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MORPHOLOGICAL ASSESSMENT OF *Pincerna globosa* (H. ADAMS, 1871) FROM WESTERN SARAWAK (MALAYSIA BORNEO)

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Abstract: *Pincerna globosa* is known as the sole species of the genus *Pincerna* distributed in Sarawak. Our preliminary morphological assessment of *P. globosa* of different populations indicated variation based on their shell shape and size. Therefore, we aimed to determine the extent of morphological variation of *P. globosa* based on these two characters. A total of 283 adult *P. globosa* were collected at limestone clusters in Bau, Padawan, and Siburan. The specimens were collected from Bau, Padawan, and Siburan and deposited into the Zoological Museum UNIMAS, Universiti Malaysia Sarawak. Based on landmark analysis, there is a significant variation in the shell shape of *P. globosa* ($p < 0.0001$) with LM10, the highest point of the lip, and LM15, the external point of the lip at the left profile of the shell, as the most variable landmarks characters. Principal Component Analysis (PCA) based on geometric morphometrics shows a total variation of 37.22% by the first two principal components. Our study has shown that the highest point of the lip and the external point of the lip at the left profile of the shell varies among populations. Furthermore, we found that populations from the limestone cluster Padawan are the largest in terms of shell size compared to populations from Bau and Siburan. We conclude that there are significant variations in the shell shape and size of *P. globosa* from the western regions of Sarawak.

Keywords: *Pincerna globosa*, morphological variation, geometric morphometric, landmarks, Principal Component Analysis.

Introduction

Morphological variations of a widely distributed taxa may indicate that the organism experiences different ecological pressures, which are mainly caused by various environmental stresses, including different habitat types, soil mineralogy, and elevation (Goodfriend, 1986; Teusch *et al*., 2002; Chiba *et al*., 2009; Kramarenko, 2016). Note that morphological studies using quantitative data analysis of measurable traits are useful to explore the patterns in size and shape changes and to understand the processes influencing morphological variation, including factors such as ecology and/or behaviour (Tellería *et al*., 2013). Qualitative assessments among invertebrates indicate morphological variations in size and shape are generally influenced by environmental conditions and associated with geographical differences (Martínez-Freiría *et al*., 2009; Fruciano *et al*., 2011).

The gastropod is an ideal model for studying morphological variation and diversification because of its high and easily quantifiable morphological diversity (Davison, 2002; Giokas *et al*., 2014). For instance, the body size of the great pond snail, *Lymnaea stagnalis* decreases with the increment of longitude from south to north, even though they share similar ecological conditions (Vinarski, 2014). Additionally, the body-size ratio may vary in response to a complex combination of environmental factors such as latitude and climate (Goodfriend, 1986), predation (Chiba, 2007; Parent & Crespi, 2009), and ecotype (Chiba, 2004). Differences in ecology and climates may affect the development of lineages, which can help explain the trends of morphological variation and adaptation of various taxa (Giokas *et al*., 2014; Stanchak & Santana, 2018). Variation in shell shape and

colour in different populations has been widely studied to understand adaptation processes under distinct environmental conditions (Cowie, 1995; Sokolova & Berger, 2000; Miura *et al*., 2007; Silva *et al*., 2013).

Consequently, geometric morphometrics has been applied to examine shell shape variation (Conde-Padin *et al*., 2007; Haase & Misof, 2009; Rao *et al*., 2018) and usually present the advantage of reducing the effects caused by the size, location, rotation, scale, and experimental bias, which are frequently seen in standard morphometric analyses (e.g., Rohlf & Marcus, 1993; Schilthuizen & Haase, 2010). Therefore, shell shape and size variation can be analysed using morphological measurements and a geometric morphometric method, which is often used to compare the shell morphology of different populations. The landmark method is useful for measuring the degree of deformations of morphometric points in coordinate space, focusing on size and shape.

In the current study, we selected *P. globosa* as our sample, a widely distributed taxa in the western regions of Sarawak. The genus *Pincerna* belongs to the family Alycaeidae. It is known to be distributed from the western Ghats (India) to Japan in the east, Gansu and Shaanxi provinces in the north of China, and the south of Indonesia (Páll-Gergely *et al*., 2020). This family is generally characterised by a sutural tube linked to numerous perpendicular microtunnels formed by the outermost shell layer (Páll-Gergely & Auffenberg, 2019). The genus *Pincerna* possesses an operculum with an external "circular cup". It can be distinguished by its globular, thinly ribbed, and white to orange-coloured shell (Páll-Gergely *et al*., 2020; Marzuki *et al*., 2021).

This study is conducted to determine the intra- and inter-population morphological variation of *P. globosa* across different locations in western regions of Sarawak. Correspondingly, we examined their shell shape and size to test whether different populations show significant shell variation based on the selected characters. This study is able to fill the gap in the literature regarding the morphological variations of *P. globosa* in the western part of Sarawak using geometric morphometrics to analyse the shell shape and size variation. We expected significant differences in the morphological features of shell size and shape towards the end of the study.

Materials and Methods

Sampling Areas

A total of 283 adult specimens of *P. globosa* were collected at limestone clusters Bau (Fairy Cave Nature Reserve, Wind Cave Nature Reserve, Gunung Soba, Gunung Tabai, Siboyuh, and Tupap), Padawan (Gunung Tok Pisu, Gua Rambus, Gunung Duai, Gunung Kayu, and Rumbang), and Siburan (Gunung Bawang, Gunung Mawah, Gunung Sewa, Gunung Mbang, Gunung Bakan, and Gua Raya) as visualised in Figure 1. An active search was conducted in a plot measuring approximately 10 m × 10 m with three people for at least 20 minutes at each sampling site. The live specimens were collected and immediately placed inside tubes containing ~100% ethanol for preservation. Subsequently, the specimens were sorted and identified. All collected specimens were deposited at the Zoological Museum UNIMAS, Universiti Malaysia Sarawak (Table 1).

