



Faculty of Resource Science and Technology

**Distribution and Morphological Assessment of the Genera *Pincerna* and
Stomacosmethis (Gastropoda, Alycaeidae) from Selected Areas in
Sarawak**

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Distribution and Morphological Assessment of the Genera *Pincerna* and
Stomacosmethis (Gastropoda, Alycaeidae) from Selected Areas in Sarawak

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.



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ABSTRACT

The genera of *Pincerna* and *Stomacosphthis* are a widely distributed genera in Sarawak. The shell variations within these two genera can be observed in certain morphological characters which required comprehensive study. Therefore, this research aimed to determine the extent of morphological variation of *Pincerna* and *Stomacosphthis* based on their shell characters. In this study, only one selected *Pincerna* species (*P. globosa*) and three species of *Stomacosphthis* (*S. hosei*, *S. sadongensis* and *S. galbanus*) can be found in Sarawak. A total of 283 adults *P. globosa* and 355 adults *Stomacosphthis* (*S. hosei* (280), *S. sadongensis* (42) and *S. galbanus* (33)) collected at limestone cluster in Bau, Padawan, Siburan, Niah and Miri. The collected specimens were deposited into Zoological Museum UNIMAS, Universiti Malaysia Sarawak. For shell shape analysis, geometric morphometric data was generated using landmarks with software tpsDig2 v. 1.1. For the shell size analysis, each shell was measured under stereo-microscope and colour images were captured using software *Toupview*. Seven measurements made were used to quantify shell size and shape: 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) with scale 1 mm as guidance. Based on landmark analysis, there is a significant variation of shell shape of *P. globosa* ($p < 0.0001$) with respected to LM10, the highest point of the lip and LM15, the external point of the lip at the left profile of the shell. Principal component analysis based on geometric morphometrics show a total variation of 37.18% by the first two principal components. Furthermore, the populations from Padawan cluster shows the largest in terms of shell size when compared with populations from Bau and Siburan. While, the genus *S. hosei* ($p < 0.0001$) shows the highest significant difference of LM13, the median lip point and the highest point of the lip (LM12). Principal component analysis based on geometric morphometrics show a

total variation of 43.78% by the first two principal components. This study also discovered *S. hosei* from Bau cluster has the tallest shells. As a conclusion, there are significant variations of shell shape and size of *Pincerna* and *Stomacosphthis* from Sarawak. From this study, it shows that these species offer valuable ecological insights as they can adapt to diverse environment condition, serve as bioindicators and species identification which highlighting their importance in both ecological and conservation. These finding highlight the potential of these land snails to offer valuable ecological insights as they can adapt to diverse environment condition, serve as bioindicators and assist in species identification underscore their importance in both ecological and conservation efforts.

Keywords: *Pincerna globosa*, *Stomacosphthis*, morphological variation, geometric morphometric, Principal component analysis (PCA)

Taburan dan Penilaian Morfologi Genera Pincerna dan Stomacosphthis (Gastropoda, Alycaeidae) dari Kawasan Terpilih di Sarawak.

ABSTRAK

Genera Pincerna dan Stomacosphthis merupakan genus yang tersebar luas di Sarawak. Variasi cangkerang dalam kedua-dua genus ini boleh diperhatikan melalui beberapa ciri morfologi yang memerlukan kajian menyeluruh. Oleh itu, kajian ini dijalankan bagi menentukan tahap variasi morfologi genus Pincerna dan Stomacosphthis berdasarkan ciri-ciri cangkerangnya. Dalam kajian ini, hanya satu spesies Pincerna yang dipilih iaitu P. globosa dan tiga spesies Stomacosphthis iaitu S. hosei, S. sadongensis, dan S. galbanus yang ditemui di Sarawak. Sebanyak 283 individu dewasa P. globosa dan 355 individu dewasa Stomacosphthis (S. hosei (280), S. sadongensis (42), dan S. galbanus (33)) telah dikutip di gugusan batu kapur di Bau, Padawan, Siburan, Niah dan Miri. Spesimen yang telah dikutip telah disimpan di Muzium Zoologi UNIMAS, Universiti Malaysia Sarawak. Untuk analisis bentuk cangkerang, data morfometrik geometri telah dijana menggunakan titik penanda (landmark) dengan perisian tpsDig2 v.1.1. Bagi analisis saiz cangkerang, setiap cangkerang diukur di bawah stereomikroskop dan imej berwarna dirakam menggunakan perisian Toupview. Sebanyak tujuh ukuran telah digunakan bagi mengira saiz dan bentuk cangkerang: diameter cangkerang (D), tinggi cangkerang (H), lebar bukaan (AW), tinggi bukaan (AH), tinggi spira (SH), lebar spira (SW), dan lebar protokon (PW) dengan skala 1 mm sebagai panduan. Berdasarkan analisis titik penanda, terdapat variasi bentuk cangkerang P. globosa yang signifikan ($p < 0.0001$), terutamanya pada LM10 iaitu titik tertinggi bibir bukaan dan LM15 iaitu titik luar pada profil kiri cangkerang. Analisis komponen utama (PCA) berdasarkan morfometrik geometri menunjukkan jumlah variasi sebanyak 37.18% pada dua komponen utama pertama. Tambahan pula, populasi dari gugusan Padawan

menunjukkan saiz cangkerang yang paling besar berbanding populasi dari Bau dan Siburan. Manakala, genus *S. hosei* ($p < 0.0001$) menunjukkan perbezaan signifikan yang paling tinggi pada LM13 (titik tengah bibir bukaan) dan LM12 (titik tertinggi bibir). Analisis PCA menunjukkan jumlah variasi sebanyak 43.76% pada dua komponen utama pertama. Kajian ini juga mendapati bahawa *S. hosei* dari gugusan Bau mempunyai cangkerang yang paling tinggi. Kesimpulannya, terdapat variasi bentuk dan saiz cangkerang yang signifikan bagi genus *Pincerna* dan *Stomacosmethis* dari Sarawak. Kajian ini menunjukkan bahawa spesies ini menawarkan maklumat ekologi yang bernilai kerana keupayaannya untuk menyesuaikan diri dalam pelbagai keadaan persekitaran, berfungsi sebagai bioindikator, dan membantu dalam pengecaman spesies. Penemuan ini menekankan kepentingan mereka dalam aspek ekologi dan pemuliharaan.

Kata kunci: *Pincerna globosa*, *Stomacosmethis*, *variasi morfologi*, *morfometrik geometri*, *Analisis komponen utama (PCA)*

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AH	Aperture height
AW	Aperture width
D	Shell diameter
et al.	And others
GM	Geometric morphometric
H	Shell height
LM	Landmarks
NP	National Park
PCA	Principal component analysis
PW	Protoconch width
SH	Height of spire
SW	Width of spire
TPS	Thin-plate splines

CHAPTER 1

INTRODUCTION

1.1 Study Background

Morphological variations in a widely distributed taxa may indicate that the organism experience different ecological pressures, which mainly caused by various environmental stress, including different habitat type, soil mineralogy, geographical differences and elevation (Goodfriend, 1986; Teusch et al., 2002; Chiba et al., 2009; Martínez- Freiría et al., 2009; Fruciano et al., 2011; Kramarenko, 2016). Morphological studies using the analysis of quantitative data of measurable traits are useful to explore the patterns in size and shape changes (Hespenheide, 1973; Klingenberg, 2010). Moreover, to understand the processes influencing morphological variation, including factors such as ecology and/or behaviour (Tellería et al., 2013).

The gastropod is an ideal model for studying morphological variation and diversification because of their high in abundance and quantifiable morphological traits (Davison 2002; Giokas et al., 2014). For instance, the body size of the great pond snail, *Lymnaea stagnalis* (Lymnaeidae) decreases with the increment of longitude from south to north, even though they share similar ecological conditions (Vinarski, 2014). Additionally, the shell size may vary in response to a complex combination of environmental factors such as latitude and climate such as *Albinaria* which tends to have smaller shell size in higher latitude and have larger area/volume during raining season (Goodfriend, 1986; Giokas et al., 2014), predation (Chiba, 2007; Parent & Crespi, 2009) and ecotype (Chiba, 2004). Differences in ecology and climates may affect the development of lineages which can help to 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 as a tool for understanding processes of adaptation under distinct environmental conditions (Cowie, 1995; Sokolova & Berger, 2000; Miura et al., 2007; Silva et al., 2013).

The family Alycaeidae Blandford 1864, is an interesting family for studying the morphological variation because many species have a wide distribution range especially in Sarawak. The genera *Pincerna* and *Stomacosmethis* which belongs to this family are widely distributed from the Western Ghats (India) to Japan in the east, Gansu and Shaanxi provinces in the north of China, and in the south of Indonesia (Páll-Gergely et al., 2020). The family Alycaeidae is defined by a sutural tube connected to multiple perpendicular, tiny microtunnels generated by the outermost shell layer (Páll-Gergely & Auffenberg, 2019). Alycaeidae have 367 valid species and subspecies currently classified in several genera which includes *Cyclorhynchus*, *Pincerna*, *Alycaeus*, *Dicharax*, *Metalycaeus* and *Stomacosmethis* (Páll-Gergely et al., 2020).

In the current study, the genus *Pincerna* was selected (represented by sole species *Pincerna globosa*) and *Stomacosmethis* (represented by *Stomacosmethis hosei*) as our studied models, two widely distributed taxa in the western regions of Sarawak. The genus *Pincerna* Preston, 1907 is an Asian land snail genus (Sang & Son, 2022) and possesses an operculum with an external "circular cup" and can be distinguished by its globular, thinly ribbed, and white to orange-coloured shell (Páll-Gergely et al., 2020; Marzuki et al., 2021). This species can be found in Kuching, Serian, Kapit and Miri, while in Sabah can be discovered in West Coast Division (Marzuki et al., 2021). This species is endemic to Borneo (Marzuki et al., 2021), means its spreading is limited in the regions which make it more vulnerable to change its environment compared to species with wider ranges. *Pincerna* is probably most closely related to *Stomacosmethis* in term of morphology, which is similar in shell size and

shape (Páll-Gergely et al., 2020). In contrast, the shells of *Stomacosmethis* Bollinger, 1918 species are considered as triangular with densely ribbed, usually brightly coloured yellow or orange, thin operculum with outer surface usually elevated (Foon & Liew, 2017; Páll-Gergely et al., 2020).

In this study, the morphological variation was identified in these two genera, *Pincerna* and *Stomacomethis*, populations collected from selected areas in Sarawak. Therefore, analysing shell shape and size variation using a geometric morphometric method is a useful tool for understanding species geometric morphometrics. This method 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 size, location, rotation, scale, and experimental bias, which are frequently seen in standard morphometric analyses (Rohlf & Marcus, 1993; Schilthuizen & Haase, 2010). In addition, analysing shell shape and size variation using morphological measurements and a geometric morphometric method are often used to compare the shell morphology of different populations. The landmark method is useful to measure the degree of deformations of morphometric points in coordinate space that focuses on size and shape.

Pincerna and *Stomacomethis* can be identified based on the size and shape, and character of their shells, which allows for detailed comparison of their morphology. Therefore, the current study is conducted to compare the variation of the morphological character (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)) of the species *Pincerna* and *Stomacomethis* from different population in selected areas in Sarawak (Chiba, 2004; Vasconcelos et al., 2021). In addition, this study aims to determine the relation of ecological factors, morphological features and distribution of the species *Pincerna* and *Stomacomethis*.

1.2 Problem Statement

There are approximately 1,000 recognised land snail species in Malaysia (Liew et al., 2021). Extensive variation in land snail shell morphology has been widely documented, although few studies have attempted to investigate the ecological and evolutionary drivers of the variation (Kotsakiozi et al., 2013). But these studies generally apply to land snail, not specifically on the genera of *Pincerna* and *Stomacomethis*. Therefore, it remains unclear if the shell and shape variation among different populations of the genera *Pincerna* as well as *Stomacosmethis* does exist. The genera *Pincerna* and *Stomacosmethis* were selected for this study due to their overlapping distribution in the selected areas in Sarawak as they are mainly can be spotted in limestone habitat such as Bau, Padawan and Siburan (Páll-Gergely et al., 2020; Marzuki et al., 2021). Despite coexisting in similar ecological habitats, these genera exhibit distinct shell morphologies, particularly in shape and size (Páll-Gergely et al., 2020). To date, there is no study has been conducted to examine the morphological variation of *Pincerna* and *Stomacomethis* although they show some variation based on shell shape and size. Hence, this study is conducted to examine the morphological variation of these two genera based on their shell shape and size. The lack of proper documentation of comparative morphology could also prove to be a limiting factor in further studies and the identification process of this genus.

1.3 Objectives

The morphology of a species can be distinguished by its distinctive characteristics which might be influenced by the ecological factors. *Pincerna* and *Stomacosmethis* can be identified based on the measurement and shape of their shells, which allows for comparison of their morphology. Therefore, the current study is embarked to achieved the following objectives:

- i. To determine the distribution of the genera *Pincerna* and *Stomacosmethis* in Sarawak.
- ii. To analyse the morphological variations of shell shape and size of the genera *Pincerna* and *Stomacosmethis* from different population in Sarawak.

1.4 Hypothesis statements

Land snails show morphological variation based on shell shape and size across different populations. However, it still remains unclear if the shell and shape variation among different population of the genera *Pincerna* and *Stomacosmethis*.

