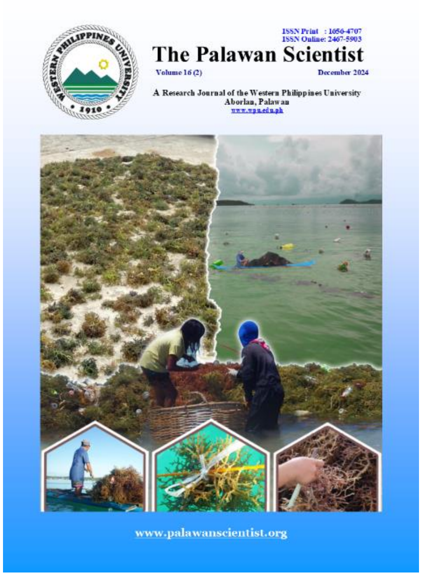
Original Article



Morphological variation among cowries (Gastropoda: Cypraeidae) using geometric morphometrics and correlation analysis based on distances

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ABSTRACT

Cowries (Cypraeidae) are popular among shell collectors because of their beauty and relative availability. Some species of cowry have high collection values among shell markets, however this has led to an increase number of species and unnecessary proliferation of taxonomic names with little information on their morphology. Thus, this study was conducted to describe morphological variations among cowry shells obtained along Sindangan Bay, Philippines. The shell morphological attributes (e.g. shell shape, color, bands, banding pattern), morphometric characters (e.g. shell length, width, height, number of teeth, etc.), and shape were characterized using the relative warp scores generated from the outline and landmark-based geometric morphometric analysis (GM) and correlation analysis based on distances (CORIANDIS). Sixteen (16) morphological and ten (10) meristic characters of 113 samples from the seven Cypraeidae species were examined and analyzed. The variations on color, banding pattern, lateral margins, dorsal/transverse line, spire, teeth, size and shape of the shell were mainly observed. Relative warp analysis showed significant shell shape variation among Cypraeidae species. Correlation analysis based on distances showed morphological, size, and shape differences among Cypraeidae species. As revealed in correlation analysis, the observed variation in size was significantly correlated with shape. The observed differences could be due to many factors including genetic, biotic and abiotic factors; developmental processes and physiology in responses of the organisms to a unique environment. Thus, geometric morphometrics and CORIANDIS helped us understand the nature of diversity in the family Cypraeidae species. Further studies on environmental heterogeneity, species position within the population's distribution, and the genetic basis of the observed phenotypic diversity are necessary. Such emphasis can lead to additional information in the systematic studies on species of family Cypraeidae.

Keywords: cowry, cluster, dendrogram, disparity, Sindangan, variation

INTRODUCTION

Cowries, marine gastropods in the family Cypraeidae, are popular among shell collectors due to their glossy shells with varied colors and patterns. Cowries are collected for its aesthetic value, consumed as food, and used in shell crafts industry. Cowries play important roles as herbivores, predators and decomposers in maintaining healthy balanced marine ecosystems (Poutiers 1998). Generally, they are widely distributed in reef-building corals (Malay and Paulay 2010). The presence of a beautiful shell in some cowry species have high collection values among shell markets (Ma et al 2023). However, commercial factors have led to an increase in the species number of cowries, which has resulted in an unnecessary proliferation of taxonomic names (Passamonti 2015). Cowries are currently classified into 55 genera and 9 subfamilies (MolluscaBase 2022) and approximately 217 species and numerous subspecies. Species level taxonomies are very subjective in contrast to stable taxonomies in higher levels. Despite well-defined shell-based genera, they have received limited attention from biologists and malacologists, resulting in few scientific studies available (Passamonti 2015).

Morphological studies rely on defining characters for species identification that may support the taxonomic validity of the species (Moneva 2012). In gastropod species-level taxonomy (Ackerly 1989), the external form of the shell is crucial for species discrimination (Kohler and Glaubrecht 2003). Cowry shells possess numerous characteristics, requiring multiple traits to describe their variability (Heiman 2011). Despite the relative disparity of shell morphologies, several conserved anatomical features and the rigid nature of their shells make gastropods well suited subjects of morphometric analysis (Smith and Hendricks 2013).

Geometric morphometrics is a tool used to quantify, interpret and analyze the size and shape of biological structures (Rohlf and Slice 1990), transforms continuous morphological data in the form of coordinate points into discrete characteristics represented by principal components of shape (Zelditch et al. 2012), integrates multivariate statistics and geometric principals to convey shape variation between specimens. This approach may add to understanding of morphological evolution, biogeography, and trait plasticity. In order to describe variability in the species, it is necessary to use a range of characters since cowry shells can be distinguished by more than one character.

Thus, this study aimed to characterized the phenotypic variations in Cypraiedae species using qualitative and quantitative morphological characters such as shell shape, color, bands, banding pattern, length, width, height, and teeth, respectively. Landmark and outline-based geometric morphometrics and correlation analysis based on distances were used to detect size and shape variation and analyze similarities and differences among species collected in Sindangan Bay, Philippines. This study is important in advancing the knowledge in the nature of variations in populations of organisms living in different ecological environments.

METHODS

Sampling Area

Sampling occurred in marine sanctuaries in Guisukan, Doña Josefa; Languyon, La Concepcion; and Dinukot, RG Macias in Sindangan Bay, (8°13'37"N; 122°54'55"E), Zamboanga del Norte, Philippines (Figure 1).

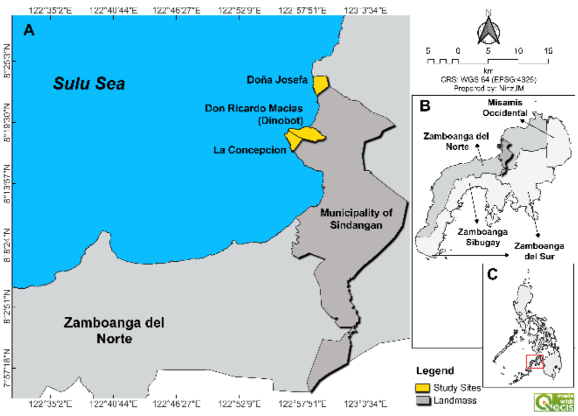

Figure 1. (A) The three study areas in Sindangan Bay, Sindangan, Zamboanga Del Norte.and (B) Zamboanga peninsula, and (C) inlet maps of the Philippines.

Sample Collection, Identification and Digital Images

Sampling was conducted during the lowest tide mark. A total of 113 specimens (Figure 2) which comprise of seven (7) *Cypraeidae* species namely; *Cypraea. caputserpentis* Linn 1758 (a), *Cypraea annulus* Linn 1758 (b), *Cypraea felina* Gmelin 1791, *Cypraea tigris* Linn 1758 (d), *Cypraea caurica* Linn 1758 (e), *Cypraea moneta* Linn 1758 (f) and *Cypraea arabica* Linn 1758 (g), were collected from reef flat shorelines up to 3 meters deep. The cowries were identified based on literature and locally available references (Poutiers 1998; Froese and Pauly 2022). Each shell was photographed where the columella is at 90° of the x-axis in aperture and dorsal views. These images were subjected to geometric morphometric analysis (e.g. TPS file, Relative Warp Analysis).