Shell Size Analysis

For imaging, the shells were positioned in the apertural view where the columellar is at 90° of the x-axis and in the orientation in which the apx is visible (Sobrepeña & Demayo, 2014). The digital images of the shells were then captured with 10× magnification and measured using XCAM Family 1030P attached to a stereo-microscope. Seven quantitative measurements of Shell Diameter (D), Shell Height (H), Apertural Width (AW), Apertural Height (AH), Height of Spire (SH), Width of Spire (SW), and Width of Protoconch (PW) were taken from the image of standardised aperture view of the shell using Toupview software [Figure 2 (b)]. Specimens were digitised and saved as Thin-Plate Splines (TPS) files.

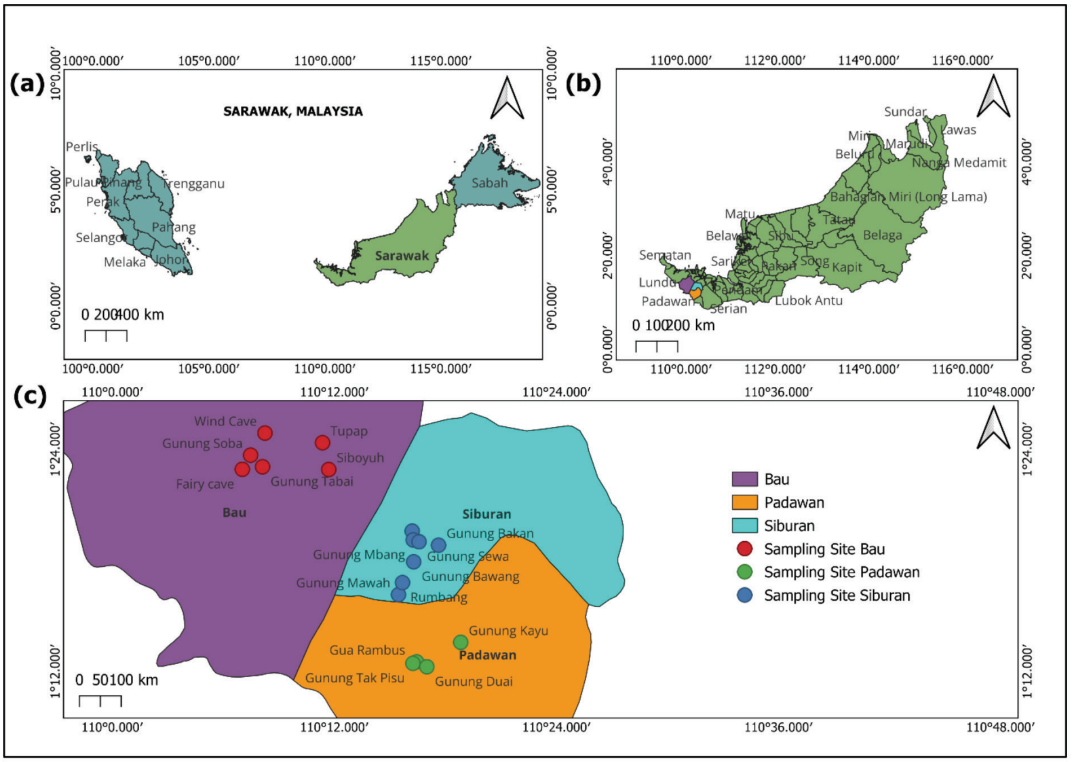


Figure 1: Geographic locations of Sarawak, Malaysia where *P. globosa* were collected. (a) Map shows a whole Malaysia's states, (b) a detailed map of Sarawak highlighting three regions: Bau, Padawan, Siburan, and (c) the sampling sites which cover a total of 17 areas of limestone cluster

Table 1: A total of 283 adult *P. globosa* based on their localities from the Zoological Museum UNIMAS collection, Universiti Malaysia Sarawak, used for measurement and geometric morphometric analysis

Limestone Clusters	Locality and Coordinate	Number of Individuals	Cat. No.
Bau	Fairy Cave Nature Reserve (= 1.38199, 110.11710)	5	MZU.MOL.19.83
	Wind Cave Nature Reserve (= 1.41459, 110.13750)	30	MZU.MOL.19.75
	Gunung Soba (=1.39472, 110.12462)	22	MZU.MOL.16.22
	Siboyuh (=1.38182, 110.19492)	1	MZU.MOL.16.86
	Tupap (= 1.4059, 110.18936)	12	MZU.MOL.19.74
	Gunung Tabai (=1.38427, 110.13532)	1	MZU.MOL.16.31

Padawan	Gunung Rambus (=1.20865, 110.27412)	5	MZU.MOL.16.185
	Gunung Duai (=1.20425, 110.28333)	3	MZU.MOL.16.70
	Gunung Kayu (=1.22622, 110.31384)	1	MZU.MOL.16.182
	Gunung Tok Pisu (= 1.20733,110.27075)	10	MZU.MOL.16.183
Siburan	Gua Raya (= 1.32638, 110.27010)	86	MZU.MOL.22.57
	Gunung Bakan (= 1.31369, 110.29383)	5	MZU.MOL.16.188
	Gunung Bawang (= 1.29873, 110.27143)	1	MZU.MOL.16.187
	Gunung Mawah (=1.26917, 110.25777)	5	MZU.MOL.16.78
	Gunung Mbang (= 1.31843, 110.27102)	25	MZU.MOL.16.186
	Gunung Sewa (= 1.31682, 110.27625)	32	MZU.MOL.16.91
	Rumbang (=1.28000, 110.26139)	39	MZU.MOL.16.109

Consequently, correlation regression on shell measurements as characters distinguishes the strength and direction of the relationship between different shell measurements (Rao *et al*., 2018; McFarland *et al*., 2023).