- i. Hypothesis 1 (H1): The distribution of the genera *Pincerna* and *Stomacosmethis* in selected areas in Sarawak is environmentally influenced with the specific environment such as limestone habitat and vegetation areas.
- ii. Hypothesis 2 (H2): There are morphological differences in shell shape and size among different populations of the genera *Pincerna* and *Stomacosmethis* in selected areas in Sarawak.

1.5 Chapter summary

This thesis is about the study of morphological variation of land snails due their diversity in ecology and morphology in variation of shell traits. Morphological studies using the analysis of qualitative and quantitative data of measurable traits are useful to explore the patterns in size and shape changes (Hespenheide, 1973; Klingenberg, 2010). In this study, the genus *Pincerna* (represented by sole species *Pincerna globosa*) and *Stomacosmethis* (represented by *Stomacosmethis hosei*) were selected as studied sample, a widely distributed taxa in the western regions of Sarawak. However, there is no study conducted to examine the morphological variation of *Pincerna* and *Stomacomethis* although they show some

variation based on shell shape and size. Therefore, this study will conduct to determine the distribution of the genera *Pincerna* and *Stomacosphthis* in Sarawak and examine the morphological variation of these two genera based on their shell shape and size.

In literature review, the existing literature will be review focusing on the family of the genera *Pincerna* and *Stomacosphthis*, Alycaeidae, with their distribution and the factors that influence the morphological variance. *Pincerna globosa* is the only known *Pincerna* in Sarawak that can be found in Kuching, Serian, Kapit and Miri, while in Sabah can be discovered in West Coast Division. Whereas, *Stomacosphthis* represented by species *S. hosei*, *S. galbanus* and *S. sadongensis* are known to be found in Kuching, Sadong and Serian divisions of Sarawak and is endemic to Borneo.

Data analysis is divided into two categories which are shell shape analysis and shell size analysis. For the purpose of analysing the morphological variation of *P. globosa* and *S. hosei*, a geometric morphometrics (GM) was employed using MorphoJ with the aim to study the shape using landmark-based method. Shape data corrected for size were analysed with Principal component analysis (PCA) to explore the morphological variation. Analysis (PCA) on collected measurement data to explore the morphological variation among *P. globosa* and *S. hosei*. Finally, regression analysis conducted to assess variances in morphological characters between regions.

CHAPTER 2

LITERATURE REVIEW

2.1 The Family Alycaeidae Blanford, 1864

The family Alycaeidae is a group of family that can be distinguished by their globular, conical shells and an exposed tube at the suture (known as breathing tube) which runs from the constriction to whorls prior to the constriction (Foon & Liew, 2017; Páll-Gergely et al., 2020). There are approximately of 363 taxa which covers 320 species or 43 subspecies within this family (Páll-Gergely et al., 2020). In Asia there are around 360 Asian species have been described and classified into 14 genera (Páll-Gergely et al., 2020). Asian Alycaeidae are extensively spread from southern India to Japan which also cover northern Vietnam, the Malay Peninsula, the eastern Himalaya region and southern Japan (Aravind & Páll-Gergely, 2018).

2.1.1 The genus *Pincerna* Preston, 1907

The genus *Pincerna* is an Asian land snail which in the superfamily of Cyclophoridae Gray, 1847 (Sang & Son, 2022). The genus *Pincerna* are widely distributed in Southeast Asia and only one species of *Pincerna* can be spotted in Sarawak. For instance, *P. globosa* (H. Adams, 1870) is the only known *Pincerna* in Sarawak that widely distributed in Kuching, Serian, Kapit and Miri, while in Sabah can be discovered in West Coast Division (Marzuki et al., 2021). This species is endemic to Borneo, means its spreading is limited in the regions which make it more vulnerable to change its environment compared to species with wider ranges. This species can be identified by possesses an operculum with an external "circular cup" and can be distinguished by its globular, thinly ribbed, and white to orange-coloured

shell (Páll-Gergely et al., 2020; Marzuki et al., 2021). The species *P. globosa* can be found on the leaves of small trees and palms at the base of the limestone cliff (Marzuki et al., 2021).

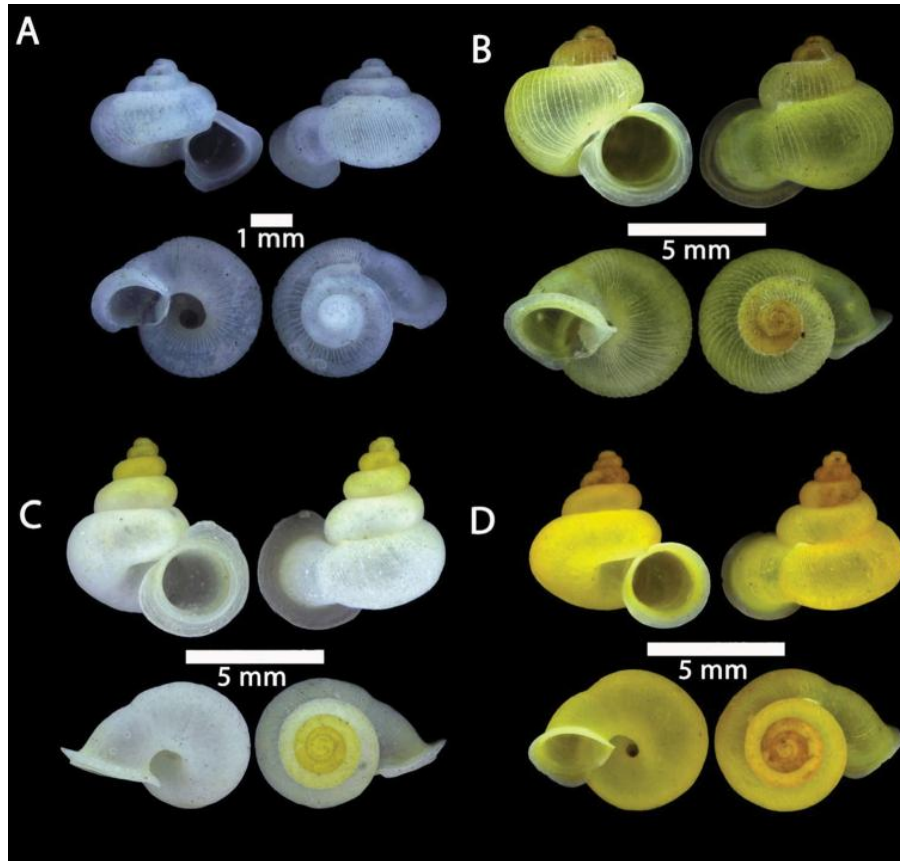


Figure 2.1: Shell morphology of A *Camalycaeus specus* B *Pincerna globosa* C *Stomacsmethis hosei* D *Stomacsmethis sadongensis* (Source: Marzuki et al., 2021)

2.1.2 The genus *Stomacsmethis* Bollinger, 1918

Stomacsmethis was defined on the basis of a pipe, tongue or cup-shaped structure on the outer side of the operculum and included two species from southern Celebes and eastern Borneo (Páll-Gergely et al., 2020). *S. hosei* (Godwin-Austen, 1889) can be identified with its protoconch has no spiral lines, weak ribs and some indication of weak spiral striae very short, with darker wider and light slimmer stripes (Páll-Gergely et al., 2020). They can be found with it shell covered with calcareous dirt and usually foraging on the wet limestone

rock surfaces that covered with lichens. While *S. sadongensis* (E. A. Smith, 1895), this species differs from other Bornean species of *Stomacosmethis* by having a shell with dense, regular, rather low riblets on the shell instead of irregular low riblets (Marzuki et al., 2021). They were observed foraging also on wet vertical limestone rock surfaces covered with mosses and lichens. *S. galbanus* (Godwin-Austen, 1889) is the only *Stomacosmethis* that only can be found at Niah hills (Páll-Gergely et al., 2020). They can be identified with their protoconch without spiral lines, matte, with irregular, rather strong ribs and very weak spiral lines, very short, with alternating darker/wider and lighter/narrower stripes, the overall surface is seemingly irregularly wrinkled (Páll-Gergely et al., 2020).

2.2 Shell variation in shape and size of land snail

Generally, the variation in shell shape and size of land snail influence by the difference environmental characteristics. For instance, larger snails tend to be found in more humid environment while smaller shells can be spotted in population with higher density (Goodfriend, 1986). These patterns suggest that different shell morphology may affected by differences adaptive responses to ecological pressure, making it a one of the traits for determine the environmental influences on species distribution and shell structures (Ashcroft et al., 2010; Proćków et al., 2018).

2.2.1 Shell variation of *Pincerna* and *Stomacosmethis*

Pincerna is known as an Asian land snail genus, which is classified in the family Alycaeidae (Sang & Son, 2022). The genus *Pincerna* have different shell shape and size. A new species has been discovered by Páll-Gergely (2017), *P. yanseni* that have founded in Medan, Indonesia. *P. constricta* is comparable in size and shape to *P. yanseni*, but has a more elevated spire, stronger and more widely spaced ribs, and weaker spiral striation (Páll-Gergely, 2017). In addition, another five species of *Pincerna* were spotted in Vietnam and

Loas which are *P. anceyi*, *P. contulosus*, *P. mouhoti*, *P. vanbuensis* and new identified species, *P. clausa*. *P. clausa* is morphologically closely to *P. vanbuensis* but have different umbilicus and have stronger spiral striations on the protoconch (Sang & Son, 2022).



Figure 2.2: Representative shell of *Pincerna*, A *P. constricta* B *P. yanseni*, showing the characteristic morphology of the genus. Scale = 1 mm (Source: Páll-Gergely, 2017)

Another new species of *Pincerna* was discovered in western Hubei, China, *P. vallis* and the Vietnamese species of *P. costulosa* is also reported in Yunan, China (Chen & Wu, 2020). Additionally, *P. yaanensis* from Yaan City, Sichuan Province, China (Chen, 2022). Consequently, this proves that the genus *Pincerna* show different shell shape and size to support adapt in different habitat conditions. Geographic distribution from this genus also highlights the ecological diversity and their adaptability, as they can be found in variety habitats across different regions.

On the other hand, *Stomacosmethis* is known for its triangular shells with a very short breathing tube (Páll-Gergely et al., 2020). In Sarawak, there are two *Stomacosmethis* that was identified by Marzuki et al. (2021), which are *S. hosei* and *S. sadongensis*. These two species labelled as endemic to Borneo and widely distributed in Sarawak specifically for species, *S. hosei*. Additionally, Páll-Gergely et al. (2020) have explained in more detailed about *Stomacosmethis* and *Pincerna*. For instance, *A. balingensis*, a species that was classified under genus *Pincerna* for its rounded shell is now reclassified as *Stomacosmethis*. This shows that these two genera are not a mutually monophyletic where they may share overlapping evolutionary histories (Páll-Gergely et al., 2020). Although *Stomacosmethis* and *Pincerna* are widely distributed in Southeast Asia, a detailed studies focusing on their morphological variation still remain limited.



Figure 2.3: Shell morphology of *Alycaeus balingensis*, A–E Shell of syntype, NHMUK 1948.10.2.4 F–J Shell of BOR/MOL 8357 K–M Close up of shell of BOR/MOL 8357 N–Q Operculum of BOR/MOL 8357 R–V Shell of BOR/MOL 8361. Scale bars: A–J, R–V, K, L 1 mm; M, N–Q 0.5 mm.

Photographs A–E by NHM staff (copyright of *NHM*, reproduced with permission). Photographs F–V by Junn Kitt Foon. (Source: (Foon & Liew, 2017).

2.2.2 The factors influencing shell variations

There are several factors that contribute to the variation in shell shape and size in land snail. These factors can be divided into two which are biotic and abiotic factors. Biotic factors refer to the influences of living organism such as predation, competition and availability of resources which directly affect the abundance, morphology and life history of land snails (Anderson et al., 2007; Wehner et al., 2019). On the other hands, abiotic factors refer to environment or non-living characters such as the availability of calcium, pH, temperature and humidity which also can influence the distribution and habitat preferences and the shell formation of land snails (Gärdenfors et al, 1995; Olkeba et al., 2020). Altogether, these two factors' interactions can influence the shell morphology of land snails in term of shape and size.

2.2.2.1 Biotic factors

The measurable shell traits of land snails focus on shell size measurements which also correlated with the environment factors such as resource abundance and competition. These factors play crucial role in shaping the shell shape of snails to adapt in their habitats. For example, *Chondrina clienta*, shows variation on shell height in different population where they found out that the shell height tends to be higher in vegetation areas (Baur, 1988). In contrast, the shell height is lower when the other land snail, *Balea perversa* coexist in the same population since they compete for resources. This highlights two type of competition affecting shell height variation which are interspecific competition between different species (*C. clienta* and *B. perversa*) and intraspecific competition within the same species. Similarly,

intraspecific competition can be observed in *Cepaea nemoralis* at Druridge Bay where they show negative correlation between shell size and population density which means the shell size tends to decrease (Perry & Arthur, 1991). This might be due to competition for resource, prey and predator or stress factors due to highest density in the single species colony.