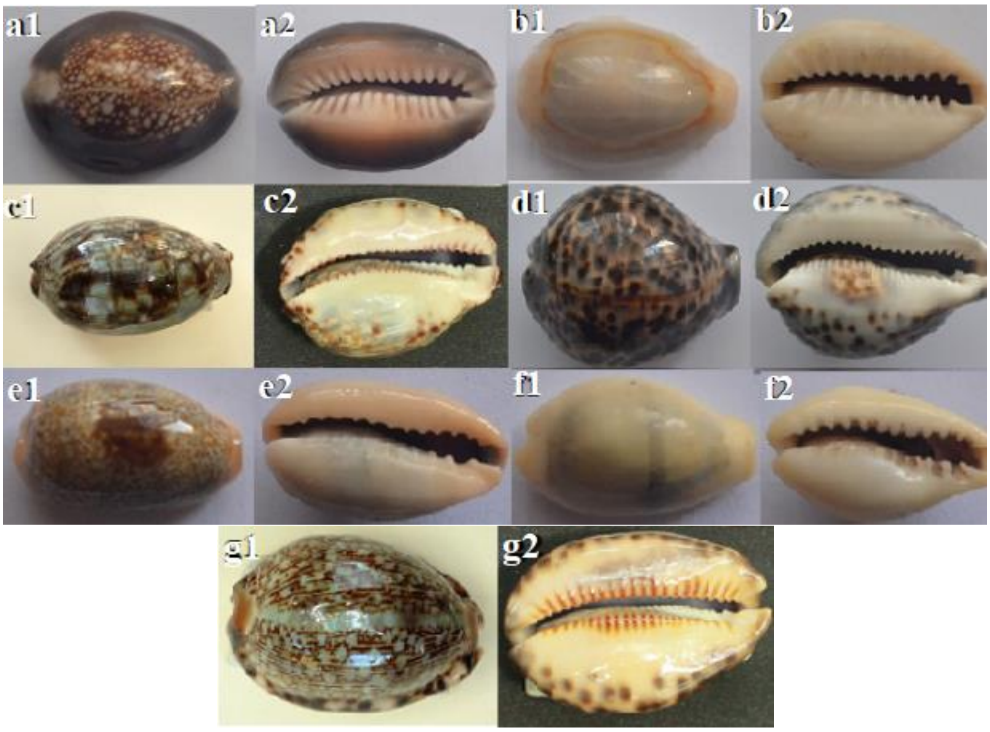

Figure 2. An illustration showing the dorsal (a1-g1) and ventral (a2-g2) shell of the Cypraeidae species. Legend: *Cypraea cataputserpentis* (a), *Cypraea. annulus* (b), *Cypraea felina* (c), C*ypraea tigris* (d), *Cypraea caurica* (e), *Cypraea moneta* (f) and *Cypraea arabica* (g).

Morphological (Qualitative) and Morphometric (Quantitative) Characterization

A matrix summarizing 26 characters (16 qualitative and ten quantitative) was created for data analysis (Table 1). Morphometric measurements, including shell dimensions, aperture size, and weight, were obtained using a digital Vernier caliper and weighing scale, respectively (Figure 3A, 3B and 3C). Columellar and labial teeth (CTR and LTR) (Figure 3D) were normalized using the formula, where total length or TL (in mm) of the shells served as the primary input variable (Heiman 2011):

$$LTR = 7 + (LT - 7) \times \left(\frac{25}{L} \times \frac{1}{2}\right)$$
$$CTR = 7 + (CT - 7) \times \left(\frac{25}{L} \times \frac{1}{2}\right)$$

Table 1. List of shell characters used in the analyses of the morphology and morphometrics characterization.

Characters	Scoring
1. Shape-dorsal	(0) ovate; (1) deltoidal-ovate; (2) elongated/cyclindrical
2. Shape-ventrall	(0) broad, flat, slightly concave; (1) flat-convex; (2) concave
3. Color- dorsal part	(0) creamy- white; (1) light fawn; (2) dark brown; (3) yellowish-green; (4) grey
4. Color- ventral part	(0) creamy grey/purplish pink; (1) creamy-light fawn/brown; (2) cream white/whitish
5. Color- lateral margins	(0) grayish-white; (1) creamy-light fawn; (2) dark brown; (3) cream-white/white
6. Color-columellar and labial teeth	(0) reddish-brown; (1) light fawn/brown; (2) cream-white/whitish
7. Axial bands- line situated along the axis	(0) none; (1) 1; (2) dissolve; (3) 3; (4) 4
8. Banding patterns- irregular arrangement of lines and space on the dorsal portion of the shell	(0) numerous; (1) few; (2) no white rounded spaces; (3) numerous white spots; (4) numerous smaller brown spots; (5) transverse bands; (6) yellow ring; (7) black/brown spot; (8) regular brown flecking crossed by four darker bands that run along at the dorsal surface

Characters	Scoring
9. Dorsal line- line around the shell body	(0) indistinct; (1) visible; (2) partially visible
10. Spire	(0) partially covered by a callus; (1) visible, slightly protruding; (2) depressed
11. Lateral margins	(0) distinctly calloused at proximity; (1) flat and smooth;(2) rounded; (3) wide angulate
12. Lateral spots	(0) absent; (1) present
13. Basal spots	(0) absent; (1) present
14. Terminal spots	(0) absent; (1) present
16. Outer lip	(0) declivous; (1) constricted
17. No. of Labial teeth	(0) 8-12; (1) 13-17; (2) 18-22; (3) 23-27
18.No of Columellar teeth	(0) 8-12; (1) 13-17; (2) 18-22; (3) 23-27
19. LTR	(0) 8-12; (1) 13-17; (2) 18-22; (3) 23-27
20. CTR	(0) 8-12; (1) 13-17; (2) 18-22; (3) 23-27
21. Shell Length (mm)	(0) 15-26; (1) 27-38; (2) 39-50; (3) 51-62; (4) 63-74; (5) ≥75
22. Shell Width (mm)	(0) 9-19; (1) 30-30; (2) 31-41; (3)42-52; (4) ≥53
23. Shell Height (mm)	(0) 7-17; (1) 18-28; (2) 29-39; (3) 40-50
24. Aperture length (mm)	(0) 13-23; (1) 24-34; (2) 35-45; (3) ≥46
25. Aperture width (mm)	(0) 1-2; (1) 3-4; (2) 5-6
26. Ave. weight (g)	(0) < 10; (1) 11-21; (2) 22-32; (3) ≥33

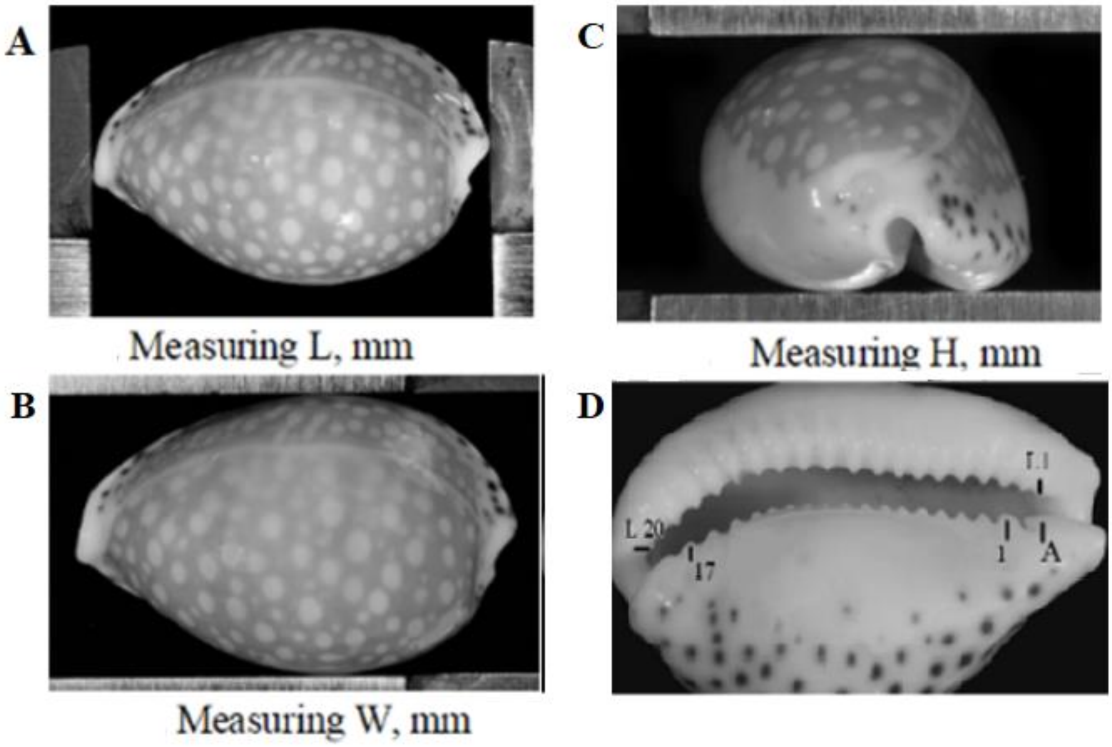

Figure 3. Morphometric and meristic measurements of *Cypraea* species shell size (in mm) such as length (A), width (B), height (C), and (D) - counting of teeth designations: A - a terminal ridge; L1 - the first labial tooth; 20-the last labial tooth; 1 the first columellar tooth; 17 - the last columellar tooth.