Shell Shape Analysis

The TPS files were used for landmarking for geometric and morphometric analysis. A total of 16 landmarks were predetermined specifically to examine the shape of the shell and aperture outline of *P. globosa* in the programme tpsDig2 (v. 1.1) [Figure 2 (a)]. LM1 represents the apex of the shell. Meanwhile, LM2 and LM3 are on the left and right border of the shell profile at the end of the upper suture of the penultimate whorl while LM4 and LM5 are on the lower suture. LM6 is the most external point in the last whorl at the left profile of the shell. LM7 is the middle point of the last suture and the start point of the lip. LM8 is the end of the suture. LM9 is the start of the external part of the lip.

LM10 is the highest point of the lip. LM11 is the external point of the lip at the right profile of the shell. LM12 is the longest of the lips. LM13 is the lowest point at the base. LM14 is the middle point of LM15 and LM16. LM15 is the external point of the lip at the left profile of the shell and LM16 is the end of the lip. LM1 and LM8 are type I, which is known as developmentally homologous while six landmarks (LM2-LM7) represent the suture line and are considered type II, which is geometrically homologous. The remaining landmarks (LM9-LM16) were categorised as type III, either located at extreme points on surfaces or positioned midway between other landmarks (Giokas *et al*., 2014). Correspondingly, the file is saved as a new TPS file to be analysed for geometric morphometrics.

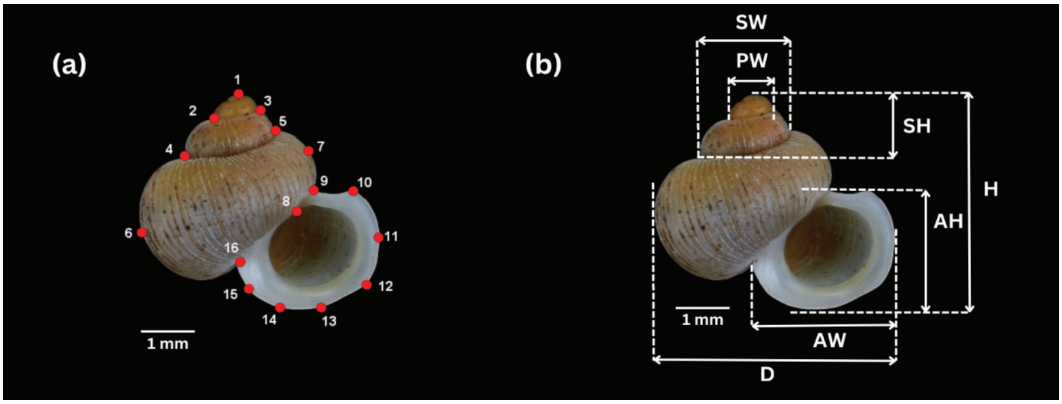


Figure 2: (a) Shell of *P. globosa* with the 16 landmarks used in the geometric morphometry analysis. (b) Measurements of the shell of *P. globosa*: Shell Diameter (D), Shell Height (H), Apertural Width (AW), Apertural Height (AH), Height of Spire (SH), and Width of Spire (SW), and Width of Proto Conch (PW)

Results

Geometric Morphometric Analysis

The comparison results among populations within the same region show significant differences in shape and size except for the limestone cluster Padawan [Figure 3 (b)]. Padawan displays no significant difference in size ($F = 2.18$, $p > 0.05$) but shows a significant difference in shell shape ($F = 1.69$, $p = 0.0005$). However, when comparing limestone clusters (Padawan vs. Bau, $F = 14.64$, $p < 0.0001$ (size), $F = 2.35$, $p < 0.0001$ (shape). Padawan vs. Siburan, $F = 67.65$, $p < 0.0001$ (size), $F = 4.90$, $p < 0.0001$ (shape) (Appendix,Tables A1 and A2), they display significant different in shell size and shape. Consequently, there are significant differences in both shell size ($F = 46.07$, $p < 0.0001$) and shell shape ($F = 4.58$, $p < 0.0001$) when combined with all localities [Figures 4 (a) and (b)].

In Principal Component Analysis (PCA) based on landmark data for all combined localities (Figure 4), the first two principal components (PC1 and PC2) accounted for 37.22% of the total shape variation in *P. globosa*. PC1 described the highest amount of variation, 21.03% whereas PC2 is 16.19%, respectively.

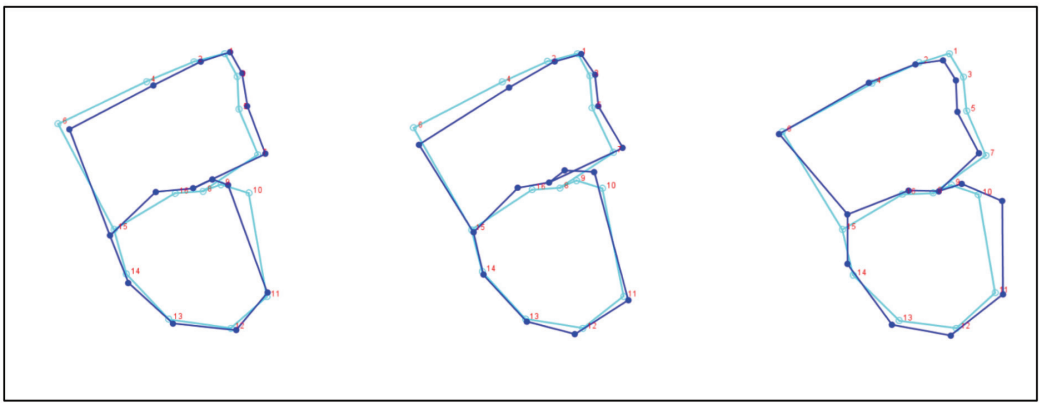


Figure 3: Shape changes along the PC1 based on landmark data for each locality: (a) Bau, (b) Padawan, and (c) Siburan

Since the present analysis intended to further classify specimen *P. globosa*, those landmarks with the highest PC coefficients were chosen. The length of the line shows the magnitude of the loading while its direction indicates the direction of the relationship between the variables and the PCA.

