture (+).	- Variation in shell width; wider body whorl, spire, and aperture width in shell of (-) warp. - Difference in aperture outer margin shape; wider lower part of the aperture giving off a longer length (+) and wider upper portion making a wider aperture (-).
3	- Variation in aperture length; longer aperture in (+) warp. - Difference in LM 2 making a more pronounce inner aperture margin shape in shell of (-) warp. Difference in LM 18 and 19 making a rounder looking body whorl shape in shell of (-) warp.	- Variation in shell width; wider size in shell of (+) warp. - Difference in aperture size; narrow longer aperture (-) and wider but shorter aperture (+).
4	- Variation in shell orientation; leaning to the left (+) and to the right (-) direction. - Difference in aperture size; wide shorter aperture (-) and narrow longer aperture (+).	- Variation in shell orientation; leaning to the left (-) and to the right (+) direction. - Slight difference in aperture length with shell in (+) warp having longer aperture length.
5		- Variation in spire-body whorl length aspect; longer spire shorter body whorl (+) and shorter spire longer body whorl (-).

Table 2 (cont.). Variability in the shells of *A. fulica* shell as explained by the significant relative warp

RW	Pattern 9	Pattern 10
1	- Variation in spire-body whorl length aspect; shorter spire-longer body whorl length (+) and longer spire-shorter body whorl (-). - Variation in aperture length; longer aperture in shell of (+) warp. - Difference in LM 2 and 3 giving more pronounce inner aperture margin in shell of (-) warp.	- Variation in spire-body whorl length aspect; shorter spire-longer body whorl length (+) and longer spire-shorter body whorl (-). - Variation in aperture length; longer aperture in shell of (+) warp.
2	- Variation in body whorl width; wider body whorl size in shell of (-) warp. - Variation in aperture outer margin shape; elongate lower portion (+) and wider upper part (-) of the aperture margin.	- Variation in shell width; wider shell size in (+) warp. - Variation in aperture outer margin shape; wider lower part (-) and wider upper part (+).
3	- Variation in shell shape; narrow or elongated shell shape (+) and rounder-looking shell (-). - Difference in the spire-body whorl length aspect; longer spire but shorter body whorl (+) and shorter spire-longer body whorl (-). - Slight difference in aperture size; longer narrower opening making slender shape aperture (+) and shorter wider opening with larger lower part making a rounder shape aperture (-).	- Variation in aperture outer margin shape; concave (-) and more elongated (+). - Difference in LM 18 and 19 making a pronounce body whorl and umbilicus shape (+).
4	- Variation in shell orientation; leaning towards the left (-) and towards the right (+) direction. - Slight difference in aperture length with shell in (+) warp have longer aperture.	- Variation in spire orientation; leaning to the left (+). - Difference in shell width; shells are wider in (-) warp than shells in (+) warp. - Difference in aperture outer margin shape; elongated margin (+) and concave margin (-).

RW	Pattern 11	Pattern 12
1	- Variation in shell shape; slender shaped shell (+) and round-looking shell shape (-). - Difference in spire-body whorl length aspect; longer spire – shorter body whorl (+) and shorter spire – longer body whorl (-). - Variation in aperture size; longer and wider opening in shell of (-) warp.	- Difference in spire-body whorl length aspect; longer spire – shorter body whorl (+) and shorter spire – longer body whorl (-). - Difference in shell width; wider shell in (-) warp. - Difference in aperture size; bigger aperture opening in (-) warp.
2	- Variation in shell width; rounder looking body shape with wider width (+) and slender body shape (-). - Difference in the aperture outer margin shape; elongated lower portion with longer length (-) and wider in the lower part (+) of the margin.	- Variation in shell width; wider spire, body whorl and aperture size in (+) warps than those in (-) warp. - Variation in aperture shape; elongated or narrow opening (-) and round-looking or concave outer margin (+).
3	- Variation in shell orientation; leaning towards the left (-) and toward the right (+) direction.	- Variation spire orientation; leaning to the left (-) and leaning to the right (+). - Variation in aperture outer margin shape; slender or narrow opening (+) and bloated lower portion of the margin (-).
4		- Variation shell orientation; leaning to the left (-) and leaning to the right (+). - Variation in aperture outer margin shape; slender or narrow opening (+) and bloated lower portion of the margin (-).