Landmark and Outline-based Geometric Morphometric Analysis

Shell shape analysis was done using a landmark and outline-based methodology to eliminate variations in location, orientation, and scale. One hundred anatomical landmarks along the dorsal portion (Figure 4A) and 17 along the ventral or apertural outline (Figure 4B, Table 2) were defined. The TpsDig freeware 2.12 (Rholf 2008a,) and Tps Util program 1.4 (Rholf 2009) facilitated the data collection and statistical analysis of landmark data in morphometrics. The landmark coordinates were transferred to Microsoft Excel application for data organization into groups. Then the generalized orthogonal least squares Procrustes average configuration of landmarks was computed using the generalized Procustes Analysis (GPA) superimposition method. GPA was performed using the software tpsRelw, ver. 1.46 (Rholf 2008b). Histograms were generated using Paleontological Statistics [PAST] software (Hammer et al. 2009). The pairwise comparisons (Demayo et al. 2011), Kruskal-Wallis tests, and Canonical Variance Analysis (CVA) were used to analyze differences in shell shape among species and population variations.

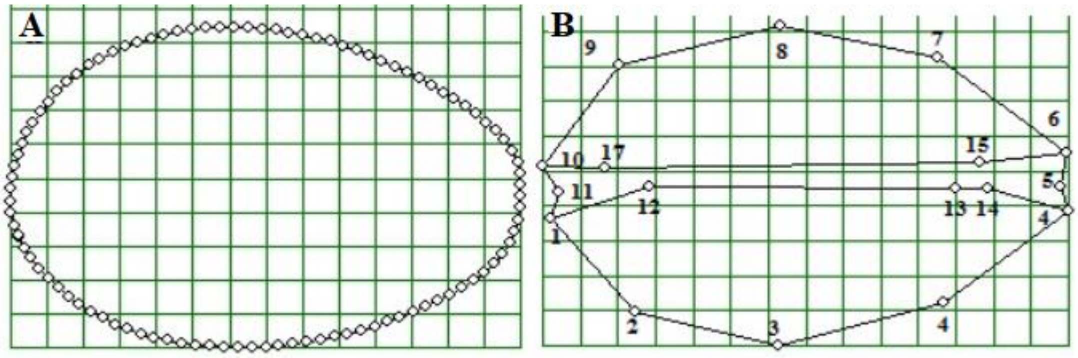

Figure 4. Landmarks used to describe the shape of the (A) dorsal and (B) ventral/apertural view of the shell of seven *Cypraea* species collected from Sindangan, Philippines.

Table 2. Descriptions of the anatomical landmark (LM) points in seven *Cypraea* species.

Landmark (LM) No.	Description of landmarks
1	last columellar tooth
2	first columellar tooth
3	terminal ridge
4	fore top of the inner lip
5	medial point between points 4 and 6 at the left shell margins
6	most prominent point of the left shell margins
7	medial point between points 6 and 8
8	hind top of the inner lip
9	junction point of the posterior canal
10	hind top of the outer lip
11	last labial tooth
12	first labial tooth
13	junction point of the anterior canal
14	fore top of the outer lip
15	medial point between points 14 and 16
16	most prominent point of the right shell margins
17	medial point between points 16 and 10

Correlation Analysis Based on Distances

Morphological and morphometric datasets that include landmarks for shell shape were analyzed using the Correlation analysis based on distances (CORIANDIS) 1.1 beta (Marquez and Knowles 2007). Associations among cowry shells were determined using multivariate datasets, compromise space projections, trait variance, unity, and multivariate covariance measures. The option "Projections on compromise space" was selected; this was done to plot all specimens/groups and traits/sets in the same space, obtained by projecting each dataset plus their weight average ('compromise') onto the compromise space. The stacked bar graphs visualized differences among the species (Tabugo et al. 2010 and Gutierrez et al. 2011). Cluster analysis was performed using the obtained scores. Correlation analysis examined the relationship between shell shape (dorsal and ventral) and size (length, thickness, aperture length).

RESULTS

Morphological (Qualitative) and Morphometric (Quantitative) Analysis of Shell Morphology

Morphological and morphometric analyses identified three clusters based on 16 morphological characteristics (Figure 5A). Clusters 1 and 2 differed in shell shape, axial bands, banding patterns, color, and lateral margins while cluster 3 consisted of four species subgroups with axial bands and banding pattern variations. Each species exhibited unique morphological traits. However, the morphometric analysis identified six clusters (Figure 5B), each representing different species based on labial and columellar teeth, shell dimensions, apertural length, and average weight. Clusters identified include Cluster 1 (two closely-related species 7), Cluster 2 (species 2, 4, 5, and 6), Clusters 3, 4, and 5 (species 1 and 3), and Cluster 6 (sole species 1). The seven Cypraeidae species exhibit distinct variations in labial and columellar teeth, shell dimensions, and average weight within each cluster.

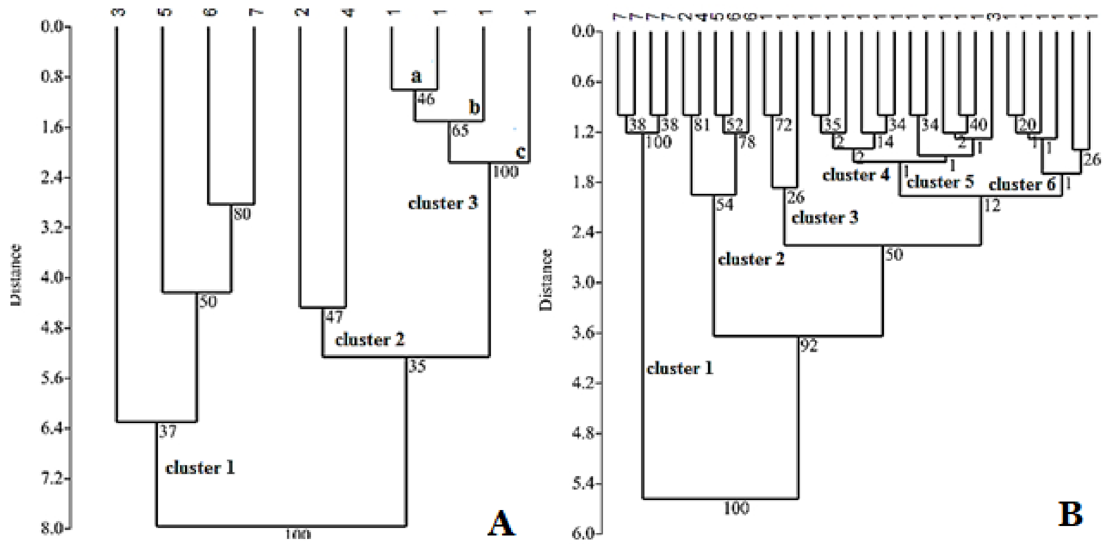

Figure 5. Hierarchical clusters of individuals of various qualitatively described (A) morphological (B) morphometric characters of *Cypraea arabica* (1), *Cypraea caputserpentis* (2), *Cypraea felina* (3) *Cypraea caurica* (4), *Cypraea moneta* (5), *Cypraea annulus* (6) and *Cypraea tigris* (7) collected in Sindangan Bay, Philippines.

Cypraea tigris had the highest shell weight and size measurements, such as length, width, thickness, apertural length, and apertural width (Table 3). *Cypraea felina* had multiple labial and columellar teeth. *Cypraea moneta* had the most petite average shell weight and size measurements except apertural width. *Cypraea annulus* had the least apertural width (1.08 mm). *Cypraea annulus*, *Cypraea moneta*, *Cypraea caputserpentis*, had measurement values very close to each other while *Cypraea arabica* had values more comparable to *Cypraea felina* and *Cypraea tigris* had the highest size measurements among the group.