Other than competition, the availability of resources (calcium) also plays crucial roles in distribution and abundance of land snails. For instance, the amount of calcium in soil from two different sites significantly influenced the abundance of land snails. Study done by Juříčková et al. (2007), shows that Tufa site, where the soil is high in calcium strongly controlled species richness and abundance. However, at the Boulder site, where calcium comes from vegetation, only species composition is affected by calcium availability. This suggest that the availability of calcium is the main resources for the land snails which give impact on their ecology, distribution and shaping species composition (Jubb et al., 2006; Juříčková et al., 2007; Charrier et al., 2013; Rygało-Galewska et al., 2023). The more the calcium they consumed, the more their body weight and shell length increase while the snails that lack calcium tend to have high mortality rates and unable to produce eggs (Egonmwan, 2008).

2.2.2.2 Abiotic factors

The descriptive characteristic of land snails focuses on the features or characteristics that provide information of their shell morphology, behaviour and habitat preferences. Shell morphology (size and shape) is important indicator to show how they interact with the environment. For example, the shell structure of land pulmonated snail varies in several ways and the variations are crucial for classification and differentiate between species (Suvorov, 2002). In this paper, it explained the range shell size can be small to large which can be differ based on several factors such as the diameter of umbilicus, number of coils and height-width

ratio. The gastropod *Bostryx torally* from different localities also shows the same aspects where it shows variation on their shell shape and colouration (Miranda, 2020). The findings found significant differences in shell shape even from small geographic area.

Shell shape of land snail can also be different based on their habitat, for example land snail at a rocky environment tend to have high-spired shells to provide support and protection while snails in bushy or soils area have a low-spired shell (Heller, 1987). Chiba and Davison (2007) also mentioned that *Mandarina polita* with high-spired shells can be found in wet and sheltered areas while low-spired shells usually found in dry and exposed areas. These finding shows that ecological interaction and microhabitats can influence the shape morphology of land snails.

Other than shell shape, shell colour and pattern also play crucial role on land snail as a character used to recognised and identify the species (Williams, 2016). It stated that predation and environment factors contribute to the evolution of shell colour. For example, the land snail *Cepaea nemoralis* across British Isles and Europe shows different shell colour which affected by predation which resulting them to evolved shell colour and pattern to help them blend into their habitats which leading to the morphological variation (Jones et al., 1980). Same case for the land snail *Mandarina* (Pulmonata), the sympatric species, experienced shell colour variation caused by the competition among the snail species. It shows that snails who lives in wet and sheltered areas tend to have dark shells while snails in dry and exposed areas have bright shells colour. From the interaction among species, it helps to maintain the colour polymorphism in the snail population and contribute to the variation of their ecological and morphological characters (Gefaell et al., 2022).

2.3 The geographical distribution of the genus *Pincerna* and *Stomacosmethis*

The distribution of the genus *Pincerna* is widely spread from the southeastern Himalaya to northern Vietnam and northern Laos, and Sumatra, the southern part of the Malay Peninsula, and northern Borneo while the genus *Stomacomethis* is spreading in Thailand, Myanmar and also Sumatra, the southern part of the Malay Peninsula, and northern Borneo (Páll-Gergely et al., 2020). Since this study only focus in Sarawak, the genus *Pincerna*, represented by *P. globosa* can be spotted in Kuching, Serian, Kapit and Miri (Marzuki et al., 2021). Interestingly, despite being widely reported in Sarawak by Marzuki et al. (2021), *P. globosa* was not observed in limestone cluster Miri or Niah during this study. While for the genus *Stomacosmethis*, represented by species *S. hosei*, *S. galbanus* and *S. sadongensis* are known to be found in Kuching, Sadong and Serian divisions of Sarawak and is endemic to Borneo (Páll-Gergely et al., 2020; Marzuki et al., 2021). Furthermore, according to Páll-Gergely et al. (2020), *S. galbanus* has been recorded in Niah National Park where they are known to be highly abundant.

Limestone karst in Southeast Asia is rich in calcium environment which make them as a hotspot for the land snail diversity (Schilthuizen et al., 2005; Von Oheimb et al., 2019; Boonmachai et al., 2023). For instance, Schilthuizen et al. (2003) studied that the environmental between limestone and non-limestone habitats in Sabah suggest the potential for significant in shell morphology were influenced by calcium and habitat conditions. It shows that snail abundance in Tabin (only limestone outcrop) was higher compared to Danum (limestone and sandstone) which conclude how substrate can drive the abundance correlates with the morphology and ecological process in land snail population. This suggest that limestone conditions, such as calcium availability and pH could similarly influence *Pincerna* and

Stomacosmethis distribution and abundance in Sarawak since these two genera found in different habitat type (vegetation areas and limestone karst).

A study done by Mujiono and Priawandiputra (2020) highlight that different geographical and ecological areas within limestone karst in Java influence the species abundance and species richness. It shows that *Landouria rotatoria* and *Japonia ciliocinctum* are widely distributed within Java's karst habitat proves that they are able to adapt to multiple karst environments. Similar to how these two species widespread distribution in Java, the genus *Pincterna* and *Stomacosmethis* are also widely distributed throughout Sarawak. This wide-ranging distribution underscores the adaptability of these genera to diverse limestone habitats and emphasizes the ecological significance of limestone karst in Sarawak as reservoirs of biodiversity.

2.4 Geometric morphometric analysis of shells

Geometric morphometric (GM) analysis can be used to analyze shell size and shape data (Haase et al., 2014) and can identify the causes of variation within and between the individuals (Lawing & Polly, 2009). In geometric morphometrics, the examination of 3D structures often involves the use of 2D images. Most studies on geometric morphometrics of mollusc shells, rely on 2D images, with 3D images being infrequently employed (Márquez and Averbuj, 2016; Márquez et al., 2011; Scalici et al., 2016).

Geometric morphometric (GM) analysis is commonly used in gastropod shells since they grow by adding layers and not changing shape. This tool can explore and understand the shape and form of shells by examining the outline-based shape which can provide information on their variation (Sobrepeña & Demayo, 2014). This study shows that *Achatina fulica* from different localities in the Philippines exhibit variations in shell shape both intra- and inter-population suggesting the species is experiencing phenotypic plasticity where their

physical traits change in response to different environment conditions. Geometric morphometric (GM) analysis also has been applied on the size and shape *Placostylus* on the Isle of Pines, New Caledonia, which discuss either their shell differences might be influenced by genetic factors, rather than environmental differences (Quenu et al., 2020).

This analysis also can be used to analyze shell size and shape data (Haase et al., 2014). For instance, GM analysis used to examine the shell shape variation among localities of species *Bostryx torallyi* (Miranda, 2020). It shows a variation on shell between low and high latitude suggesting latitude as the one of the factors for the morphometric differences. The specimens from different localities within a small geographic area there are still have a high level of morphological variability in shell shape which due to environmental factors or microhabitat differences. Giokas et al. (2014) also supporting the fact that latitude plays significant role in shaping the morphological such as size and shape. Geometric morphometric analysis is very useful in identifying the shape variation of genus *Albanaria* by analysing specific landmarks on shell surface which allow precise measurement and compare morphological differences across different localities from different latitude.

The classification of specimen into different categories based on their shell shape and size also can use the same tool, geometric morphometric (GM) analysis (Quenu et al., 2020). This study uses *Placostylus* as sample that have both geometric and genetic information from previous study (Dowle et al., 2015) and the results show the snails were divided into two groups based on their shell shape supported by the genetic data where these groups are two separate species. Other than that, geometric morphometric (GM) methods also an effective tool in identifying variances on shell shape and size between different sexes (Minton & Wang, 2011; Moneva et al., 2012; Cabuga et al., 2017; Phung et al., 2022). For instance, sexual dimorphism in shape exists in *Viviparus subpurpureus* where the shell shape shows

differences between male and female (Minton & Wang, 2011). Females tend to have rounded shell with wider aperture which helps them to have better brooding since they have larger shells size. In Table 2.1 is the summarize of the previous studies that used geometric morphometrics for various research purposed.

Table 2.1: Overview of studies using geometric morphometrics.

Species (Genus/ Family/Class)	Scope of study	Number of land- marks	Study by
<i>Placostylus</i> (Gatropoda, Bothriembryontidae)	To investigate the size and shape variation of the shells of the endemic land snail <i>Placostylus</i> .	40	Quenu et al. (2020)
<i>Albinaria</i> (Gatropoda, Clausiliidae)	To identify the shell variation influenced by stressful environmental factors.	16	Giokas et al. (2014)
<i>Theba</i> (Gatropoda, Helicidae)	To restructure the ancestral shapes and sizes at the using geometric morphometric methods.	10	Haase et al. (2014)
<i>Achatina fulica</i> (Gatropoda, Archatinidae)	To quantitatively describe the differences in shell shape of <i>Achatina fulica</i> changes based on its banding patterns and geographical locations in Philipines.	199	Sobrepeña & Demayo (2014)
<i>Bostryx torallyi</i> (Gatropoda, Bulimulidae)	To evaluate the shell shape variation and provide the anatomical information of <i>B. torallyi</i> .	9	Miranda (2020)
<i>Placostylus</i> (Gatropoda, Bothriembryontidae)	To determine the factors that influence the diversity of <i>Placostylus</i> by using geometric	28	Dowle et al. (2015)

	morphometric and genetic structure to examine the shell shape.		
<i>Viviparus</i> (Gastropoda, Vivipariidae)	To test the shape differences between male and female <i>Viviparus</i> (sexual dimorphism) by using geometric morphometric.	19	Minton & Wang (2011)
<i>Pomacea canaliculata</i> (Gastropoda, Ampullariidae)	To explore the sexual dimorphism in <i>P. canaliculata</i> by using geometric morphometric.	21 LMs on aperture view and 17 LMs on dorsal view.	Moneva et al. (2012)
<i>Pomacea canaliculata</i> (Gastropoda, Ampullariidae)	To describe shell variations and sexual dimorphism in <i>P. canaliculata</i> by using geometric morphometric.	16 LMs on dorsal view and 21 LMs on apertural view.	Cabuga et al. (2017)
<i>Leptopoma perlucidum</i> (Gastropoda, Cyclophoridae)	To identify the differences in shell size and shape between male and female of <i>Leptopoma perlucidum</i> .	9	Phung et al. (2022)
<i>Crotalus adaman-teus</i> (Reptilia, Viperidae) and <i>Gennetta</i> spp., (Mammalia, Viverridae)	In this paper it discusses how geometric morphometric can be use in different objective such as identifying hybrid zones and hybrid individuals in a group, to identify the shape characters that most linked to geographic cline and to explore how deformation or changes on morphometric structures.	-	Lawing & Polly (2009)

CHAPTER 3

MATERIALS AND METHODS

3.1 Sampling Areas

Sampling was conducted along the Western part Sarawak, from Bau to Miri, between October 2022 and May 2023. The adult specimens of *Pincerna* and *Stomacsmethis* were collected from different limestone clusters in Bau, Padawan, Siburan, Miri and Niah. Each cluster is known for its unique geological formation and limestone abundance. The sampling areas were specifically chosen due to the known habitat preference of these genera for limestone environments. These limestone cluster is abundance with shaded rocks and variety habitat condition make it perfect for the species to thrive (Schilthuizen et al., 2003; Clements et al., 2008; Marzuki et al., 2021). The sampling areas are illustrated in Figure 3.1.

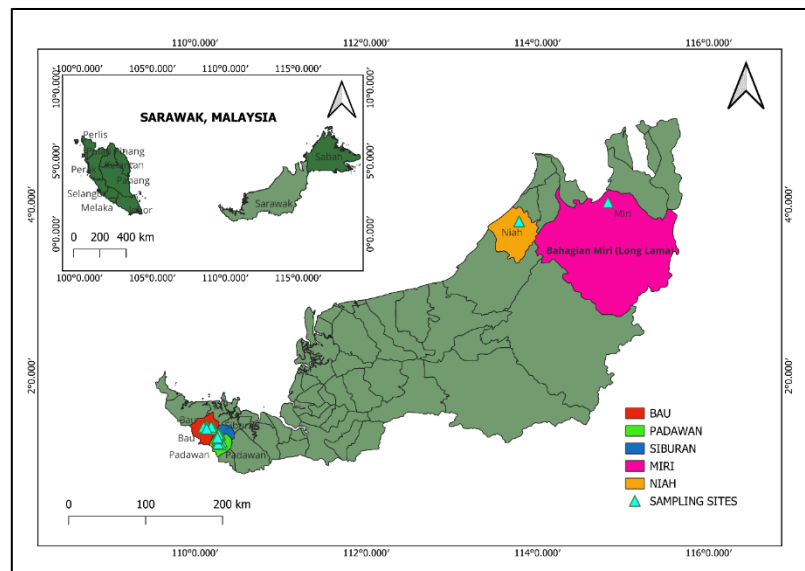


Figure 3.1: Map showing the highlights the sampling sites of the studied populations of *Pincerna* and *Stomacmethis* which cover limestone cluster Bau, Padawan, Siburan, Miri and Niah.

3.2 Fieldwork

Active search was conducted in a plot measuring approximately 20 m × 20 m with three people for at least 45 minutes at each sampling site. The live specimens were collected and immediately placed inside tubes containing ~96% of ethanol for preservation. The soil sample and leaf litter collected for each sampling site then undergo floatation method where the soil put in the basin full of water and the snails would float on the water. After collecting all the land snails from the soil samples, each specimen is magnified, followed by sorting using a stereomicroscope for identification and cataloguing of the snail shells. All collected specimen deposited into Zoological Museum UNIMAS, Universiti Malaysia Sarawak.