The transformation grid dimensions of PC1 [Figure 4 (b)] are used to visualise the shape variation during shape transformation. Thus, PC1 [Figure 4 (c)] was highly affected by the highest point of the lip (LM10) and the external point of the lip at the left profile of the shell (LM15). In contrast, PC2 [Figure 4 (d)] was mainly influenced by the most external point in the last whorl at the left profile of the shell (LM6) and the left profile of the lower suture (LM4). Thus, the highest point of the lip was pondered as the main parameter of PC1 and the most external point in the last whorl at the left profile of the shell for PC2.

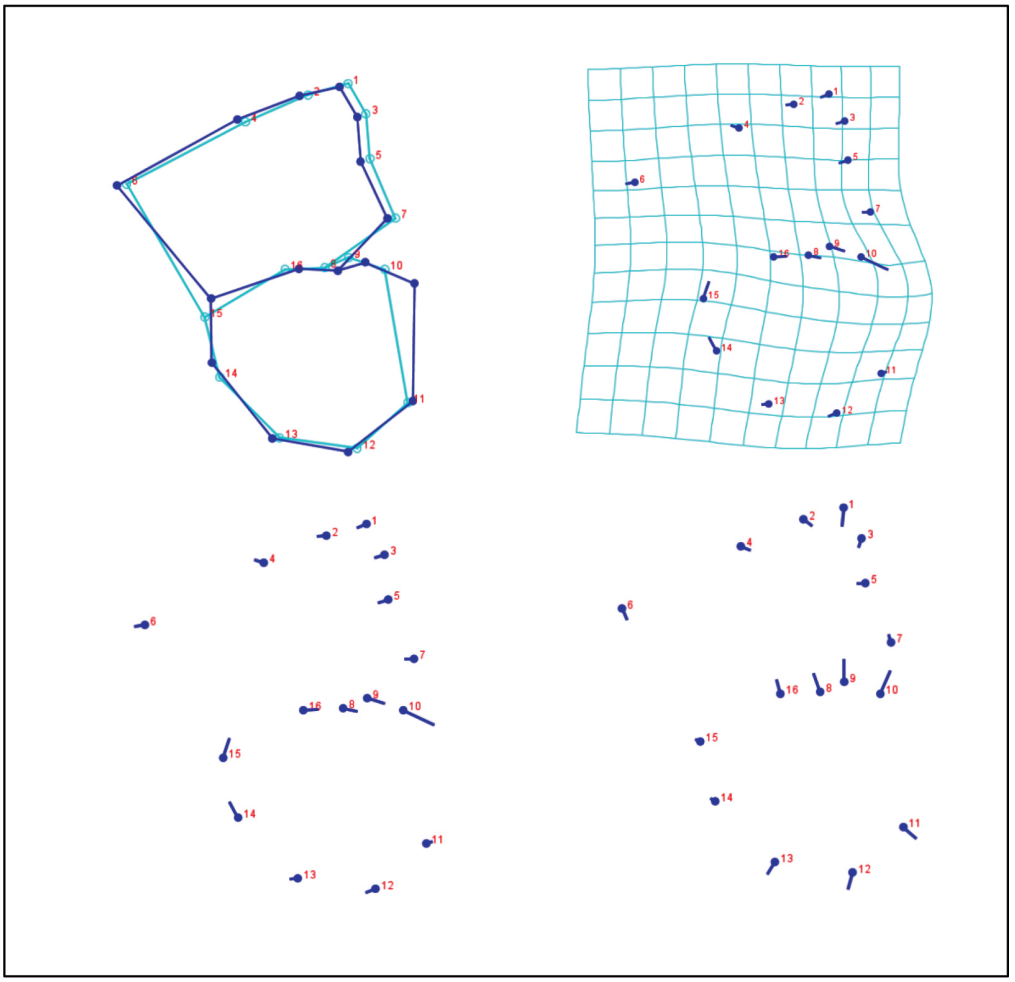


Figure 4: Shape changes of the *P. globosa* based on landmark data for all combined localities. (a) The mean shape is represented by the wireframe graph averaged across all individual shapes. (b) The transformation grid dimensions of PC1 show the number of grids used to construct the deformation grid. The lollipop graph is based on PCA, which illustrates the differences between (c) PC1 and (d) PC2, where they display the loadings of each variable

Shell Measurement Analysis

PCA on shell measurement shows 82.5% variation on the first principal axis (PC1) with inertia coefficients, indicating that populations with high factor scores on PC1 have positive values for all measurements. Meanwhile, PC2 (9%) with inertia coefficients revealed Shell Diameter (D), Shell Height (H), Apertural Width (AW), and Apertural Height (AH), indicating negative coefficients on the shell (Appendix, Table A3). This shape component describes the specimen that tends to have larger shell sizes. The arrows indicate the strength of the correlation where the variables are strongly correlated within the dataset (Figure 5), Analyses of Variance (ANOVA) for seven shell measurements was shown in Appendix, Table A4.

In addition, PCA shows that apertural width is the most contributing variable on both dimensions, indicating its significant impact across all components, which suggests it could be used as a proxy for overall shell size (Appendix, Table A5). Thus, we used apertural width as a covariate in the regression analysis of all remaining linear metrics of shell size. All six shell measurements showed different relationships with the apertural width when comparing the *p*-value for the difference in slope between the region and the entire dataset. Note that the combined data of the three regions is represented by the black regression line, showing that all regression slopes for the metrics were positive and significant, suggesting that apertural width strongly influences other shell characteristics (Figure 6).