Landmark and Outline-based Geometric Morphometric Analysis

Figure 6 presents the geometric morphometric analysis summary of 113 shells, showing consensus morphology (ventral/apertural and dorsal portion) based on relative warps (RW) from Table 4. Two significant RWs described the dorsal shape (Figure 6A), and four described the ventral shape (Figure 6B). Histogram projections display negative and positive deviations from the mean shape. The mean shape of the shell is shown in the topmost image. Negative RW1 and RW2 show depressed right lateral margins on the dorsal side, while positive RW1 and RW2 show depressed left and right lateral margins. On the ventral side, negative RW1-4 scores indicate a slightly depressed anterior extremity, while positive scores show heavily depressed anterior and posterior extremities. Kruskal-Wallis test confirmed differences in dorsal and ventral shell shapes among the seven species (Table 5).

Significant differences in shell shapes of the seven species were observed, such as the number of landmarks used, species differentiation based on the distribution of the samples along the first canonical variate axes of RW scores of the dorsal and ventral/apertural view of the shell (Table 6). The distribution of individual cowries in the CVA scatterplot of the dorsal and ventral shell is shown in Figure 7A and Figure 7B, respectively. While overlaps were observed, the differences could also be observed on those variant forms that are outside the overlaps.

Table 3. Average measurements in shell size such as shell length (SL), shell width (SW), shell thickness (ST), shell aperture length (SAL), shell aperture with (SAW); shell weight (SWt) and number of normalized labial (LTR) and columellar (CTR) teeth of the seven *Cypraea* species.

Species	SL (mm)	SW (mm)	ST (mm)	SAL (mm)	SAW (mm)	SWt (g)	LTR (no.)	CTR (no.)
Cypraea arabica	43.05	27.78	22.66	40.51	3.35	14.88	19.16	18.49
Cypraea caputserpentis	27.67	21.28	13.92	26.40	1.68	5.94	15.00	11.00
Cypraea felina	39.80	25.20	21.13	38.63	2.88	7.64	22.00	20.00
Cypraea caurica	20.99	11.57	9.65	20.19	1.18	0.94	14.00	13.00
Cypraea moneta	16.48	9.58	7.66	14.97	1.23	0.49	13.00	11.00
Cypraea annulus	19.67	13.80	10.04	18.59	1.08	1.84	13.00	10.00
Cypraea tigris	73.39	51.93	39.10	68.82	5.84	64.46	17.00	16.00

Table 4. Percentage variance and overall shape variation in the dorsal and ventral shell of Cypraeidae as explained by significant relative warps.

RW	% Variation	Dorsal shell	% Variation	Ventral shell
1	73.13	High (+) RW scores show heavily depressed left and right lateral margins near the anterior and posterior parts of the right shell margins. Low (-) RW1 scores indicate heavily depressed right lateral margins near the anterior part of the shells.	52.08	High (+) RW scores: Heavily depressed anterior and posterior extremities. Low (-) RW scores: Slightly depressed anterior extremity of the shell.
2	17.66	High (+) RW scores: Heavily depressed left and right lateral margins near the anterior part, slightly depressed posterior part of right shell margins. Low (-) RW1 scores: Heavily depressed right lateral margins near the anterior part of shells.	16.77	High (+) RW scores: Heavily depressed anterior and posterior extremity. Low (-) RW scores: Slightly depressed anterior extremity of the shell.
3			10.11	High (+) RW scores: Heavily depressed anterior and posterior extremity. Low (-) RW scores: Slightly depressed anterior extremity of the shell.
4			7.27	High (+) RW scores: Heavily depressed anterior and posterior extremity. Low (-) RW scores: Slightly depressed anterior extremity of shell.

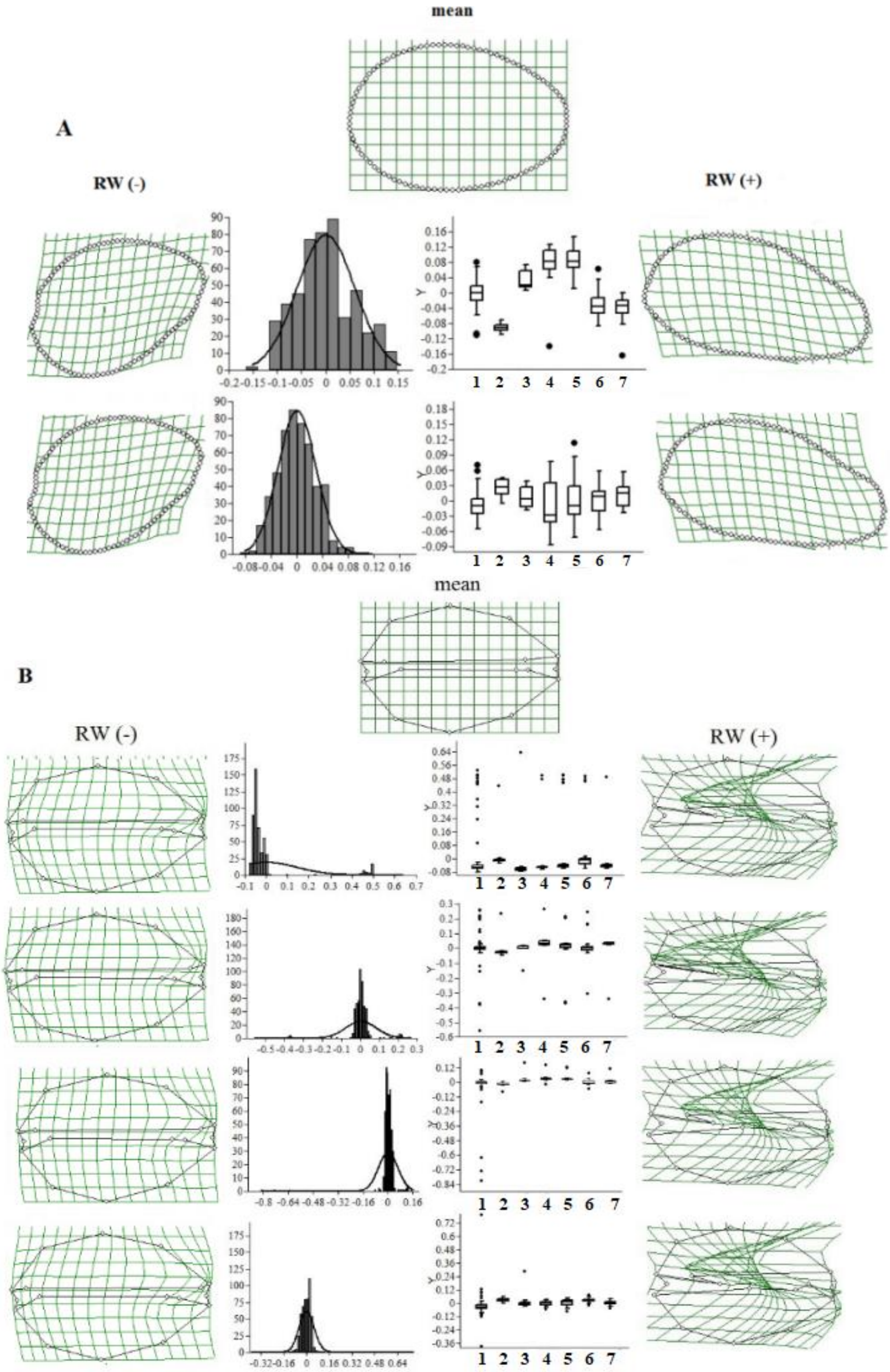

Figure 6. Relative warp (RW), box plot and histogram showing variations in the (A) dorsal and (B) ventral/aperture shell shape of seven *Cypraea* species. Legend: (1) *Cypraea arabica*, (2) *Cypraea caputserpentis*, (3) *Cypraea felina*, (4) *Cypraea caurica*, (5) *Cypraea moneta*, (6) *Cypraea annulus* and (7) *Cypraea tigris*.

Table 5. Pairwise comparison of the mean shapes of the dorsal and ventral side of the shell of the seven *Cypraea* species from Kruskal-Wallis Test.