3.3 Species identification

In species identification, the shell features of each species were examined closely in these two genera, *Pincerna* and *Stomacosmethis* as they share several similarities due to belonging to the same family, Alycaeidae. A detailed species identification was conducted in distinguishing them based on noticeable differences in their shell characteristics (Páll-Gergely et al., 2020; Marzuki et al., 2021). This identification was carried out mainly through direct observation with the naked eyes, aided with microscope focusing on the key morphological traits that distinct between these species. This method allowed us to characterise and distinguish these species despite their overall similarities. Moreover, from species identification also provides information in the population dynamics and detecting changes in their distribution and abundance over time (Cha et al., 2023). *Pincerna* and *Stomacosmethis* hold ecological importance as bioindicators and contribute to nutrient cycling within limestone ecosystems as like other land snail (Nation, 2005; Gheoca et al., 2021; Salih et al., 2021). Their limited distribution and habitat specificity also highlight their conservation value in

Sarawak. Additionally, their morphological diversity is important for future biodiversity research.

3.3.1 Identification of species of the genus *Pincerna* (Preston, 1907)

So far, only one species of *Pincerna* is currently recognised in the region and known as endemic to Borneo which is *P. globosa* (Figure 3.2). It can be identified by the following characteristics: the shell is dextral, thick, solid, sub-glossy with an orangish to yellowish. The overall texture is robust and smooth, suture deep. It consists of two whorls with a short breathing tube, have denser ribs and smooth protoconch sculpture. The shape of the aperture rounded and not descending. The peristome expanded and equally reflected, internally thickened and boundary between inner and outer peristomes visible and also have open, narrow umbilicus. The species dimensions include a diameter of 3.90 - 5.32 mm, a height of 3.7 - 5.2 mm, a width aperture of 2.26 - 3.22 mm, an aperture height of - 2.00 - 2.93 mm and a protoconch size of 0.94 - 0.96 mm. The distribution of *P. globosa* is limited to specific regions within Sarawak including Bau, Padawan and Sibul, particularly areas with abundant vegetation and limestone karst.

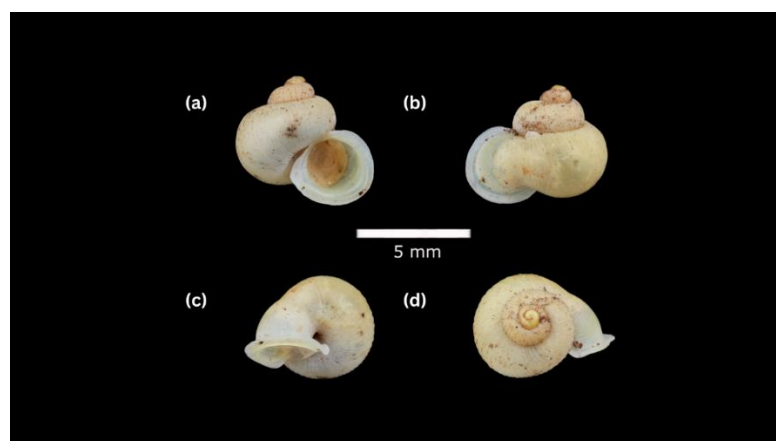


Figure 3.2: The (a) aperture view, (b) lateral view, (c) ventral view and (d) dorsal view of *Pincerna globosa*. Scale = 5 mm.

3.3.2 Identification of the species of the genus *Stomacosmethis* (Bollinger, 1918)

Only three species of *Stomacosmethis* is currently recognised in Sarawak and known as endemic to Borneo (Marzuki et al., 2021). First is *S. hosei* where they can be identified with these characters: they have shell dextral with pear-shaped, thick, solid and sub-glossy with orangish to yellowish colour (Figure 3.3). The overall texture is triangle and smooth and consist of four whorls with a very short breathing tube, have denser ribs and smooth protoconch sculpture. Their aperture is rounded and not descending while the peristome expanded and equally reflected, internally very thickened and boundary between inner and outer peristomes visible. Umbilicus: open, narrow. This species dimensions include a diameter of diameter 3.93 - 6.08 mm; a height of 4.57 - 6.80 mm; a width aperture of 2.28 - 3.64 mm; a height aperture of 0.33 - 3.92 mm and a protoconch of 0.35 - 0.80 mm. The distribution of *S. hosei* is widely spread within Sarawak (Bau, Padawan, Siburan and Miri), particularly areas with abundant of limestone karst.

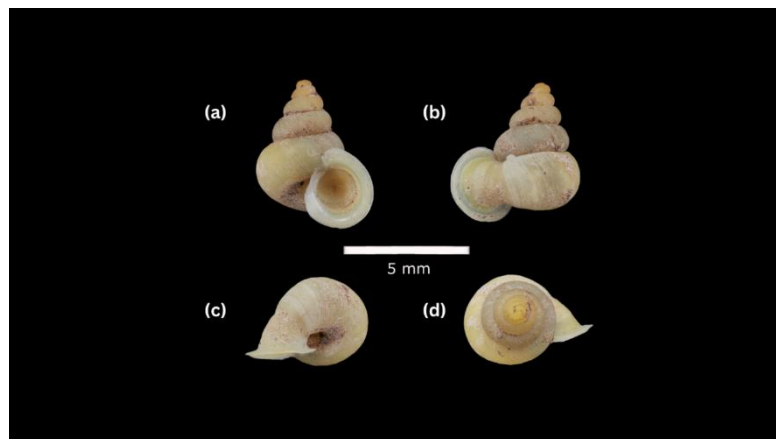


Figure 3.3: The (a) aperture view, (b) lateral view, (c) ventral view and (d) dorsal view of *Stomacosmethis hosei*. Scale = 5 mm.

Next is *S. sadongensis* where they can be identified with these features: the shell dextral with pear-shaped, thick, solid, sub-glossy, orangish to yellowish, have darker strips

and light strips in between (Figure 3.4). The overall texture is triangle and smooth and consist of four whorls with very short breathing tube, have denser ribs and smooth, elevated protoconch sculpture. They have rounded and not descending aperture. On the other hand, the peristome is expanded, internally very thin and boundary between inner and outer peristomes visible and have open, narrow umbilicus. This species dimensions include a diameter of 4.49 - 5.67 mm, a height of 4.33 - 5.48 mm, a width aperture of 2.16 - 2.95 mm, a height aperture of 1.93 - 2.69 mm and a protoconch of 0.32 - 0.69 mm. This *S. sadongensis* has a distribution limited to specific regions within Sarawak (Bau, Sadong and Siburan), particularly areas with abundant vegetation and limestone karst.

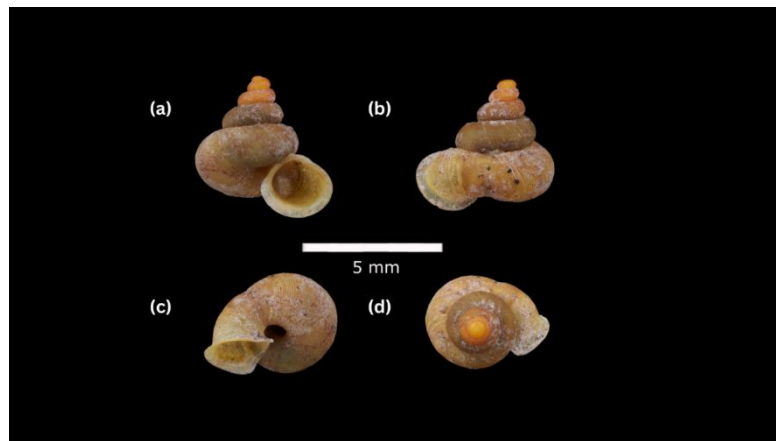


Figure 3.4: The (a) aperture view, (b) lateral view, (c) ventral view and (d) dorsal view of *Stomacosmethis sadongensis*. Scale = 5 mm.

The only species of *Stomacosmethis* that can be found in Niah National Park and have the largest shell among them is *S. galbanus* (Figure 3.5). This species can be identified with these characteristics: the shell dextral, thick, solid, matte, the colour pattern with darker areas being wider and lighter areas being narrower. The overall texture is uneven texture, suture deep and consist of four whorls with very short breathing tube, have denser ribs and smooth protoconch sculpture. The aperture is rounded, tending toward an oval shape and have

expanded and equally reflected peristome, internally thickened and boundary between inner and outer peristomes visible. Umbilicus: open, narrow. This species dimensions include a diameter of 5.86 - 6.73 mm, a height 4.92 - 6.41 mm, a width aperture 3.08 - 3.75 mm, a height aperture 2.52 - 3.72 mm and a protoconch of 0.41 - 0.81 mm. This species has a distribution limited to only one specific regions in Sarawak (Niah National Park), particularly areas with abundant of limestone karst with not exposed to sunlight.

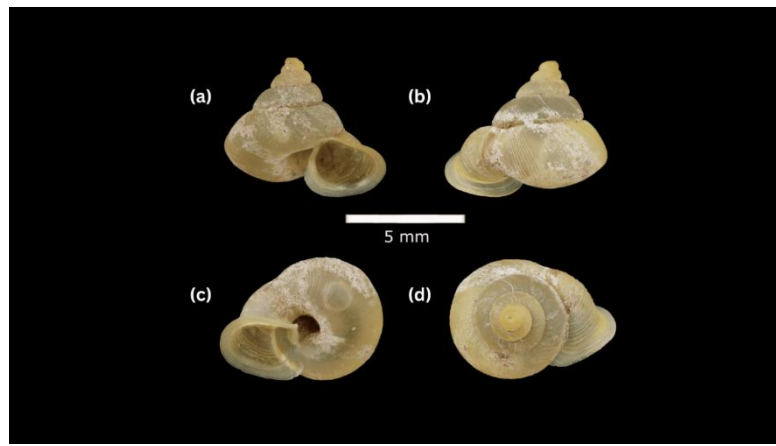


Figure 3.5: The (a) aperture view, (b) lateral view, (c) ventral view and (d) dorsal view of *Stomacosphis galbanus*. Scale = 5 mm.

3.4 Statistical analysis

3.4.1 Normality test

In order to avoid errors in ANOVA which can lead to inaccurate results, the Shapiro-Wilk test was used to determine the normality (Shapiro & Wilk, 1965; Rahman & Govindarajulu, 1997). Nevertheless, the precision depends on the sample size as W-statistic can be affected by how many observations are included (De Souza et al., 2023). Therefore, the Shapiro-Wilk normality test was applied to assess whether the shell data of individual specimens of *Pinctura* and *Stomacosphis* from each limestone cluster followed a normal distribution.

Raw data of *Pincerna* and *Stomacosmathis* that were collected per regions were entered in Excel and the Shapiro-Wilk test was run using RStudio v. 4.2.2. The W-statistics indicates the measure of normality with closure to 1 and p-value more 0.05 ($p > 0.05$) suggesting to more likely normally distributed (Shapiro & Wilk, 1965).

3.4.2 Shell shape analysis

Pincerna globosa and *S. hosei* was used as a representative to analyse the morphological variation using a geometric morphometrics (GM) with the aim to studying the shape through landmark-based method (Conde-Padin et al., 2007; Haase & Misof, 2009) as they are widely distributed and high abundance in Sarawak. The geometric morphometrics (GM) usually present the advantage of reducing the effects caused by size, location, rotation, scale, and experimental bias, which are frequently seen in standard morphometric analyses (Rohlf & Marcus, 1993; Schilthuizen & Haase, 2010). This method consists in studying the covariation between shape variables among individuals (Rohlf & Marcus 1993). Digital images were taken from shells at each locality then *TpsUtil* software was used to create the input file (Rohlf, 2004). On each image, the shells' geometric morphometric size and shape parameters were generated using 16 landmarks (LMs) for *P. globosa* (Table 3.1) and 20 landmarks (LMs) for *S. hosei* (Table 3.2) describing the shell and aperture outline (Figure 3.6). Although the number of landmarks is different between these two species, each landmarks shows the natural variances in shell structure between them. The geometric morphometric analyses were performed independently for each genus to ensure the landmark homology and consistency. From this method, it allows more understanding of shape variation within species. Digitisation of landmarks was conducted using the programme *tpsDig2* v. 1.1 (Rohlf, 2015) and occupied the same positions over the sum of the specimens.