When region effects were examined, specimen from limestone cluster Siburan showed the most statistical differences ($p < 0.05$), where the result demonstrates there are significant correlations in all the relationships with the apertural width except for the relationship between apertural width and spire width [Figure 6 (e)].

Population from Bau represents a significant correlation with the apertural width [Figures 6 (a), (c), and (f)] while not significantly correlated in [Figures 6 (b), (d), and (e)]. Despite variations in statistics, the species regression lines intersected at their slopes. Meanwhile, for the limestone cluster Padawan population, no significant correlation was observed between apertural width and all six measurements (Figure 6).

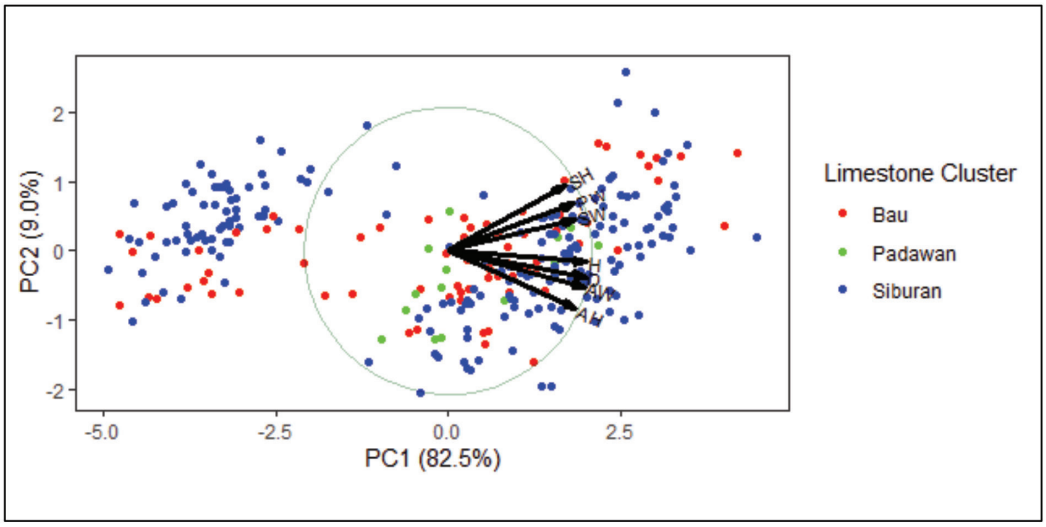
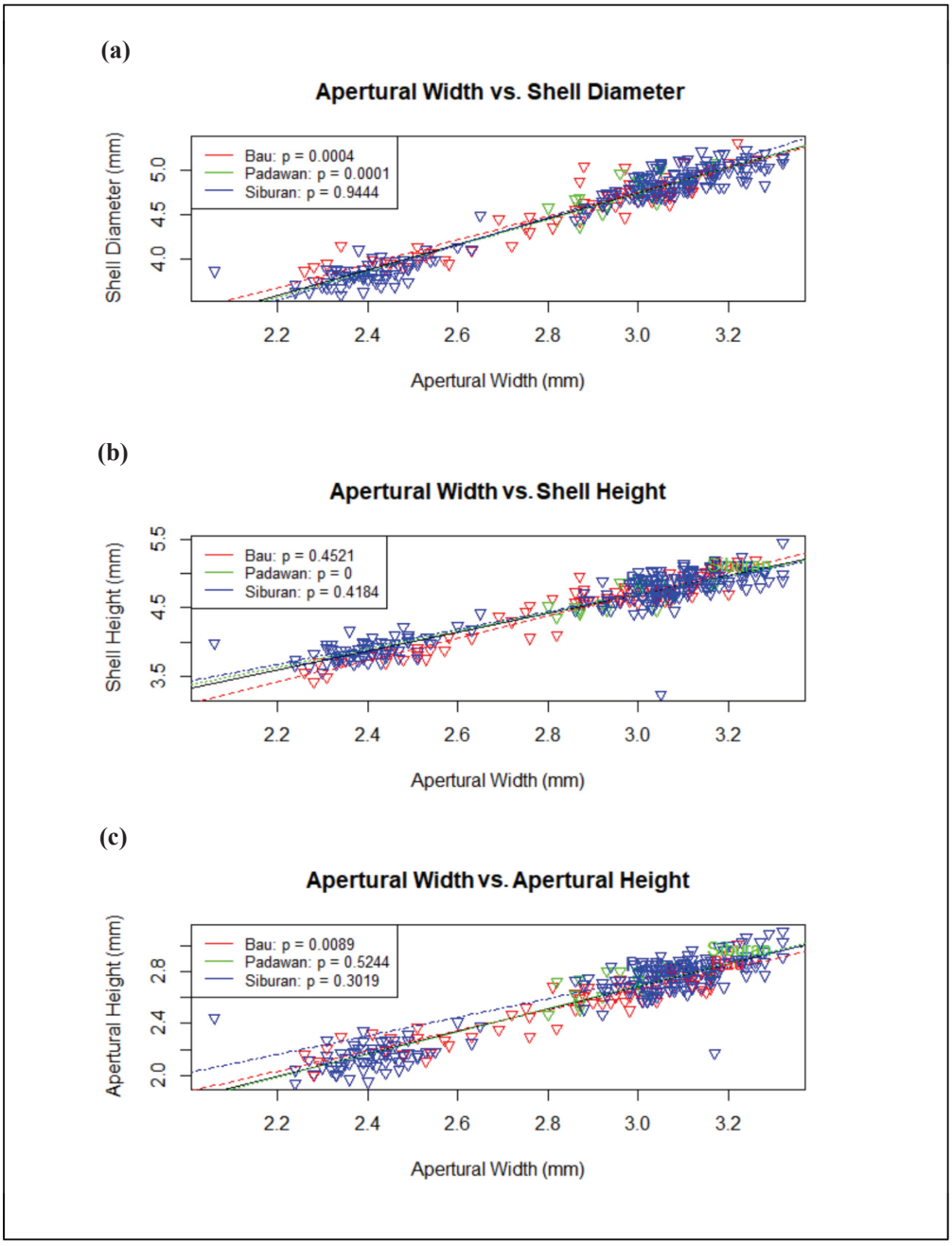