RW	Sp.	**Dorsal**						
		1	2	3	4	5	6	7
1	1		4.365E-30	2.908E-08	1.766E-15	1.585E-31	1.569E-14	7.551E-13
	2	9.168E-29		1.371E-14	6.698E-13	1.072E-22	5.75E-24	2.02E-15
	3	6.107E-07	2.88E-13		4.113E-07	1.398E-10	1.97E-12	1.121E-2
	4	3.709E-14	1.407E-11	8.637E-06		0.6957	5.248E-14	2.893E-11
	5	3.328E-30	2.251E-21	2.937E-09	1		8.06E-26	3.436E-18
	6	3.295E-13	1.208E-22	4.138E-11	1.102E-12	1.693E-24		0.8937
	7	1.586E-11	4.242E-14	2.354E-11	6.076E-10	7.215E-17	1	
2	1		6.174E-21	0.0001107	0.2131	0.2954	5.375E-05	1.317E-07
	2	1.297E-19		1.428E-05	5.856E-05	3.608E-06	7.91E-08	0.01201
	3	0.002325	0.0002999		0.02236	0.07839	0.7623	0.3131
	4	1	0.00123	0.4696		0.1734	0.08118	0.005793
	5	1	7.576E-05	1	1		0.2599	0.01551
	6	0.001129	1.661E-06	1	1	1		0.05106
	7	2.765E-06	0.2522	1	0.1217	0.3257	1	
RW	Sp	Ventral/Apertural						
		1	2	3	4	5	6	7
1	1		1.776E-21	1.749E-07	0.9235	3.521E-07	3.656E-19	0.00127
	2	3.729E-20		6.698E-13	20525E-11	2.121E-08	0.2912	1.079E-15
	3	3.673E-06	1.407E-11		6.067E-06	6.539E-12	3.473-13	1.357-08
	4	1	5.303E-10	0.0001274		0.0001309	2.087E-09	0.0003621
	5	7.394E-06	4.454E-07	1.373E-10	0.002748		1.709E-05	0.4282
	6	7.678E-18	1	7.294E-12	4.383-08	0.0003589		2.603E-10
	7	0.02667	2.266E-14	2.849E-07	0.007604	1	5.467E-09	
2	1		1.913E-23	0.001159	2.48E-12	3.252E-10	0.8862	1.051E-15
	2	4.018E-22		3.998E-12	3.763E-12	5.479E-16	4.411E-16	9.703E-15
	3	0.02434	8.397E-11		5.573E-10	0.01113	0.02106	1.392E-11
	4	5.207E-11	7.903E-11	1.17E-08		8.897E-05	3.634E-11	0.9244
	5	6.83E-09	1.151E-14	0.2337	0.001868		2.885E-07	1.586E-06
	6	1	9.264E-15	0.4423	7.631E-10	6.059E-06		5.996E-15
	7	2.208E-14	2.038E-13	2.924E-10	1	3.331E-05	1.259E-13	
3	1		1.913E-23	0.001159	2.48E-12	3.252E-10	0.8862	1.051E-15
	2	4.018E-22		3.998E-12	3.763E-12	5.479E-16	4.411E-16	9.703E-15
	3	0.02434	8.397E-11		5.573E-10	0.01113	0.02106	1.392E-11
	4	5.207E-11	7.903E-11	1.17E-08		8.897E-05	3.634E-11	0.9244
	5	6.83E-09	1.151E-14	0.2337	0.001868		2.885E-07	1.586E-06
	6	1	9.264E-15	0.4423	7.631E-10	6.059E-06		5.996-15
	7	2.208E-14	2.038E-13	2.924E-10	1	3.331E-05	1.259E-13	
4	1		5.146E-27	1.402E-09	3.283E-09	3.485E-17	3.668E-33	8.855E-14
	2	1.081E-25		8.188E-10	2.634E-09	4.078E-08	0.8696	5.206E-10
	3	2.943E-08	1.72E-08		0.4553	0.05558	5.881E-10	0.1596
	4	6.894E-08	5.531E-08	1		0.1445	1.746E-09	0.5689
	5	7.321E-16	8.565E-07	1	1		4.727E-09	0.2755
	6	7.702E-32	1	1.235E-08	3.666-08	9.926E-08		2.742E-10
	7	1.859E-12	1.093E-08	1	1	1	5.758E-09	

Table 6. Canonical variance analysis (CVA) of relative warp scores among seven *Cypraea* species.

	DORSAL	**VENTRAL**
Wilks Lambda	0.1556	0.6977
df1	12	24
df2	984	1711
F	125.8	7.748
P(same)	3.721E-189	2.061E-25

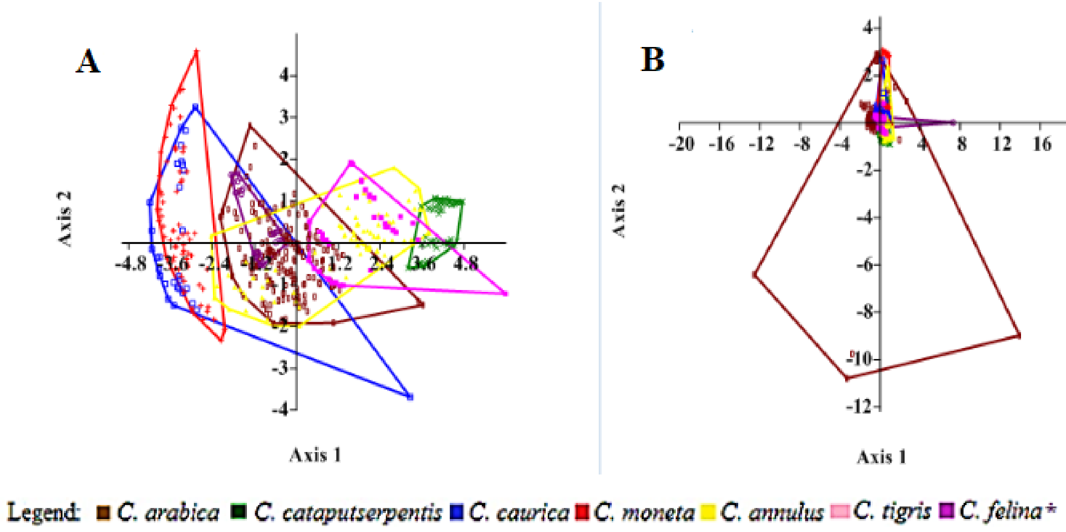

Figure 7. Canonical variance analysis (CVA) scatter plot of the (A) dorsal and (B) ventral shell of the seven *Cypraea* species.

Correlation Analysis Based on Distances (CORIANDIS)

The heights of the stacked bar graphs (Figure 8) and the interspecific locations of traits/characteristics (represented as colored points) in compromise space (Figure 9) indicate morphological and meristic differences among Cypraeidae species. The result shows low congruence and high disparity in the species' average shapes (dorsal and ventral/apertural) morphology and size measurements. As shown in the figure, species 2, 4, 5, and 6 had higher tendencies to cluster together, followed by species 1 and 3 in terms of their morphological and meristic similarities, except species 7, which inferred great differences in the characters between clusters.

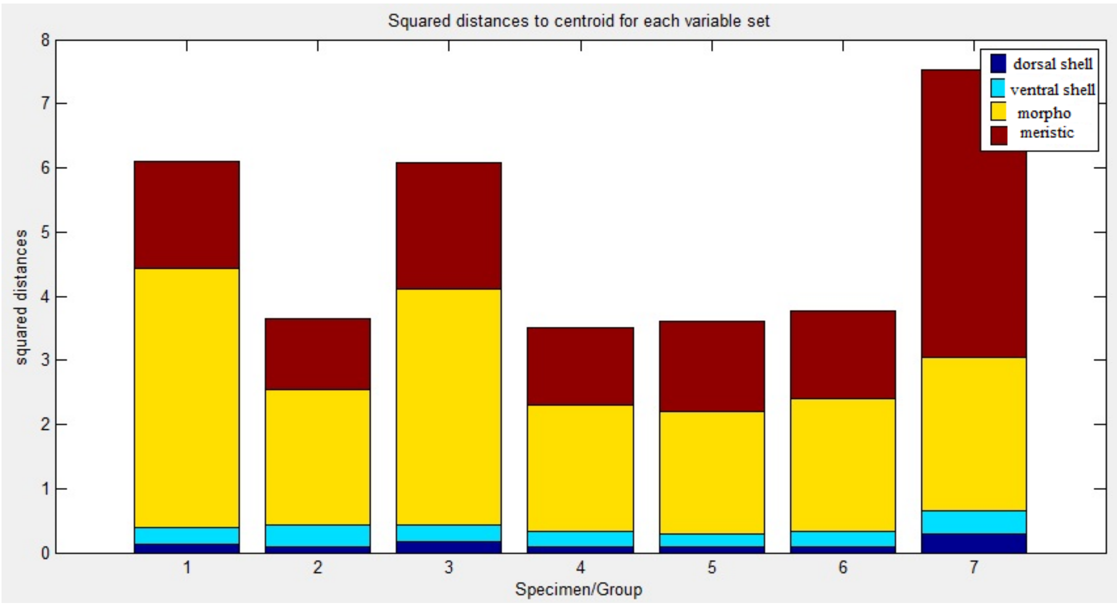

Figure 8. Stacked bar graphs showing disparity among the seven *Cypraea species* with regards to the shape (dorsal and ventral), morphological and morphometrics characters. Legend: (1) *Cypraea arabica*, (2) *Cypraea caputserpentis*, (3) *Cypraea felina*, (4) *Cypraea caurica*, (5) *Cypraea moneta*, (6) *Cypraea annulus* and (7) *Cypraea tigris*.