RW	Pattern 13	Pattern 14
1	- Variation in spire-body whorl length aspect; longer spire – shorter body whorl length (+) and shorter spire – longer body whorl length (-). - Slight difference in shell size with wider width in shell of (-) warp. - Variation in aperture length and width; bigger aperture opening in shell of (-) warp than the one in (+) warp.	- Variation in spire-body whorl length aspect; longer spire – shorter body whorl (+) and shorter spire – longer body whorl (-). - Difference in shell width; wider upper body whorl and spire in shell of the (-) warp. - Difference in aperture size; longer and wider aperture opening in (+) warp.
2	- Variation in body whorl width, rounder body with wider width (+) and slender body shape (-). - Difference in LM 18 and 19 making a more concave left margin of the body whorl - Difference in the aperture outer margin shape; wider in the lower portion (-) and a concave margin (-).	- Variation in shell size; wider body whorl, spire and aperture I shell belong to the (+) warp. - Slight difference in the shell length with shell in the (-) warp is longer than in (+) warp.
3	- Variation in aperture shape; elongated and slender looking opening (+) and a concave aperture outer margin (-).	- Variation shell orientation; leaning to the left (-) and leaning to the right (+). - Difference in aperture shape; slender or narrow opening (+) and wider lower portion of of the outer margin (-).
4	- Variation in body whorl size; wider body in shell of the (+) warp. - Variation in aperture length with longer opening the shell of (-) warp.	- Variation spire orientation; leaning to the left (+. - Variation in aperture shape; round looking opening (-) and concave outer margin (+). - Difference in LM 2; pronounce inner aperture margin (-).

Comparing the shapes of the different banding patterns based from the pooled and the most important warp scores,the generated CVA scatter plot and scores revealed a randomly distributed shell shape distributionsuggesting high intrapopulational variation in shell shapes (Figure 5). It is important to note however that from the result of the non-parametric Kruskal–Wallis test ($p=1.737^{-69} <0.05$), it was shown that there were significant differences between the medians of at least two populations. Table 3 shows the result of the Bonferroni corrected Mann-Whitney pairwise comparison of the most significant warp scores (first and second) of the shell shape for all populations of the different banding patterns. Pairwise comparisons showed that Pattern 4 is significantly different to the other banding patterns.