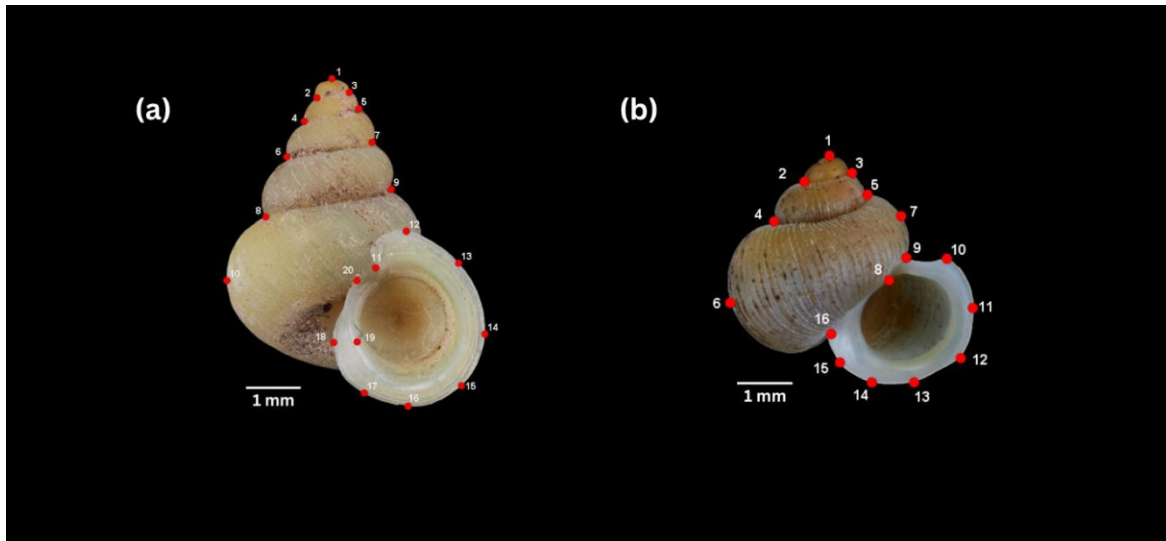


Figure 3.6: Shell with aperture view of (a) *Stomacosmethis hosei* with 20 LMs and (b) *Pincerna globosa* with 11 LMs used in the geometric morphometry analysis.

Table 3.1: Landmarks (LMs) description *Pincerna globosa*.

LM(s)	Descriptions
LM1	The apex of the shell
LM2	The left and right border of the profile of the shell
LM3	The left and right border of the profile of the shell
LM4	The lower suture
LM5	The lower suture
LM6	The most external point in the last whorl at the left profile of the shell
LM7	The middle point of the last suture and the start point of lip
LM8	The end of the suture
LM9	The start of the external part of the lip;
LM10	The highest point of the lip
LM11	The external point of the lip at the right profile of shell
LM12	The longest of the lip
LM13	The lowest point at the base
LM14	The middle point of LM15 and LM16
LM15	The external point of the lip at the left profile of the shell
LM16	The end of the lip

Table 3.2: Landmarks (LMs) description *Stomacosphthis hosei*.

LM(s)	Descriptions
LM1	The apex of the shell
LM2	The left and right border of the profile of the shell
LM3	The left and right border of the profile of the shell
LM4	The left and right border of the profile of the shell
LM5	The left and right border of the profile of the shell
LM6	The left and right border of the profile of the shell
LM7	The left and right border of the profile of the shell
LM8	The lower suture
LM9	The lower suture
LM10	The most external point in the last whorl at the left profile of the shell
LM11	The start of the external part of the lip
LM12	The highest point of the lip
LM13	The median lip point (middle point between LM12 and LM14)
LM14	The external point of the lip at the right profile of shell
LM15	The longest of the lip
LM16	The lowest point at the base
LM17	The base midpoint (middle point between LM16 and LM18)
LM18	The external point of the lip at the left profile of the shell
LM19	The perpendicular lip-point (perpendicular to LM18)
LM20	The end of the lip

In geometric morphometric, the landmarks (LMs) in both *P. globosa* and *S. hosei*, they were categorised into three parts. Type I, known as developmentally homologous, for example LM1 and LM8 for *P. globosa* and LM1 and LM11 in *S. hosei*. Type II is geometrically homologous which were represented the suture line, comprise LM2-LM7 *P. globosa* and LM2-LM10 in *S. hosei*. The remaining landmarks (LM9-LM16 in *P. globosa* and LM12-LM20 in *S. hosei*) were categorised as type III, as they are either located at the extreme points on surfaces or positioned midway in between other landmarks (Giokas et al., 2014). Then the file saved as a new thin-plate splines (TPS) files to analyse for geometric morphometric.

Shape data corrected for size were analysed with Principal component analysis (PCA) to explore the morphological variation. Principal component analysis (PCA) was executed on the covariance matrices of the aligned landmark coordinates using MorphoJ

software (Klingenberg, 2010). The morphological traits were subjected to analysis of variance to find the morphological character that differs between localities. Principal Component Analysis (PCA) is very important as it shows major shape variation and one of the most widely used methods for multivariate analysis. In MorphoJ, PCA is calculated from the covariance matrix and showed three results: PC shape Changes, Eigenvalues and PC Scores. However, as MorphoJ presents this result only in a graphic form. Therefore, a PCA was conducted using RStudio v. 4.2.2 to support and confirm the finding obtained from MorphoJ. It is mainly suitable for landmark-based geometric morphometric data, as it allows the identification patterns of variation without assuming group structures (Zelditch et al., 2012). This method has been widely applied in geometric morphometric studies to assess shape variation and species differentiation.

3.4.3 Shell size analysis

A total of 283 adults *P. globosa* 355 adults *Stomacosmethis* (*S. hosei* (280), *S. sadongensis* (42) and *S. galbanus* (33)) were measured under stereo-microscope and colour images were captured using software *Toupview*. Seven measurements made were used to quantify shell size and shape: 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) with scale 1 mm as guidance (Figure 3.7). Each shell was measured in axial view where the axis of coiling was perpendicular to the line of sight.

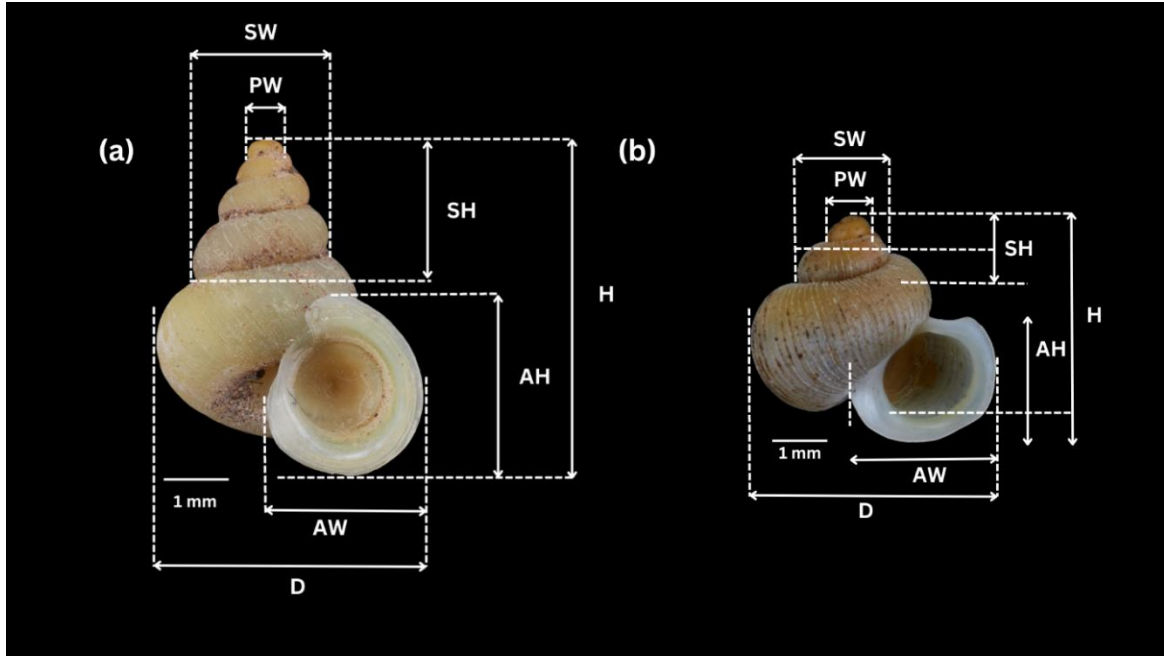


Figure 3.7: Measurements of the shell of *Pincerna globosa* and *Stomacosmethis hosei* used in the analysis: shell diameter (D), shell height (H), apertural width (AW), apertural height (AH), height of spire (SH) and width of spire (SW), and width of proto conch (PW).

Capture One 20 Pro's software was used during the photo session. Snail shells' structure causes them to naturally rest with their umbilici or apertures downward, which is precisely the position where identification is most difficult. It is required to balance diagnostic features in sometimes difficult postures in order to observe them. During the photoshoot session, Open Visionary Digital Passport used to focus the location of snails that photographed. *Helicom* software also used to operate the lens motor in the system of a lens from the computer. This software allows to choose the number of shots in the stack as well as the distance to move the focus over. Finally, the photoshop software used to for measurement and scaling. Then, the Principal Component Analysis (PCA) was employed using RStudio v. 4.2.2 on collected measurement data to explore the morphological variation among *Pincerna* and *Stomacosmethis*. By applying PCA, the principal components can identify the greatest amount of variance among morphological traits of these two taxa. Additionally, we

can identify the most contributing variables influencing both the first and second dimensions. This analysis provides understanding into which morphological traits are most indicative of difference between *Pincerna* and *Stomacosmethis*. PCA is very useful as it minimises the complex morphological data into principal component that capture the majority of shape variation between *Pincerna* and *Stomacosmethis*. Moreover, PCA also helps reveal the morphological traits that contribute the most, helping better understanding of differences on shell shape and size (Rohlf & Marcus, 1993; Adams et al., 2004).

Finally, the regression analysis was conducted using RStudio v. 4.2.2 to assess variances in morphological characters between regions. This analysis is suitable for determine how the specific morphological features vary in response to geographic factors. The correlation regression on shell measurements as characters for distinguish the strength and direction of the relationship between different shell measurements (Rao et al., 2018; McFarland et al., 2023). This analysis also helpful to quantify how these morphological features vary in relation to geographic regions or among populations. Moreover, it is suitable to distinguish the correlations and measure the strength and direction of the relationships among the shell traits (Zhao et al., 2014). PCA and regression analysis both provide important information on morphological analysis as PCA explores overall shape variation (Giokas et al., 2014; Doyle et al., 2018), while regression shows how specific shell measurements are related (Salkeld, 1995). This analysis compared the slopes of the regression lines for each variable across different species and localities in Sarawak. The p-values ($p \leq 0.05$) obtained suggesting that environment factors could influence the morphological traits of these genera from difference regions.

CHAPTER 4

RESULTS

4.1 Morphological assessment for the genus *Pincerna*

4.1.1 The distribution of the genus *Pincerna* in Sarawak

A total of 283 adult specimens of *Pincerna globosa* were collected at limestone cluster 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 4.1 and Table 4.1.

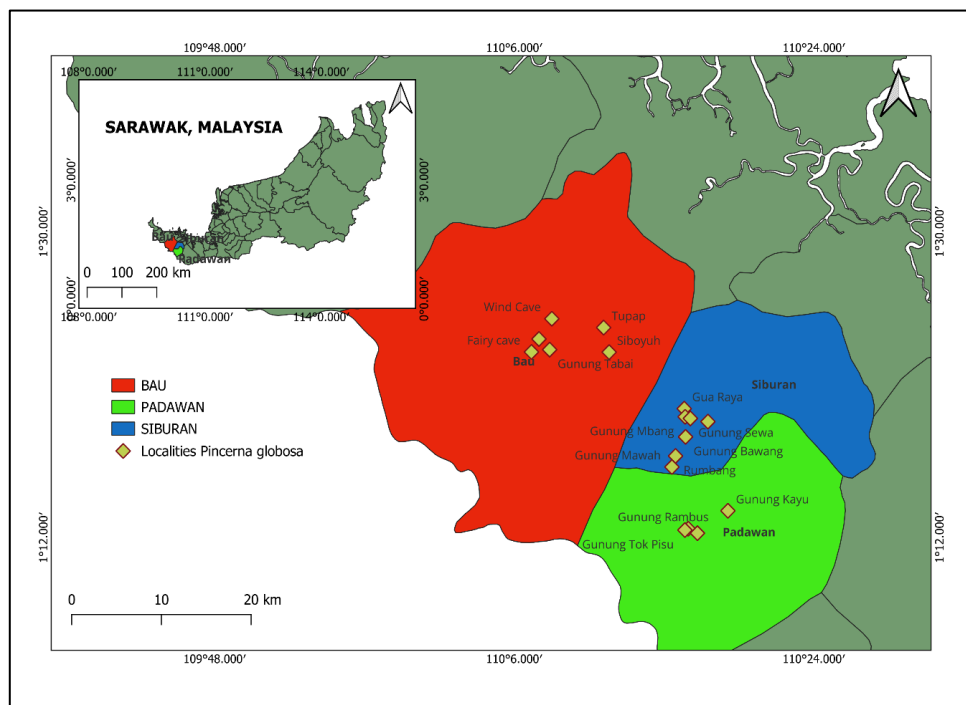


Figure 4.1: A map illustrating the sampling locations in Sarawak, focusing on five limestone clusters: Bau, Padawan, Siburan, Miri and Niah. The map highlights the species distribution, with *Pincerna globosa* represented by diamond brown.

Table 4.1: A total of 283 adult *Pincerna globosa* based on their localities from in 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 1.40590, 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

A brief comparison of the colour variation and slight shell shape differences among *Pincerna globosa* across the three limestone clusters reveals significant differences (Figure 4.2). For instance, *P. globosa* from cluster Bau shows more striking orange colour whereas specimen from cluster Padawan and Siburan displaying a pale white to yellowish colour shell. These colour variances as shown in (Figure 4.2) indicates that individuals from different localities or regions shows different features, though the underlying causes remain uncertain.