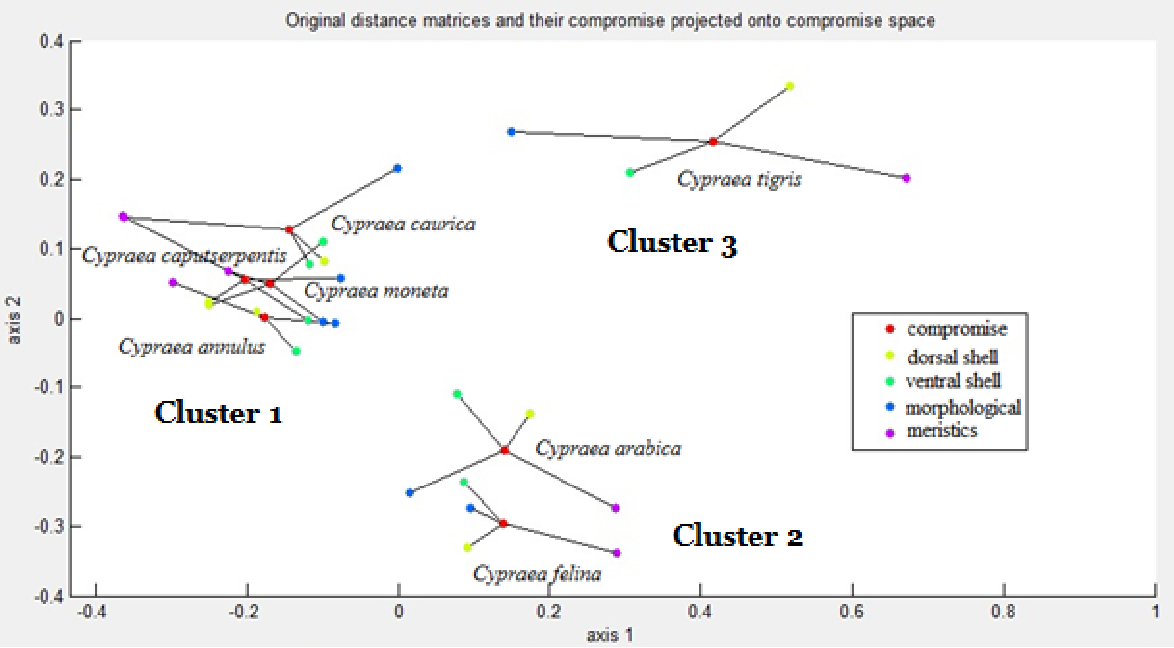

Figure 9. Plot of the principal components of "compromise" space axis of the seven *Cypraea* species.

Figure 10 displays a dendrogram of the overall relationship among the seven Cypraeidae shells in compromise space. The results revealed three clusters based on the shell characters examined. Cluster 1, comprised of *C. annulus* was 71% similar to *C. caurica*, while *C. moneta* was 29% identical to *C. caputserpentis*, and 38% similar to *C. caurica*. The size, color, dorsal and ventral shape, and depressed spire were the main points of similarity. Cluster 2 C. *arabica* and *C. felina* were 34% similar in the visible spire, dorsal line, rounded lateral margins, and present lateral spots. Clusters 1 and 2 are 65% identical while species *C. tigris* solely clustered at 100% with the rest of Cypraeidae species because of its apparent large size and numerous black spots.

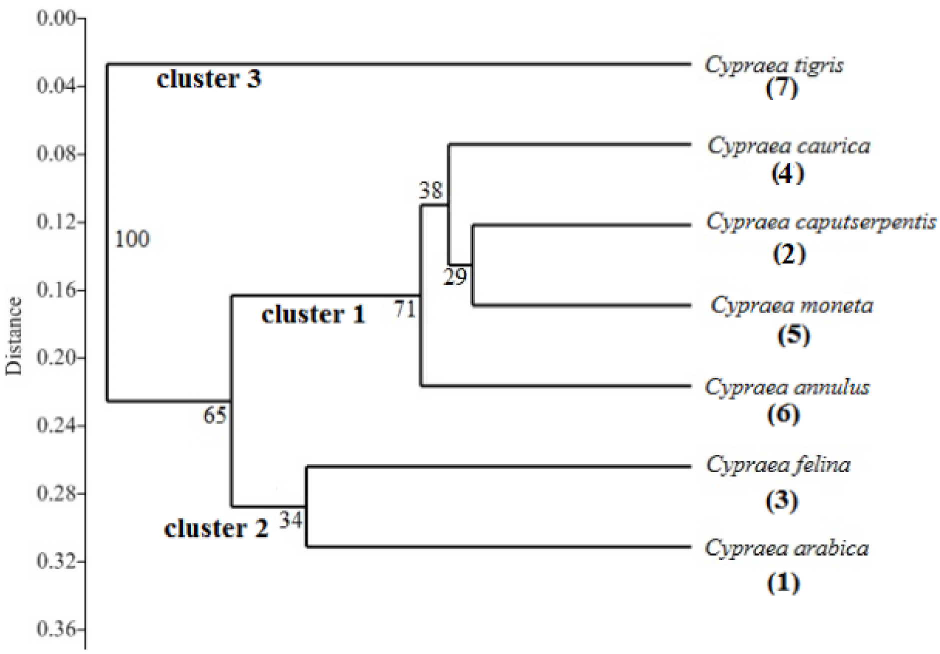

Figure 10. Plot showing the degree of similarity of characters among the seven *Cypraea* species.

A correlation analysis was performed to determine the observed shell shape (Figure 11B) and size (Figure 11A) differences between groups based on the computed centroid size. Results show that shell length (Figures 12 and 13A), width (Figures 12B and Figure 13C), aperture length (Figures 12 and 13D) and width (Figures 12 and 13E) were significantly correlated with shell shape ($P < 0.05$) except for the average weight (Figures 12 and 13F; Table 7).

DISCUSSION

Morphological (Qualitative) and Morphometric (Quantitative) Analysis of Shell Morphology

Morphological differences observed among cowries (such as color, shape, banding pattern, lateral margins, dorsal/transverse line, spire, and teeth) are likely influenced by phenotypic plasticity, developmental plasticity, genetic composition, and environmental conditions (Miner et al. 2005). Phenotypic plasticity is an adaptive process that allows organisms to adjust their traits in response to environmental pressures (Moneva 2012). However, it is difficult to identify specific environmental factors responsible for shell morphology differences (Patiluna and Demayo 2015). Several studies revealed that plasticity in shell morphology has implications for its adaptation to different environments and evolutionary processes (Trussell and Etter 2001). The morphological differences of the snail, *A. fulica* in size, shape and color can be attributed to environmental conditions (Mead 1961), variability in shape was due to genetic anomalies (Bequaert 1950). In addition, the morphological expression of characteristics (phenotypic) in other *Cypraea* species (i.e. *Cypraea annulus)* varies from juvenile to post-adult development of shells (Laimeheriwa 2017). According to Irie and Iwasa (2003), the variety of phenotype characters are strongly influenced by genetic material or genotype and environment or ecotype. Furthermore, the environmental factors such as climate, humidity and temperature may cause physiological changes which could affect snail's development (Vinic et al. 1988), especially the shell length-width relationship (Albuquerque et al. 2009) and influence the phenotypic character of a cowry shell (Oliver 2004).