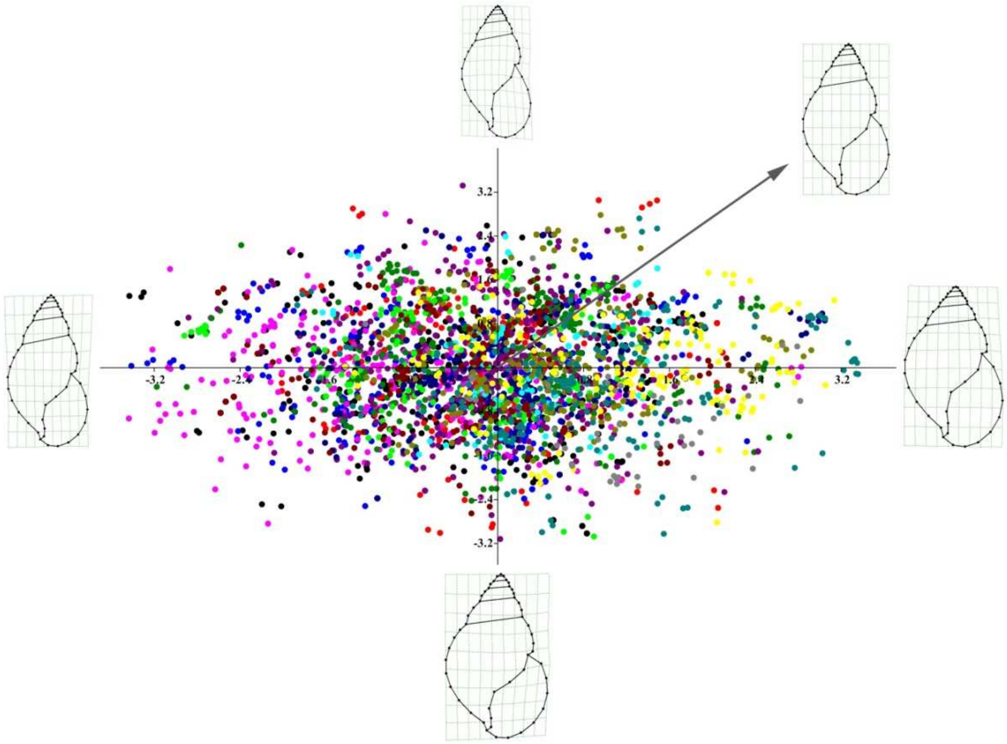

Figure 5. CVA scatter plot showing the distribution of shell shapes of different patterns of samples from the different provinces of the Philippines based on landmark geometric morphometric analysis with corresponding shapes of each axis and the mean shape indicated by the arrow Results of MANOVA test for significant variation in the shell shape: Wilk's Lambda= 0.7981, df1= 26, df2= 6842, F= 31.41, and p (same) =3.652^{-146}

Pattern 1 (black), Pattern 2 (red), Pattern 3 (blue), Pattern 4 (pink), Pattern 5 (green), Pattern 6 (violet), Pattern 7 (yellow green), Pattern 8 (navy blue), Pattern 9 (sky blue), Pattern 10 (brown), Pattern 11 (maroon), Pattern 12 (blue green), Pattern 13 (yellow), and Pattern 14 (gray)

Table 3. Result of Kruskal-Wallis test on shell shape of different banding pattern based on the first and second relative warp score. Mann-Whitney pairwise comparison (Bonferroni corrected) of the shell shape

Bold numbers indicate significant difference (0.05 level of significance)

Pattern	Pattern													
	1	2	3	4	5	6	7	8	9	10	11	12	12	14
1	-													
2	1	-												
3	1	1	-											
4	**1.58^{-09}**	**9.19^{-06}**	**0.01179**	-										
5	**0.04824**	**3.083^{-04}**	**3.44^{-06}**	**5.56^{-14}**	-									
6	0.1462	**2.702^{-03}**	**8.41^{-06}**	**2.48^{-26}**	1	-								
7	1	1	1	**4.19^{-06}**	**0.02371**	**0.07456**	-							
8	1	1	0.08545	**5.11^{-13}**	0.909	1	1	-						
9	0.2488	**5.401^{-03}**	**3.126^{-04}**	**6.82^{-22}**	1	1	**2.81^{-07}**	1	-					
10	**9.06^{-09}**	**2.11^{-11}**	**7.10^{-14}**	**9.68^{-31}**	0.1626	**1.522^{-03}**	**8.46^{-08}**	0.03552	0.9777	-				
11	1	1	1	0.101	0.0218	0.08344	1	**6.10^{-09}**	**3.45^{-06}**	**2.72^{-08}**	-			
12	**4.66^{-09}**	**8.97^{-11}**	**1.55^{-13}**	**2.44^{-46}**	0.0648	**7.24^{-04}**	**8.11^{-16}**	1	1	1	**3.97^{-08}**	-		
13	**3.19^{-07}**	**5.64^{-10}**	**1.24^{-10}**	**4.21^{-16}**	**0.01644**	**1.643^{-04}**	**5.95^{-4}**	**2.02^{-08}**	**3.44^{-06}**	1	**1.08^{-07}**	1	-	
14	**3.06^{-10}**	**3.49^{-10}**	**9.76^{-14}**	**7.47^{-56}**	**3.819^{-05}**	**1.86^{-05}**	**1.03^{-28}**	**4.80^{-08}**	**1.56^{-06}**	1	**6.02^{-09}**	1	1	-