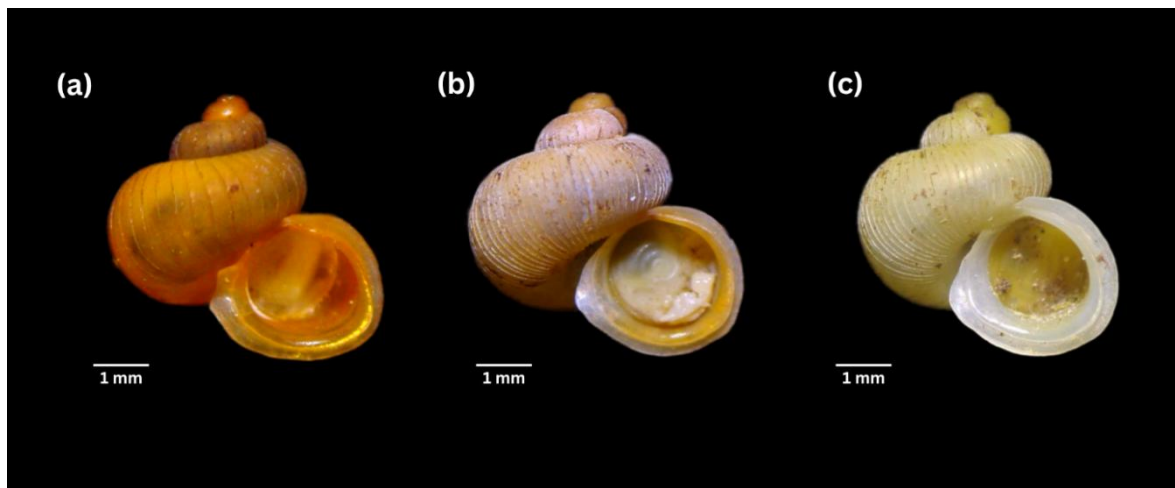


Figure 4.2: Aperture views of *Pincerna globosa* from various limestone clusters: (a) Bau, (b) Padawan, (c) Siburan. Scale = 1mm.

Based on the Shapiro-Wilk normality test, the data on *P. globosa* distribution were normally distributed ($p > 0.05$), shows that there are geographically spread (Table 4.2). The total number of *P. globosa* collected indicates that they are dominantly widespread in the western part of Sarawak which makes the data suitable for further statistical analysis.

Table 4.2: The Shapiro-Wilk normality test on *Pincterna globosa* from various limestone clusters. The W-static indicates the measure of normality with closure to 1 and p-values more 0.05 ($p > 0.05$) suggesting to more likely normally distributed.

Limestone clusters	W	p-values
Bau	0.885	0.295
Padawan	0.949	0.712
Siburan	0.850	0.122

4.1.2 Geometric morphometric analysis of *Pincterna globosa*

The results of the comparison morphological variation among populations within the same region show there are significant differences in both shape and size except for limestone cluster Padawan (Figure 4.3b). Padawan cluster shows there is no significant different in size ($F = 2.18, p > 0.05$) but shows significant different in shell shape ($F = 1.69, p = 0.0005$) (Table 4.3). However, when comparing between limestone cluster (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)) (Table 4.3 and 4.4), they show significant different in shell size and shape. Also, 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 (Figure 4.4ab).

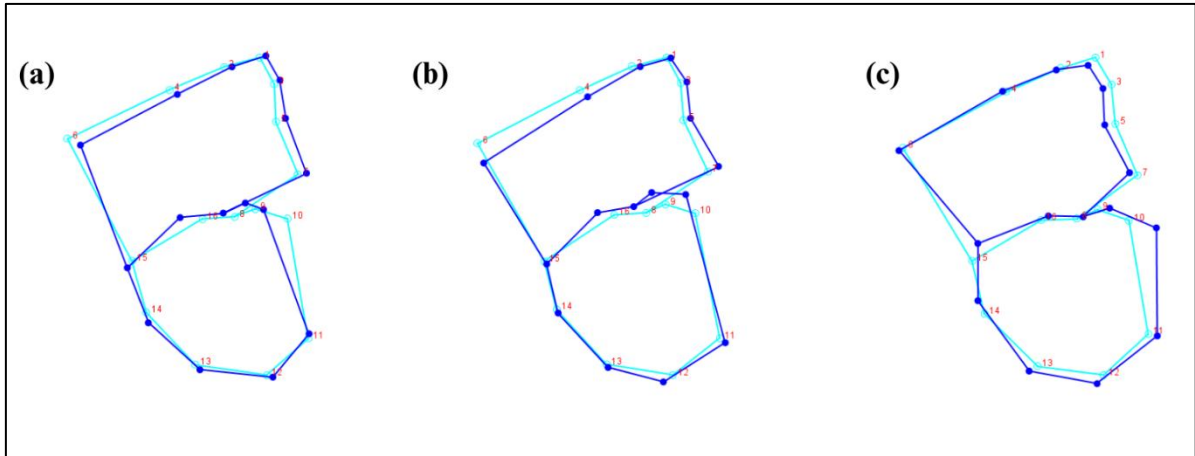


Figure 4.3: Shape changes along the PC1 based on landmark data for each locality: (a) Bau (b) Padawan (c) Siburan.

Table 4.3: The centroid size of shell of *Pincerna globosa* using based on their locality using Procrustes ANOVA ($p < 0.0001$).

Limestone clusters	Effect	SS	MS	d.f	F	P
Bau	Individual	3.13×10^5	6.27×10^4	5	21.55	<0.0001
	Residual	1.89×10^5	2.91×10^3	65		
Padawan	Individual	8.40×10^3	2.80×10^3	3	2.18	>0.1331
	Residual	1.93×10^4	1.28×10^3	15		
Siburan	Individual	1.16×10^6	1.94×10^5	6	106.94	<0.0001
	Residual	3.44×10^5	1.81×10^3	190		
Bau-Padawan	Individual	3.43×10^5	3.81×10^4	1	14.64	<.0001
	Residual	2.08×10^5	2.60×10^3	88		
Bau-Siburan	Individual	1.47×10^6	1.22×10^5	12	58.66	<.0001
	Residual	5.24×10^5	2.09×10^3	251		
Padawan-Si-buran	Individual	1.19×10^6	1.19×10^5	10	67.65	<.0001
	Residual	3.54×10^5	1.76×10^3	201		
Overall	Individual	1.50×10^6	9.40×10^4	16	46.07	<.0001
	Residual	5.43×10^5	2.04×10^3	266		

Table 4.4: The shell shape of shell of *Pincterna globosa* using based on their locality using Procrustes ANOVA ($p < 0.0001$).

Limestone clusters	Effect	SS	MS	df	F	P
Bau	Individual	3.03×10^{-2}	2.16×10^{-4}	140	2.63	<0.0001
	Residual	1.49×10^{-1}	8.21×10^{-5}	1820		
Padawan	Individual	9.40×10^{-3}	1.12×10^{-4}	84	1.69	0.0005
	Residual	2.78×10^{-2}	6.62×10^{-5}	420		
Siburan	Individual	8.54×10^{-2}	5.08×10^{-4}	168	6.40	<0.0001
	Residual	4.23×10^{-1}	7.95×10^{-5}	5320		
Bau-Padawan	Individual	4.68×10^{-2}	1.86×10^{-4}	252	2.35	<0.0001
	Residual	1.77×10^{-1}	7.91×10^{-5}	2240		
Bau-Siburan	Individual	1.43×10^{-1}	4.24×10^{-4}	336	5.30	<0.0001
	Residual	5.62×10^{-1}	8.00×10^{-5}	7028		
Padawan-Siburan	Individual	1.07×10^{-1}	3.84×10^{-4}	280	4.90	<0.0001
	Residual	4.41×10^{-1}	7.83×10^{-5}	5628		
Overall	Individual	1.63×10^{-1}	3.63×10^{-4}	448	4.58	<0.0001
	Residual	5.90×10^{-1}	7.92×10^{-5}	7448		

In PCA, based on landmark data for all combined locality (Figure 4.4), the first two principal component (PC1 and PC2) accounted 37.18% of the total shape variation in *P. globosa*. PC1 described the highest amount of variation, 21.02% whereas PC2 is 16.16%, respectively (Table 4.6). Since the present analysis intended to further classify on specimen *P. globosa*, those landmarks with 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 4.4b) is used to visualize the shape variation during shape transformation. Thus, PC1 (Figure 4.4c) 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), while PC2 (Figure 4.4d) 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.

Table 4.5: Eigenvalues of Principal Components for *P. globosa*.

Component	Eigenvalue	Component	Eigenvalue	Component	Eigenvalue
Comp 1	5.632557e-04	Comp 11	6.946771e-05	Comp 21	1.463730e-05
Comp 2	4.330032e-04	Comp 12	5.773531e-05	Comp 22	1.305501e-05
Comp 3	2.646961e-04	Comp 13	4.097425e-05	Comp 23	1.076731e-05
Comp 4	2.355978e-04	Comp 14	3.776250e-05	Comp 24	8.809584e-06
Comp 5	2.073505e-04	Comp 15	2.899476e-05	Comp 25	8.368910e-06
Comp 6	1.779761e-04	Comp 16	2.308807e-05	Comp 26	7.291082e-06
Comp 7	1.142116e-04	Comp 17	2.184243e-05	Comp 27	6.585895e-06
Comp 8	1.032877e-04	Comp 18	1.824658e-05	Comp 28	5.891571e-06
Comp 9	9.317025e-05	Comp 19	1.807105e-05		
Comp 10	8.006833e-05	Comp 20	1.586263e-05		

Table 4.6: Percentage of Total Variance Explained by Principal Components *P. globosa*

Component	% Variance	Component	% Variance	Component	% Variance
Comp 1	21.02%	Comp 11	2.59%	Comp 21	0.55%
Comp 2	16.16%	Comp 12	2.15%	Comp 22	0.49%
Comp 3	9.88%	Comp 13	1.53%	Comp 23	0.40%
Comp 4	8.79%	Comp 14	1.41%	Comp 24	0.33%
Comp 5	7.74%	Comp 15	1.08%	Comp 25	0.31%
Comp 6	6.64%	Comp 16	0.86%	Comp 26	0.27%
Comp 7	4.26%	Comp 17	0.81%	Comp 27	0.25%
Comp 8	3.85%	Comp 18	0.68%	Comp 28	0.22%
Comp 9	3.48%	Comp 19	0.67%		
Comp 10	2.99%	Comp 20	0.59%		

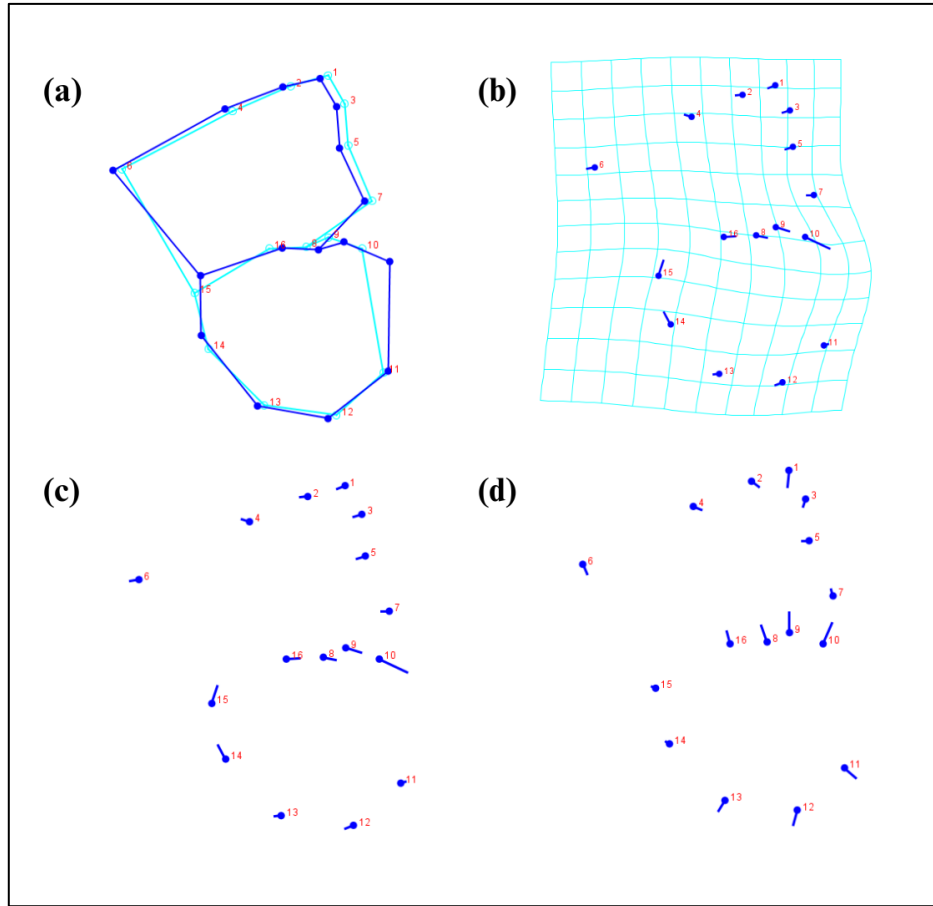


Figure 4.4: Shape changes of the *Pincerna globosa* based on landmark data for all combined locality. (a) The mean shape represented by the wireframe graph that is averaged across all individual shapes. (b) The transformation grid dimensions of PC1 shows the number of grids used to construct the deformation grid. Lollipop graph based on Principal Component Analysis (PCA) illustrating the differences between PC1 (c) and PC2 (d) where they display the loadings of each variable.