Figure 5: The PCA of shell metrics demonstrated that the first principal axis accounted for 82.5% of the variation in the first principal axis and 9% in the second principal axis

When we compared the *p*-values for differences in slopes between each pair of regions, we found that all six shell measurements had strong evidence that the relationships between the shell measurements and apertural width differ across all regions. The *p*-values obtained from these analyses are close to 0, indicating strong evidence that these variables' relationships (slopes) vary across regions. This finding could be important in understanding the impact of different factors on the connection between shell measurements and aperture width in various geographical or ecological regions.



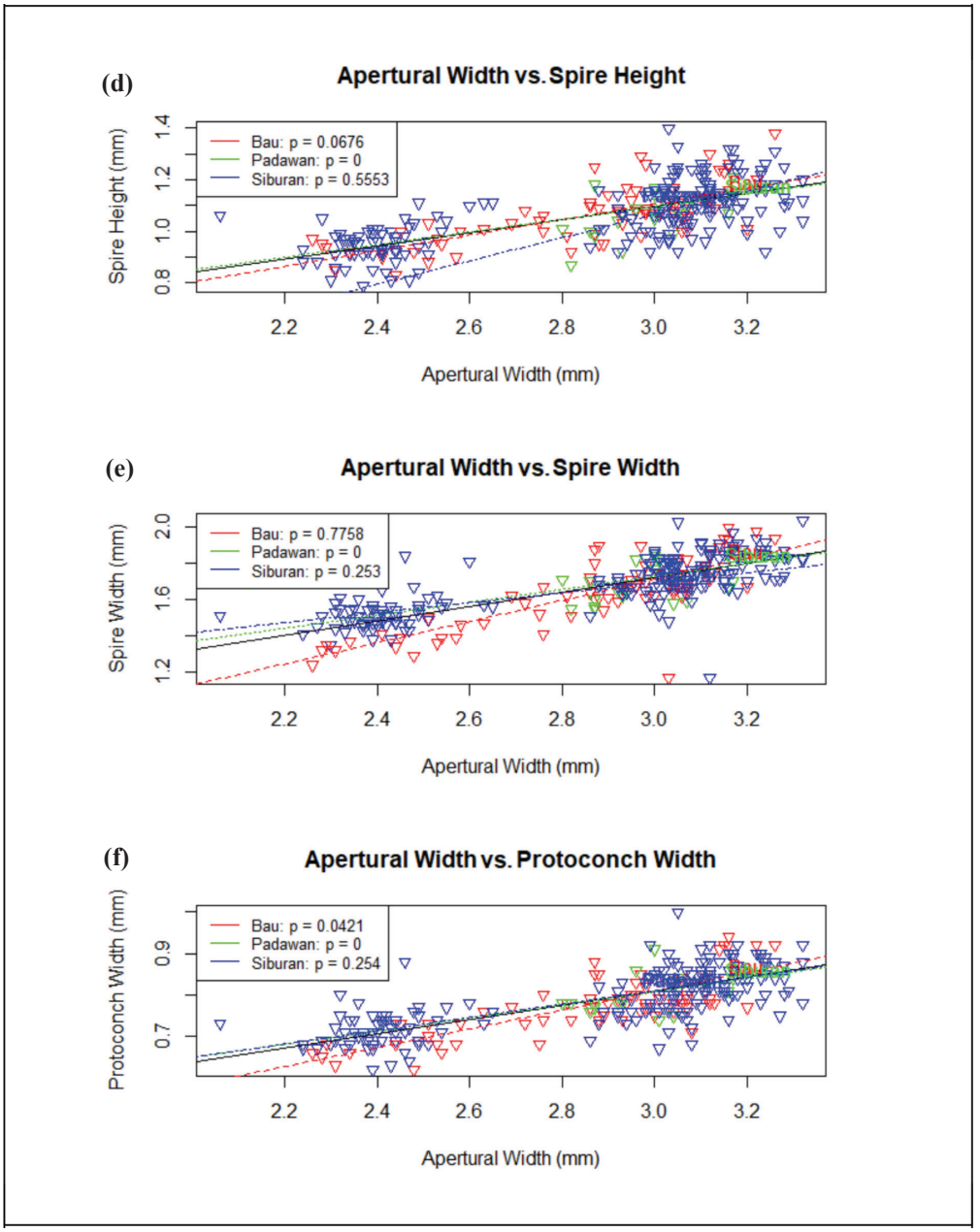


Figure 6: Regressions analyses of shell characters of *P. globosa* from different regions, with the dependent variable being apertural width. (a) Apertural Width vs. Shell Diameter, (b) Apertural Width vs. Shell Height, (c) Apertural Width vs. Apertural Height, (d) Apertural Width vs. Spire Height, (e) Apertural Width vs. Spire Width, and (f) Apertural Width vs. Protoconch Width

Discussion

Shell Shape Variation of Pincerna Globosa

The geometric morphometric analyses show variations among populations of *P. globosa*, with landmark analysis revealing significant variation across limestone clusters of Bau, Padawan, and Siburan (Figure 4). The obtained results demonstrate clear size and shape differences between shells, specifically from the limestone cluster Padawan, where they tend to have a taller aperture. In contrast, limestone clusters Siburan and Bau have rounded to wider apertural openings (Figure 3). The PCA analysis from landmark analysis also shows highly affected by the highest point of the lip (LM10) and the external point of the lip at the left profile of the shell (LM15) on PC1, which suggests a correlation with the width of the aperture. The aperture index is significant for size (Appendix, Table A6), which supports the importance of LM10 and LM15 for shape.