Growth rate and population density may also influence shell variations, indicating the presence of either phenotypic plasticity or genetic differentiation (Dela Rosa et al. 2010). While genetics is commonly associated with ecology-to-evolution effects, evidence is inconsistent (Hendry 2013). Understanding the specific mechanisms and other potential factors involved is crucial (Trussell and Etter 2001). A recent study in the reconstruction of the Cypraeidae's mitogenomic phylogeny showed the usefulness of pairwise genetic distance analysis in the identification of species (Ma et al. 2023), especially in cases of phenotypic plasticity or convergence (Abalde et al. 2017a, b; Yang et al. 2018). Despite the progress made in genetic analysis, the conventional classification of Cypraeidae, which mostly relied on shell and anatomical traits, remains relevant.

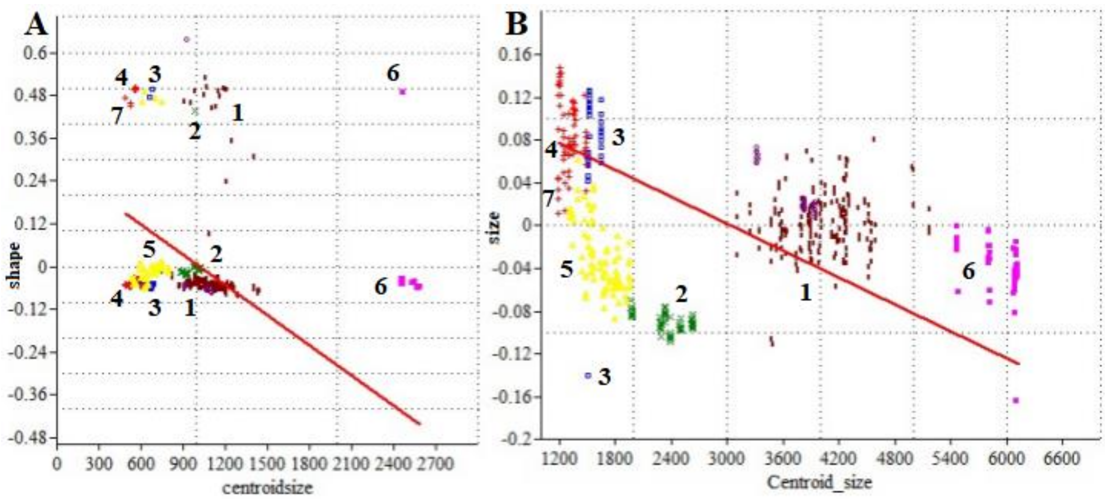

Figure 11. Centroid size (A) vs shape correlation (B) of the shell of the seven *Cypraea* species. Legend: (1) *Cypraea arabica;* (2) *Cypraea caputserpentis,* (3) *Cypraea caurica;* (4) *Cypraea moneta; (5) Cypraea annulus;* (6) *Cypraea felina* and (7) *Cypraea tigris*.

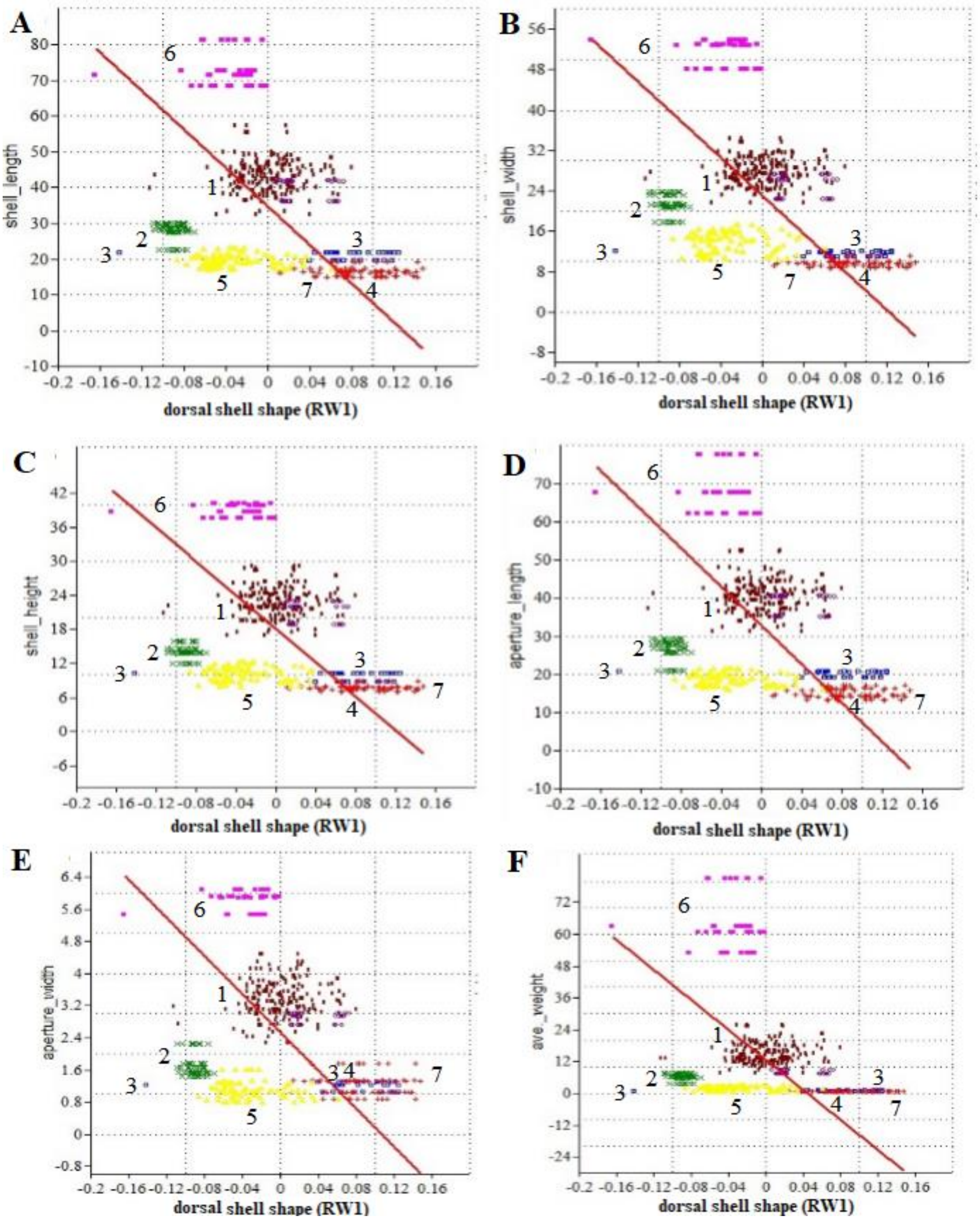

Figure 12. Correlation of shell dorsal shape with different morphological measurements such as (A) shell length, (B) shell width, (C) shell height, (D) shell aperture length, (E) width and (F) average weight of the seven *Cypraea* species. Legend: (1) *Cypraea arabica;* (2) *Cypraea caputserpentis*, (3) *Cypraea caurica;* (4) *Cypraea moneta; (5) Cypraea annulus;* (6) *Cypraea felina* and (7) *Cypraea tigris*.