4.1.3 Shell measurement analysis of *Pincerna globosa*

PCA on shell measurement shows 82.5% variation on the first principal axis (PC1) with inertia coefficients indicating that populations with high factor score 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) shows negative coefficients on shell (Table 4.7). This shape component described the specimen

tends to have larger shell sizes. The arrows indicate the strength of the correlation where the variables are strongly correlated with each other within the dataset (Figure 4.5).

Table 4.7: Relative contribution of each variable to the inertia of first two principal component analysis component in PCA for *Pincerna globosa*.

Measurements	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 4.8: Eigenvalues and Variance Explained by Principal Components of *P. globosa*.

Principal Component	Eigenvalue	% Variance Explained
PC1	5.7756	82.51%
PC2	0.6305	9.01%
PC3	0.2584	3.69%
PC4	0.1578	2.25%
PC5	0.0869	1.24%
PC6	0.0593	0.85%
PC7	0.0315	0.45%

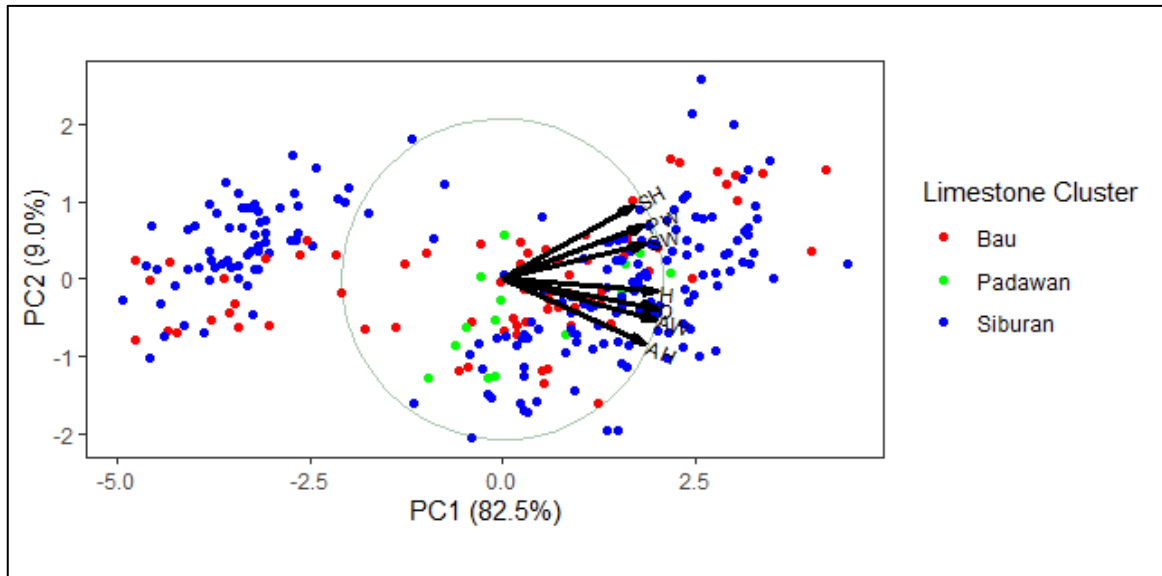


Figure 4.5: The Principal Component Analysis (PCA) of shell metrics demonstrated that the first principal axis, accounted for 82.5% of the variation in the first principal axis and 9% in the second principal axis.

In addition, from PCA it shows that apertural width is the most contributing variable on both dimensions, indicating its significant impact across all components, which suggested it could be used as proxy for overall shell size (Table 4.9). Thus, the apertural width was used as covariate in regression analysis of all remaining linear metrics of shell size. All six shell measurements showed different relationships with the apertural width when comparing p-value for the difference in slope between region and the entire dataset. 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 4.6).

Table 4.9: 'cos2' values for each variable across the first two principal components in the PCA for *Pincterna globosa*.

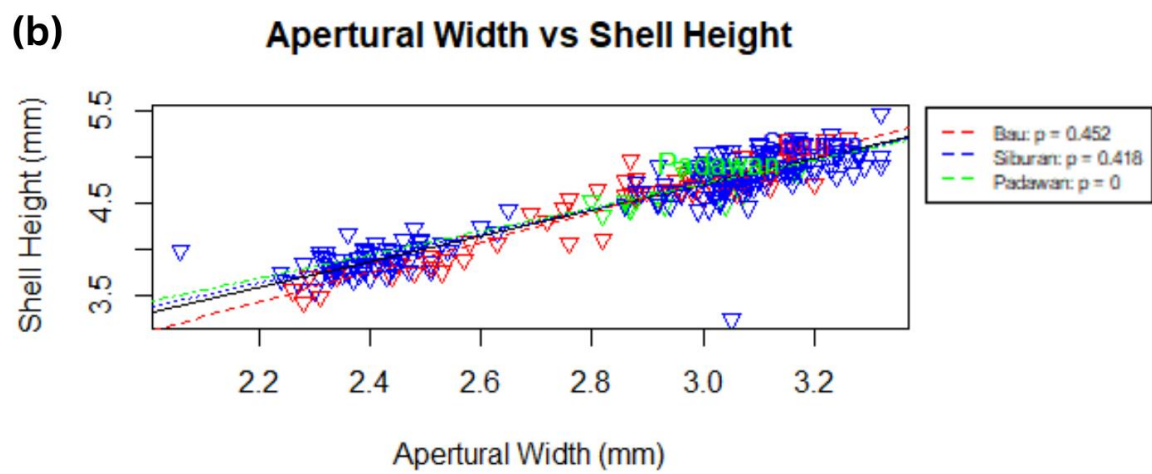
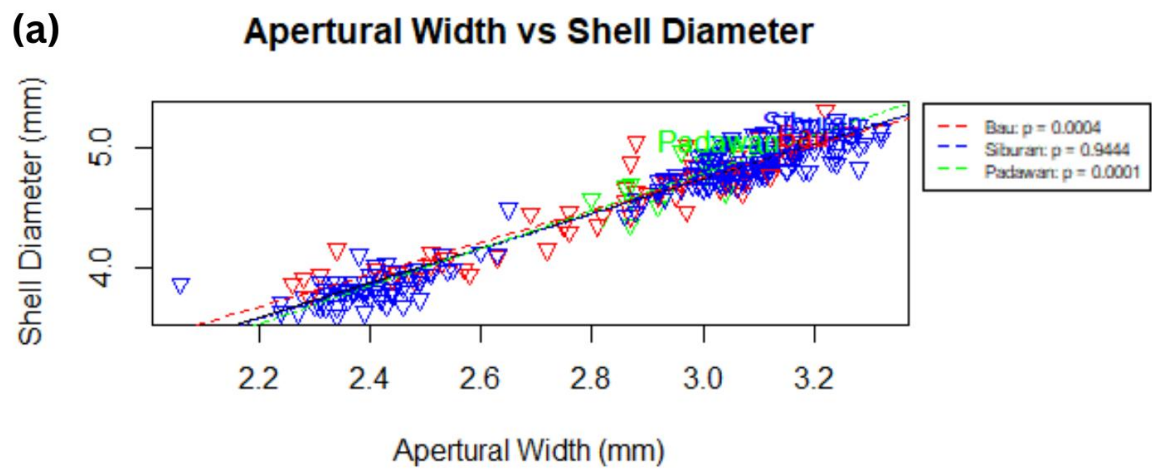
Variables	Dim 1	Dim 2	Total
Shell diameter (D)	0.9225049	0.034200280	0.95670518
Shell height (H)	0.9225622	0.006490201	0.92905240
Apertural width (AW)	0.9045218	0.062543001	0.96706480
Apertural height (AH)	0.7961837	0.164568615	0.96075231
Width of spire (SH)	0.6694439	0.207694149	0.87713805
Height of spire (SW)	0.7856048	0.047768293	0.83337309
Width of protoconch (PW)	0.7747997	0.107221487	0.88202119

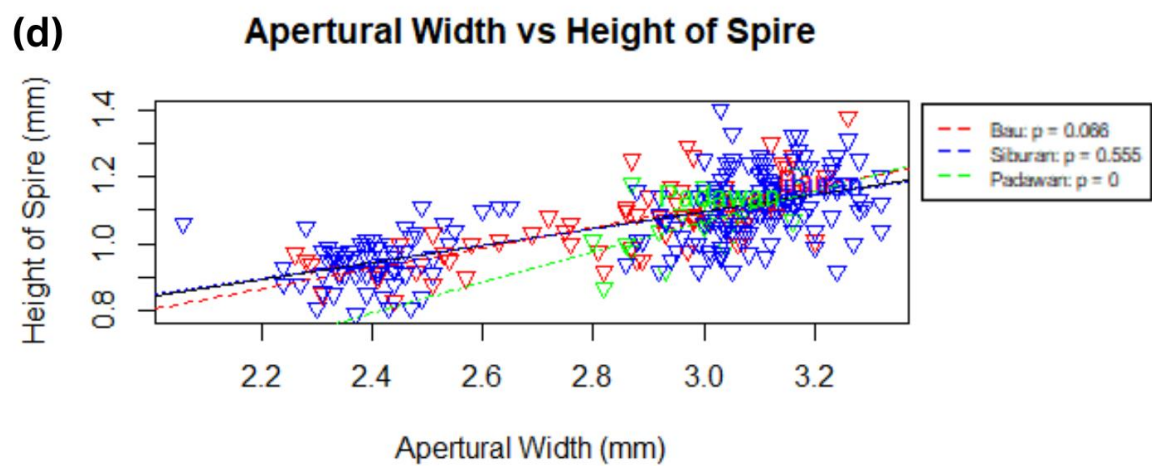
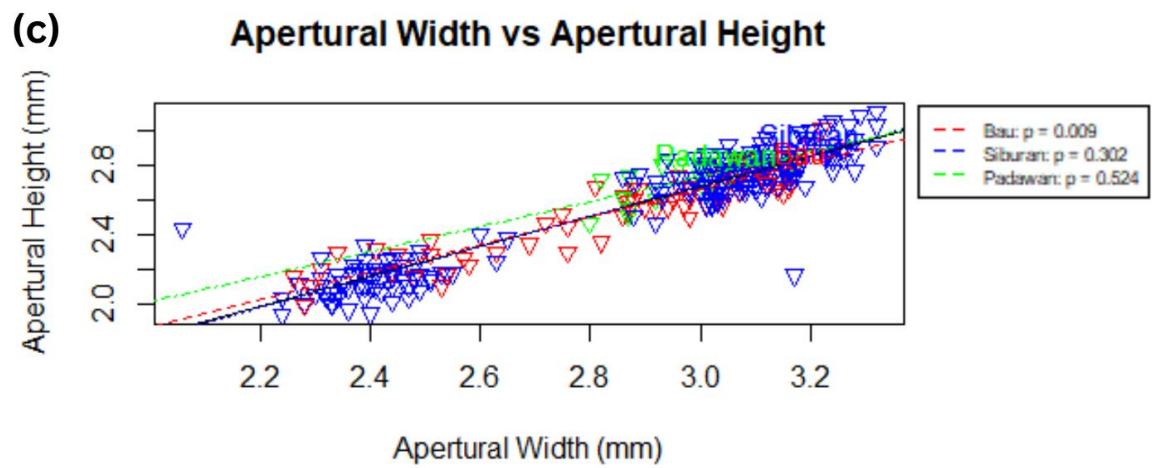
When region effects examined, specimen from limestone cluster Siburan showed the most statistical differences ($p < 0.05$) where the result shows there are significant correlation in all the relationships with the apertural width except for the relationship between apertural width and spire width (Figure 4.6e). Population from Bau shows significant correlation with the apertural width (Figures 4.6a, c and f) while no significant correlation was observed in (Figures 4.6b, d and e). Despite variations in statistics, the species regression lines all intersected at their slopes. While for limestone cluster Padawan population, shows that there is no significant correlation observed between apertural width with all six measurements (Figure 4.6).

When compared the p-values for differences in slopes between each pair of regions, it shows 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 all close to 0, indicating that there is strong evidence that the relationships (slopes) between these variables 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.

Table 4.10: R^2 and p values for linear regression of Aperture Width (AW) against other measurements across different regions.