These differences are similar to a study by Mathieu *et al*. (2003), which examined different populations of *Cornu aspersum*, a type of land snail, and exhibited significant variation based on shell shape and size. Other than geographical patterns, environmental factors including temperature and rainfall may contribute to the populations varying in size and shell shape, which could be related to niche traits (Sherratt *et al*., 2017; Khalik *et al*., 2020). The weather report from the Malaysian Meteorological Department between 2017 and 2021 reported that Bau, Padawan, and Siburan show an average of 3,400 mm to 4,600 mm of rainfall and temperature ranging from 21°C to 37°C, which indicates a moist and humid environment during this period. This condition is common in tropical regions with high rainfall and humidity levels. This environment is well-suited for land snails since they tend to be in microhabitats with low humidity and/or high temperatures to maintain moisture levels (Moreno-Rueda *et al*., 2009).

The variations in shell size and shape among populations could also be due to different temperatures and ecological conditions (Anderson *et al*., 2007; Sherratt *et al*., 2017) and the light intensity that could affect the pigmentation of the shell (Khalik *et al*., 2020). Note that morphological variation for gastropod shells also may change from genetic differences as a product of population history (Dillon & Jacquemin, 2015; Dowle *et al*., 2015) and can be influenced by environmental factors (Welter-Schultes, 2000; Anderson *et al*., 2007; Mathieu & Bellido, 2007; Stankowski, 2011; Quenu *et al*., 2020). For instance, the New Zealand land snail *Placostylus ambagiosus* displays variation in size, which is influenced by changes in temperature and moist environment (Stringer *et al*., 2014).

The observed morphological differences among species populations are probably due to random ecological variations or differences in the environments in which these populations exist. Similarly, we suspected that variation in *P. globosa* in different populations of the studied population could be due to ecological variation or cryptic diversity of the genus *Pincerna*. The different limestone habitats are characterised by dense vegetation and limited sunlight exposure, which could create conditions that influence the shell morphology and ecological adaptation within this genus.

Shell Size Variation of Pincerna Globosa

The PCA result (Figure 5) based on the measurement of *P. globosa* shows that all the variables are positively correlated along the principal component, indicating there are larger shell sizes with globose shells and wide aperture. Globose shells have been interpreted as accommodating for climbing vertical vegetation (Heller, 1987) since they mostly can be found in vegetation areas. As a result, they tend to have large feet and large-mouthed shells. Figure 5 also illustrates that the individual from all limestone clusters display overlaps based on their shell measurement, which indicates that shell size alone may not be sufficient to differentiate shell variation among populations.

The specimens could have high or low values for certain shell metrics, which causes them to appear distant from others.

The pattern of shell size and shape within specific geographic regions exposes the correlation or similarities between the physical characteristics of the shells and geographic locations to identify the process that affects the shell morphology variation. For instance, Johnson and Black (1998) and Chiba (1999) demonstrate that environmental factors and geographic isolation can influence the morphological variation in land snails, which supports the idea that specific regions can affect shell characteristics. Our findings of the regression correlation between the apertural width with all six measurements were revealed, showing that the relationship between apertural width and shell size is consistent, with no significant differences among the population in the limestone cluster of Padawan. This consistency is maintained when compared to the entire dataset. However, there could be differences in how strong the specific characteristics of this correlation may vary when examining shell measurement in different geographical regions.

In addition, the morphological shell size parameters show that *P. globosa* in the limestone cluster in Padawan has a relatively larger shell size compared to the *P. globosa* in Bau and Siburan since the Padawan population is characterised by a larger mean shell size, indicating they possess larger shells (Appendix, Table A7). Additionally, snails in higher population densities may exhibit smaller adult sizes (Goodfriend, 1986; Baur, 1988). This effect is believed to be influenced by the impact of pheromones on their growth rates, which is also observed in *P. globosa.* The limestone cluster in Padawan has the least number of specimens compared to Bau and Siburan, which indicates that the higher the population value, the more likely the size of the individual will be reduced. This can be observed in the study conducted by Anderson *et al*. (2007), where the density also affects adult *Oreohelix cooperi* by measuring the number of shells determined in the study areas. Other factors such as humidity (Goodfriend, 1986; Arad *et al*., 1993), temperature (Albuquerque *et al*., 2009; Melatunan *et al*., 2013; Benbellil-Tafoughalt & Koene, 2015), and average rainfall (Hausdorf, 2006; Rangarajan *et al*., 2013) can also affect the shell size of *Pincerna*.

Conclusions

The current study has presented that the shell shape and size of *P. globosa* varies among different limestone clusters of Bau, Padawan, and Siburan. There is a significant difference between LM10, the highest point of the lip, and LM15, the external point of the lip at the left profile of the shell, among different limestone clusters. As for shell measurement, the most variable characters are the Aperture Height (AH) and Aperture Width (AW). Future research could incorporate molecular assessment of *P. globosa* of different populations to explain the possible morphological divergent and cryptic diversity.

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Conflict of Interest Statement

The authors agree that this research was conducted in the absence of any self-benefits or commercial or financial conflicts and declare the absence of conflicting interests with the funders.