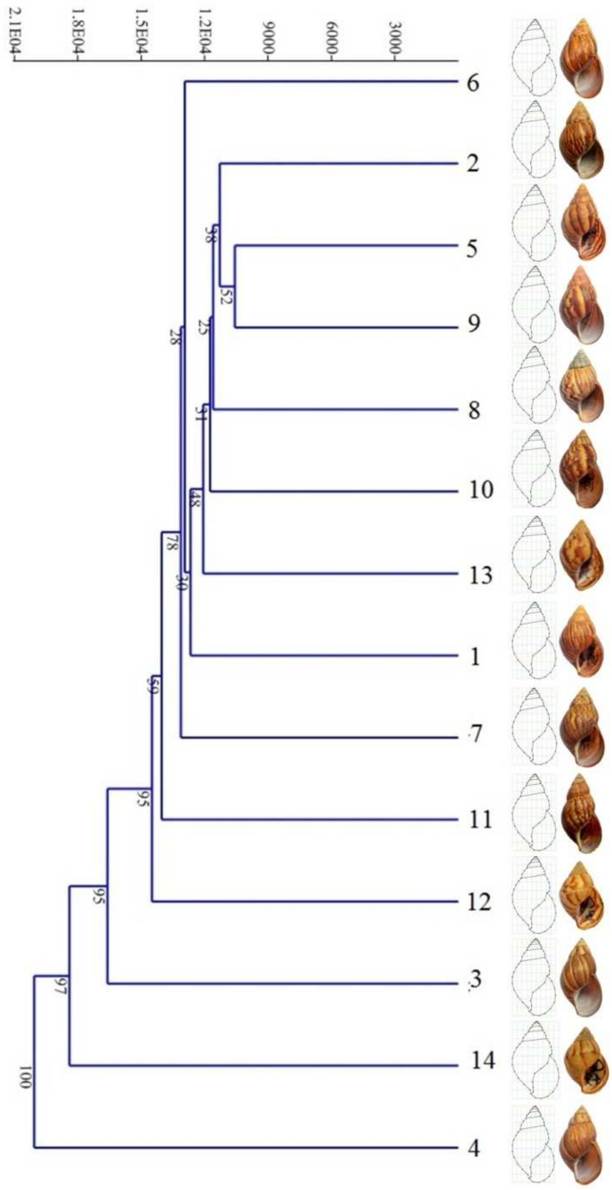

Figure 6. Cluster analysis result of the shell shape based on centroid size for all populations of different banding patterns with images of the corresponding mean shape and body whorl banding pattern categorization.

Cluster analysis of the centriod size show pattern4 form was very different from the others wherethe shells are characterized to have unmodified spreading of band pigments and the more visible ones are the dark vertical growth lines (Figure 6). Shapes of patterns 6, 2, 5,9,8,10,13 were not significantly different from each other and examination of the shells show these are variations of highly similar banding patterns. Patterns 3, 4, 7 11, 12 and 14 were observed to be not only different from their banding patterns but also their shell shapes. The variations observed in the shapes of shells of *A. fulica*with differences in banding patterns can be due tomany possible factors including genetics, biotic and abiotic factors. Some researchers claim that the variability in shape of *A. fulica* was due to genetic anomalies [7]. It was found out that there was a clear mate-choice behavior in *A. fulica*, where mate-choice criteria could be based on the reproductive stage or a size-assortative [13]. This could be a basis for the genetic effect on the shell morphology on the land snails. Some studies argue that there is an unclear factor in driving morphometric shape variation among littorinid gastropods [33]. It was suggested that differences in morphology is due to the snail's adaptation to microhabitat characteristics. A study showed that variation in shells ofA. *fulica* is not only genetic but also affected by the growth rate and population density of the snails [26]. It was suggested that variations in shell morphology is governed by genetically set allometric relationships which resulted in plasticity due to environmental constraints. It may also be possible that the diversity within populations observed [26] could be due to the numerous introduction and reintroduction of several gene pools of snails to different regions of the Philippines. This could also be possible in this study since shells were from different islands in the Philippines which have different climatic and environmental factors. The aestivation stage may also promote physiological changes in *A. fulica* and affected the snail's development [34]. It may also that climate variables, especially humidity and temperature ranged have significantly influenced the total shell length and width of *A. fulica* [6].

CONCLUSION

The findings of this study show that there is a significant relationship of shell shape and banding pattern in *A. fulica*. Some degree of intrapopulational variation was also observed indicating phenotypic plasticity or genetic differentiation in the snail population.

Acknowledgment
The senior author would like to acknowledge the Department of Science and Technology – Accelerated Science and Technology Human Resource Development Program (DOST-ASTHRDP) for the scholarship grant. Acknowledgment is also extended to Muhmin Michael Manting and Sharon Rose Tabugo for the technical assistance.

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