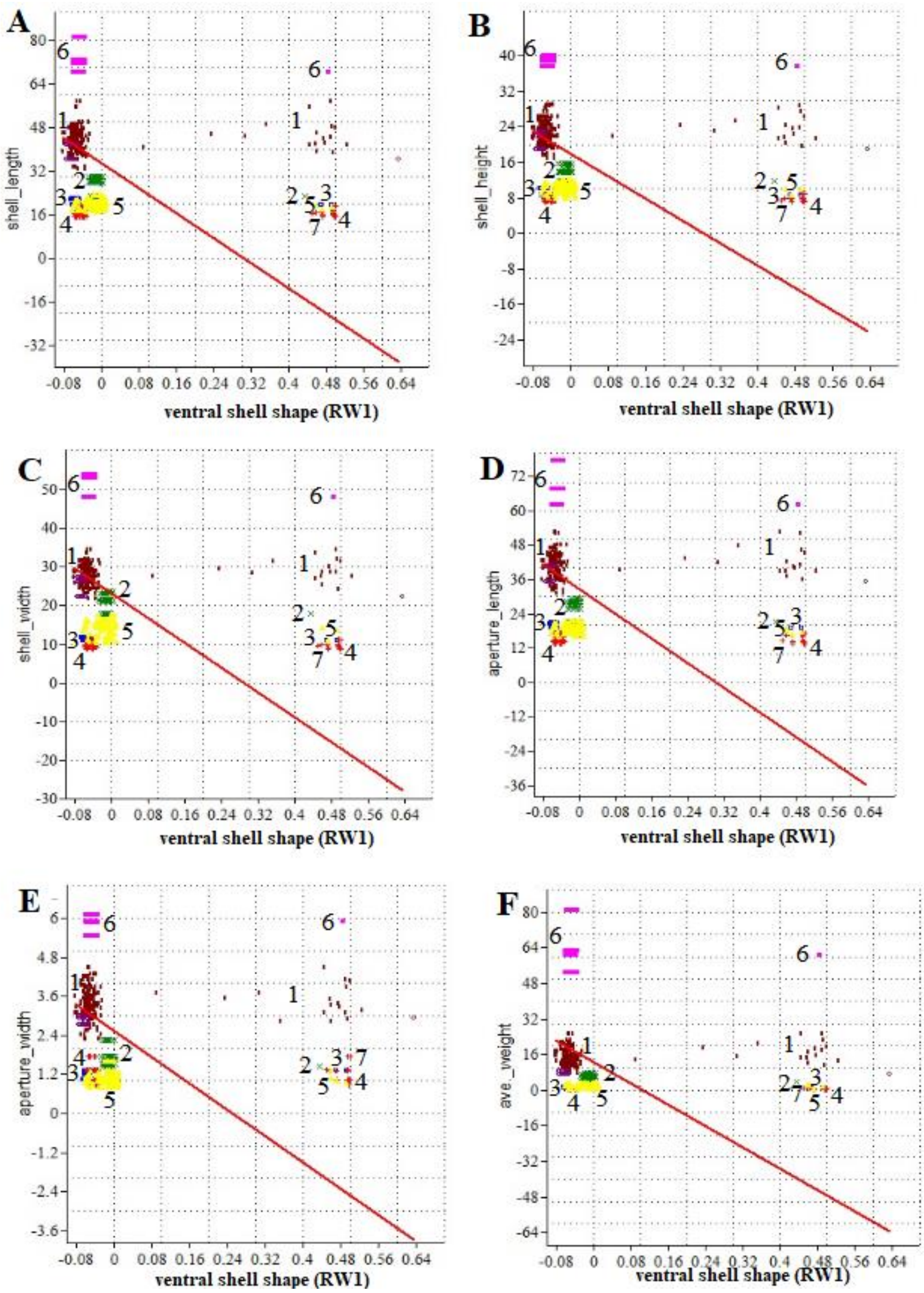

Figure 13. Correlation of shell ventral shape with different morphological measurements, such as (A) shell length, (B) shell height, (C) shell width, (D) shell aperture length, (E) width and (F) average weight of the seven *Cypraea* species. Legend: (1) *Cypraea arabica;* (2) *Cypraea caputserpentis,* (3) *Cypraea caurica;* (4) *Cypraea moneta; (5) Cypraea annulus;* (6) *Cypraea felina* and (7) *Cypraea tigris*.

Table 7. Correlation analysis of shape (significant relative warp, RW1) with centroid size and other morphological measurements, such as shell length, shell height, shell width, shell aperture length and average weight of the seven *Cypraea* species.

DORSAL			
Correlation	**Slope**	***r***	**P(uncorr)**
Shape (RW1) vs centroid size	-4.1999E-05	-0.24215	**4.1851E-08**
Shape (RW1) vs SL	-269.22	-0.24218	**4.165E-08**
Shape (RW1) vs ST	-148.53	-0.24941	**1.5805E-08**
Shape (RW1) vs SW	-188.56	-0.34053	**4.8501E-15**
Shape (RW1) vs SAL	-254.19	-0.24821	**1.8616E-08**
Shape (RW1) vs SAW	-23.763	-0.16363	**0.0002384**
Shape (RW1) vs SAve.Wt.	-281.98	-0.23462	**1.1123-07**
VENTRAL			
Shape (RW1) vs centroid size	-0.0002824	-0.12477	**0.0052096**
Shape (RW1) vs SL	-114.3	-0.1307	**0.0034148**
Shape (RW1) vs ST	-63.06	-0.13176	**0.00316**
Shape (RW1) vs SW	-80.053	-0.13417	**0.0026445**
Shape (RW1) vs SAL	-107.92	-0.13625	**0.0022636**
Shape (RW1) vs SAW	-10.089	-0.11072	**0.013244**
Shape (RW1) vs SAve.Wt.	-119.72	-0.086341	0.053679

Landmark and Outline-based geometric morphometric analysis

The observed variations in shell shape among cowries can be determined by the shell ontogenetic process, where new shell material is gradually deposited onto the existing aperture through accretionary growth. This allows the shell to expand and develop as the organism matures, maintaining its structural integrity (Liew and Schilthuizen 2016). Differences in shell shape amongst closely related species may therefore represent variations in shared developmental trajectories (Cruz et al. 2012), genetic architecture, and the influence of environmental factors (Cazzaniga 2006) such as shore exposure and zonation (Doyle et al. 2018). However, there are advantages to quantifying these shape differences that had been carried out in this study. Quantification allows for the recognition of intermediate forms; judging degrees of proximity or similarity; and extrapolation or prediction of hypothetical and experimental extremes (Cruz et al. 2012).

As a result of this study, the landmark-based method has the greatest advantage than the outline-based method because aperture shape is highly variable depending on the environmental factors where greater shell girth acts as defense against crushing predators (Doyle et al. 2018). Whereas the outline-based method can capture shells callus thickness since individuals of *Cypraea annulus* and *Littorina* species displayed larger and broader shell in lower shore and sheltered shores and with higher temperature (Irie 2005). Thus, geometric morphometrics offers a more effective approach than traditional methods for studying gastropod shell morphology. It provides a more detailed assessment of shape, identifying subtle differences and patterns missed by traditional measurements (Carvajal-Rodríguez et al. 2005).

Correlation Analysis Based on Distances (CORIANDIS)

The morphometric characters observed in this study are positively correlated with shell shape, except for weight and number of teeth (Irie 2005). Cowries exhibit explicit determinate growth, with shell size not increasing after the juvenile stage (Irie and Morimoto 2008). The shell form among cowries has, over time, varied greatly in the morphometrics selected to represent shell form (Bridges and Lorenz 2013). The observed variations can be the result from the interaction between an individual's genetic architecture and the environment. Environmental conditions are essential for understanding the differences observed in cowry shells shape (Cazzaniga 2006; Torres et al. 2011).

Several studies have pointed out that there is no reaction norm by which of these environmental cues induces could increase or decrease body size in cowries (Irie 2005). One should not discount the influence of environmental conditions in bringing about differences in shell shapes. It was reported that latitudinal difference in juvenile shell size can be explained by phenotypic plasticity based on environmental heterogeneity (Irie 2005). It is also plausible that a cue from predators affects callus thickness, because predation pressures vary at different latitudes (Irie 2005; Vermeij 1972).

This study highlights the use of GM and CORIANDIS in assessing morphological, size, and shape variations in Cypraeidae species. These variations result from the interaction between genetic architecture and the environment, indicating phenotypic diversity. Autoregulatory developmental processes may differ among species in buffering against environmental and genetic influences. Further exploration of variables like environmental heterogeneity and species distribution is needed. Understanding the genetic basis of phenotypic diversity is crucial. Consideration of juvenile size and shape as well as the genetic analysis can provide additional insights for systematic studies of Cypraeidae species.

Geometric morphometric analysis revealed significant variations in gastropod shell morphology influenced by phenotypic plasticity and environment. Geometric morphometrics outperformed traditional measurements. Future research should explore genetic and environmental mechanisms, expand the analysis to more taxa, and integrate genomics for a comprehensive understanding of shell morphology and its evolutionary implications in response to environmental changes.

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ETHICAL CONSIDERATIONS

There are no human subjects in this article and informed consent is not applicable. However, the collection procedures of shell samples were conducted in accordance with local government guidelines and approved by the office of municipal Mayor.

DECLARATION OF COMPETING INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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