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Appendix

Table A1: The centroid size of the shell based on their locality using Procrustes ANOVA ($p < 0.05$)

Regions	Effect	ss	ms	df	f	*p*
Bau	Individual	313387.851703	62677.570341	5	21.55	< 0.0001
	Residual	189050.679476	2908.471992	65		
Padawan	Individual	8395.257397	2798.419132	3	2.18	> 0.1331
	Residual	19273.297076	1284.886472	15		
Siburan	Individual	1162714.628605	193785.771434	6	106.94	< 0.0001
	Residual	344304.854143	1812.130811	190		
Bau-Padawan	Individual	343143.478220	38127.053136	1	14.64	< .0001
	Residual	208323.976552	2604.049707	88		

Bau-Siburan	Individual	1468783.054327	122398.587861	12	58.66	< .0001
	Residual	523692.830713	2086.425620	251		
Padawan-Siburan	Individual	1191135.235337	119113.523534	10	67.65	< .0001
	Residual	353915.448313	1760.773375	201		
Overall	Individual	1504625.680248	94039.105016	16	46.07	< .0001
	Residual	542966.127789	2041.226044	266		

Table A2: The shell shape of the shell based on their locality using Procrustes ANOVA ($p < 0.05$)

Regions	**Effect**	**ss**	**ms**	**df**	**f**	***p***
Bau	Individual	0.03027214	0.0002162295	140	2.63	< 0.0001
	Residual	0.14947619	0.0000821298	1820		
Padawan	Individual	0.00940204	0.0001119291	84	1.69	0.0005
	Residual	0.02781830	0.0000662341	420		
Siburan	Individual	0.08542418	0.0005084773	168	6.40	< 0.0001
	Residual	0.42268266	0.0000794516	5320		
Bau-Padawan	Individual	0.04684361	0.0001858873	252	2.35	< 0.0001
	Residual	0.17727419	0.0000791403	2240		
Bau-Siburan	Individual	0.14262045	0.0004244656	336	5.30	< 0.0001
	Residual	0.56236520	0.0000800178	7028		
Padawan-Siburan	Individual	0.10742283	0.0003836530	280	4.90	< 0.0001
	Residual	0.44073693	0.0000783115	5628		
Overall	Individual	0.16261256	0.0003629745	448	4.58	< 0.0001
	Residual	0.59016548	0.0000792381	7448		

Table A3: Relative contribution of each variable to the inertia of the first two PCA components

Variables	**PC1**	**PC2**
Shell Diameter (D)	0.3996	-0.2329
Shell Height (H)	0.3997	-0.1014
Apertural Width (AW)	0.3957	-0.3149
Apertural Height (AH)	0.3713	-0.5108
Width of Spire (SH)	0.3404	0.5739
Height of Spire (SW)	0.3688	0.2752
Width of Protoconch (PW)	0.3663	0.4124

Table A4: Analyses of Variance (ANOVA) for seven shell measurements

Measurement		df	Sum Sq.	Mean Sq.	*F* value	Pr (>F)
Shell Diameter (D)	Region	2	0.71	0.3535	1.568	0.21
	Residuals	280	63.11	0.2254		
Shell Height (H)	Region	2	0.42	0.2125	0.956	0.386
	Residuals	280	62.25	0.2223		
Apertural Width (AW)	Region	2	1.99	0.09927	1.011	0.365
	Residuals	280	27.487	0.09817		
Apertural Height (AH)	Region	2	0.397	0.19851	2.365	0.0958
	Residuals	280	23.504	0.08394		
Width of Spire (SW)	Region	2	0.005	0.2125	0.956	0.386
	Residuals	280	3.798	0.2223		
Height of Spire (SH)	Region	2	0.102	0.05096	1.924	0.148
	Residuals	280	7.416	0.02648		

Table A5: "cos2" values for each variable across the first two PCA

Variables	Dim 1	Dim 2	Total
D	0.9225049	0.034200280	0.95670518
H	0.9225622	0.006490201	0.92905240
AW	0.9045218	0.062543001	0.96706480
AH	0.7961837	0.164568615	0.96075231
SH	0.6694439	0.207694149	0.87713805
SW	0.7856048	0.047768293	0.83337309
PW	0.7747997	0.107221487	0.88202119

Table A6: t-test: Paired two sample for means

	Variable 1	Variable 2
Mean	4.559505	4.51894
Variance	0.226291	0.22224
Observations	283	283
Pearson correlation	0.918125	
Hypothesised mean difference	0	
df	282	
t Stat	3.560233	
P(T < = t) one-tail	0.000217	
t Critical one-tail	1.650275	
P(T < = t) two-tail	0.000435	
t Critical two-tail	1.968412	

Table A7: Range and mean for a sample group of *P. globosa*. Measurements of the shell of *P. globosa*: Shell Diameter (D), Shell Height (H), Apertural Width (AW), Apertural Height (AH), Height of Spire (SH), Width of Spire (SW), and Width of Protoconch (PW)

Limestone cluster	Number of Locality (N)	D	H	AW	AH	SH	SW	PW
Bau	71	5.32 - 3.90	5.2 - 3.7	3.22 - 2.26	2.93 - 2.00	1.38 - 0.83	2.00 - 1.17	0.94 - 0.96
Mean		4.571	4.493	2.868	2.556	1.067	1.634	0.778
Standard deviation		0.383	0.448	0.263	0.223	0.112	0.191	0.075
Standard error		0.045	0.053	0.031	0.026	0.013	0.023	0.009
Padawan	19	5.09 - 4.36	4.89 - 4.36	3.05 - 2.87	2.84 - 2.47	1.21 - 0.87	1.84 - 1.59	0.91 - 0.74
Mean		4.737	4.659	2.968	2.711	1.051	1.682	0.803
Standard deviation		0.208	0.202	0.105	0.110	0.086	0.099	0.043
Standard error		0.047	0.046	0.024	0.025	0.019	0.023	0.010
Siburan	193	5.24 - 3.62	5.19 - 3.63	3.18 - 2.31	3.09 - 1.94	1.33 - 0.79	2.04 - 1.38	1.00 - 0.63
Mean		4.537	4.514	2.861	2.564	1.060	1.678	0.786
Standard deviation		0.520	0.496	0.341	0.321	0.120	0.156	0.072
Standard error		0.037	0.035	0.024	0.023	0.009	0.011	0.005