Variables	Bau	Siburan	Padawan
AW vs D	0.871 (p = 0.0004)	0.005 (p = 0.9444)	0.638 (p = 0.0001)
AW vs H	0.015 (p = 0.452)	0.017 (p = 0.418)	0.448 (p = 0)
AW vs SH	0.250 (p = 0.066)	0.041 (p = 0.555)	0.309 (p = 0)
AW vs SW	0.020 (p = 0.776)	0.062 (p = 0.235)	0.085 (p = 0)
AW vs AH	0.853 (p = 0.009)	0.045 (p = 0.302)	0.030 (p = 0.524)
AW vs PW	0.632 (p = 0.042)	0.063 (p = 0.254)	0.150 (p = 0)





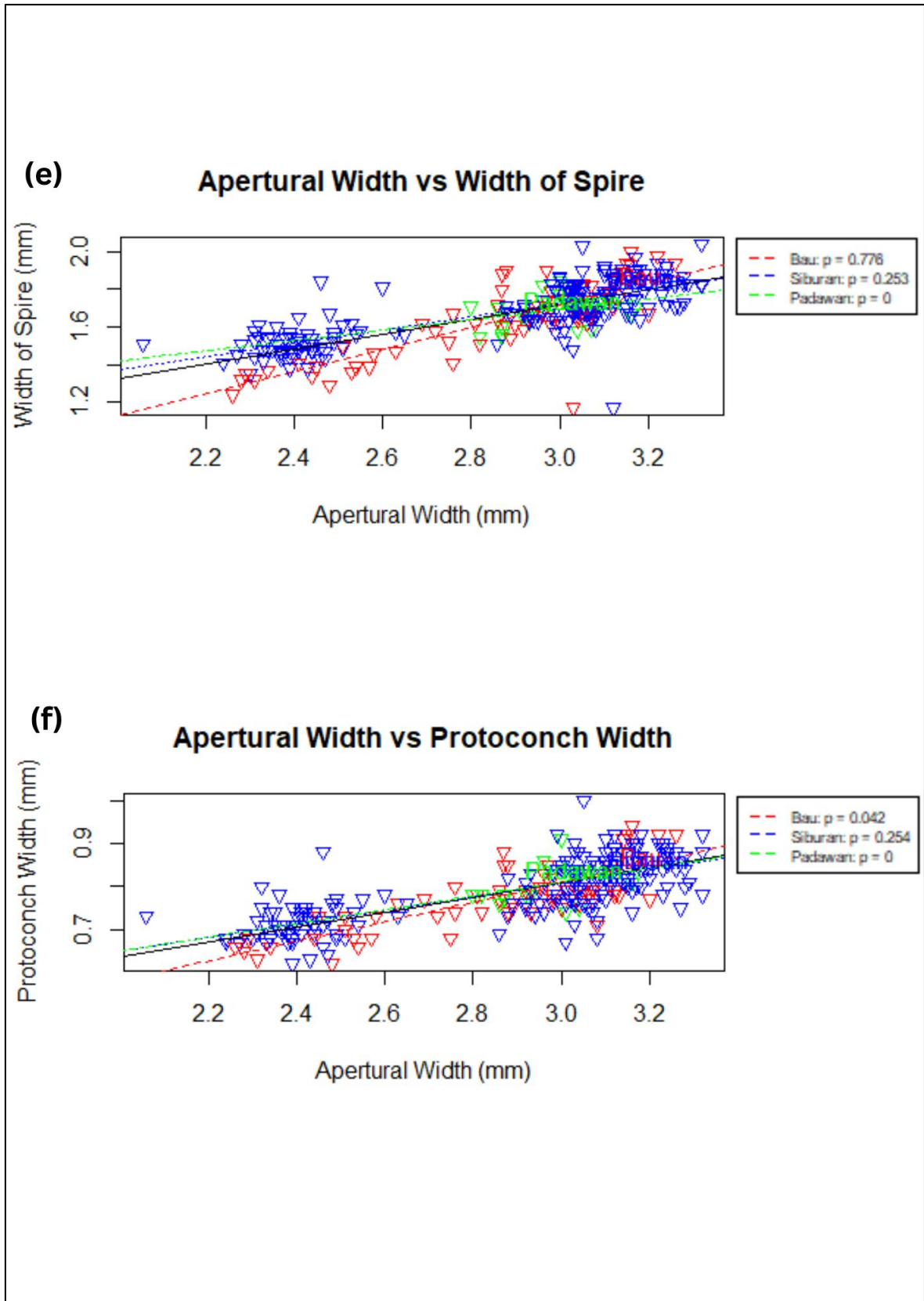


Figure 4.6: Regressions analyses of shell characters of *Pincerna globosa* from different regions with the dependent variable being apertural width. (a) Apertural Width vs Shell Diameter. (b) Apertural Width vs Shell Height. (c) Apertural

Width. vs Apertural Height (d) Apertural Width vs Height of Spire. (e) Apertural Width vs Width of Spire. (f) Apertural Width vs Protoconch Width.

4.2 Morphological assessment for the genus *Stomacoscemthis*

4.2.1 The distribution of the genus *Stomacoscemthis* in Sarawak

A total of 355 *Stomacoscemthis* were collected from 15 localities in limestone cluster Bau (Gunung Soba, Tupap, Bidi and Merembih), Padawan (Sibekam, Temurang, Kayau, Gunung Regu and Gunung Babu), Siburan (Gunung Bawang, Gunung Mbang, Gunung Bakan, and Gunung Mawah), Miri (Deer Cave Mulu) and Niah (Gua Niah National Park) as visualised in Figure 4.7 and Table 4.11.

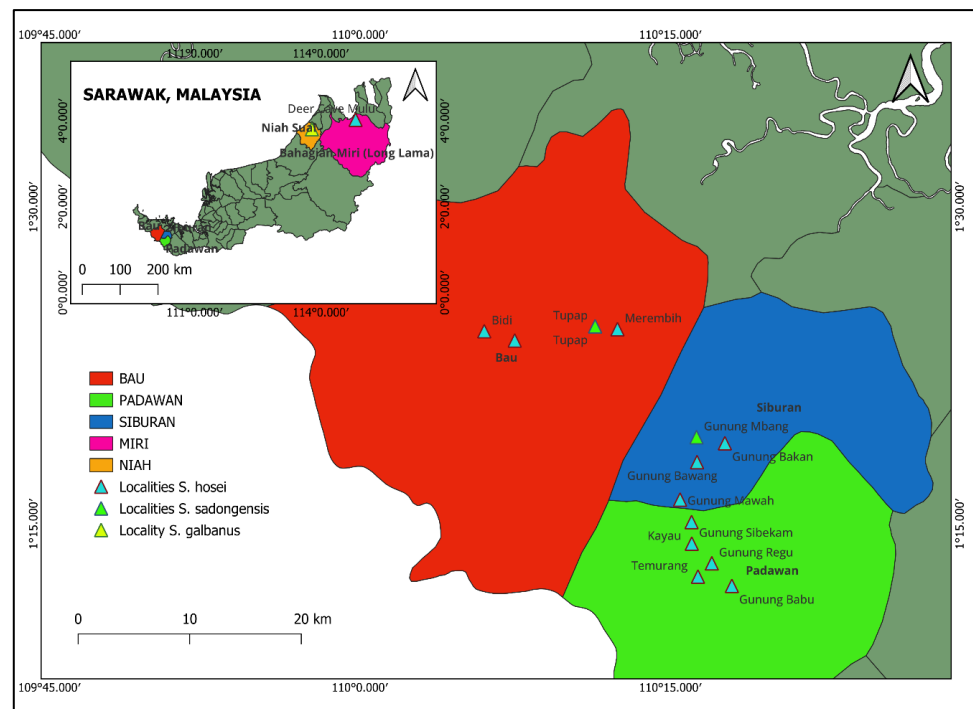


Figure 4.7: A map illustrating the sampling locations in Sarawak, focusing on five limestone clusters: Bau, Padawan, Siburan, Miri and Niah. The map highlights the species distribution, with, *Stomacoscemthis hosei* in blue triangle, *Stomacoscemthis sadongensis* in green triangle and *Stomacoscemthis galbanus* marked in yellow triangle.

Table 4.11: A total of 355 adult *Stomacosphthis* based on their localities from in Zoological Museum UNIMAS collection, Universiti Malaysia Sarawak used for measurement and geometric morphometric analysis.

Lime-stone clusters	Species	Locality and coordinate	Number of individuals	Cat. No.
Bau	<i>S. hosei</i>	Merembih 1.40371, 110.20736	2	MZU.MOL.19.67
	<i>S. hosei</i>	Tupap 1.40590, 110.18936	14	MZU.MOL.19.86 MZU.MOL.19.87
	<i>S. sadongensis</i>	Tupap 1.40590, 110.18936	9	MZU.MOL.19.180 MZU.MOL.19.181
	<i>S. hosei</i>	Bidi 1.40206, 110.10008	25	MZU.MOL.16.291
	<i>S. hosei</i>	Gunung Soba 1.39472, 110.12462	31	MZU.MOL.16.24
Padawan	<i>S. hosei</i>	Gunung Sibekam 1.23445, 110.26718	19	MZU.MOL.16.198
	<i>S. hosei</i>	Temurang 1.20843, 110.27210	50	MZU.MOL.23.53 MZU.MOL.23.147 MZU.MOL.23.148
	<i>S. hosei</i>	Kayau 1.25146, 110.26701	31	MZU.MOL.16.293
	<i>S. hosei</i>	Gunung Regu 1.21889, 110.28332	4	MZU.MOL.16.195
	<i>S. hosei</i>	Gunung Babu 1.20111, 110.29948	31	MZU.MOL.16.294
Siburan	<i>S. hosei</i>	Gunung Bakan 1.31369, 110.29383	5	MZU.MOL.16.197
	<i>S. sadongensis</i>	Gunung Mbang 1.31843, 110.27102	38	MZU.MOL.16.295
	<i>S. hosei</i>	Gunung Bawang 1.29873, 110.27143	28	MZU.MOL.16.290
	<i>S. hosei</i>	Gunung Mawah 1.26917, 110.25777	30	MZU.MOL.16.76
Miri	<i>S. hosei</i>	Deer Cave Mulu 4.02388, 114.82314	5	MZU.MOL.17.95
Niah	<i>S. galbanus</i>	Gua Niah NP 3.80164, 113.78532	33	MZU.MOL.22.364

This study focuses only on *Stomacosphthis hosei* due to its wider distribution within Sarawak compared to the other species. In *S. hosei*, slight shape variations are spotted particularly at the aperture view shape, which is observed through naked eyes (Figure 4.8).

Especially in *S. hosei* from cluster Bau where they tend to have wide lip lines compared to the other clusters. In contrast, *S. hosei* from cluster Miri shows the highest shell size but smaller aperture which could be due to geographical differences since they are located far from the other clusters. Regarding colour variation, there is minimal differences observed across the cluster where they have white to orange-coloured shell.

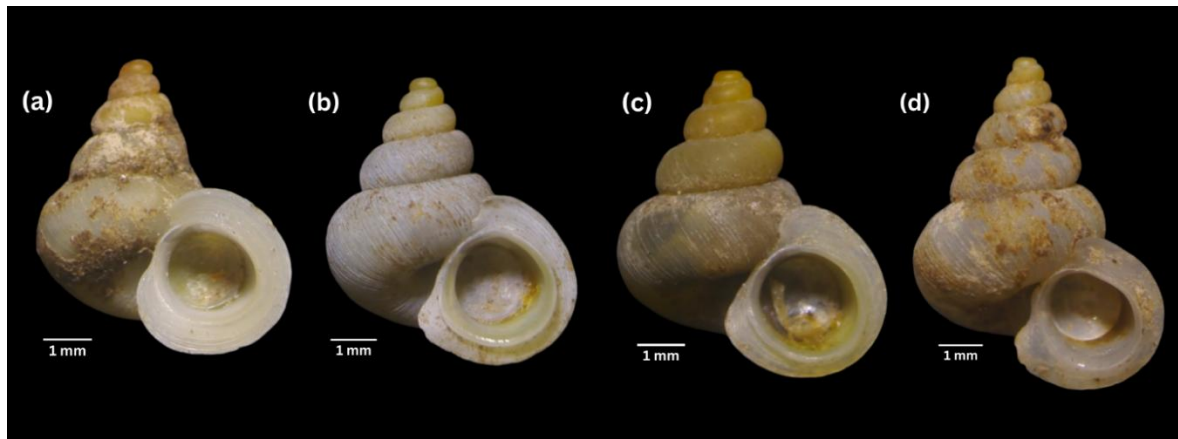


Figure 4.8: Aperture view of *Stomacosphis hosei* from different limestone cluster: (a) Bau (b) Padawan (c) Siburan (d) Miri. Scale = 1mm.

Based on the Shapiro-Wilk normality test, the data on the distribution of *Stomacosphis* species were normally distributed ($p > 0.05$), and demonstrating reliable insights into their geographic spread. In limestone cluster Miri and Niah could not be identified because they only have one locality in one cluster which resulting in NA (not available) for the p-values (Table 4.12).

Table 4.12: The Shapiro-Wilk normality test on *Stomacosphthis* from various limestone clusters. The W-static indicates the measure of normality with closure to 1 and p-value more 0.05 ($p > 0.05$) suggesting to more likely normally distributed.

Limestone clusters	W	p-values
Bau	0.984	0.924
Padawan	0.971	0.880
Siburan	0.879	0.333
Miri	NA	NA
Niah	NA	NA
Overall	0.905	0.114

4.2.2 Geometric morphometric analysis of *Stomacosphthis hosei*

The results of the comparison among populations within the same region reveal significant differences in both shell shape and size (Figure 4.9). This indicates that even within geographically close, there is distinguished variation in these morphological traits. Additionally, when comparing the population from different regions, the analysis also shows significant different which highlighting the effect of geological factors on shell morphology. Specifically, the finding display a significant difference in both shell size ($F = 12.72, p < 0.0001$) and shell shape ($F = 6.37, p < 0.0001$) when combined with all localities (Table 4.13 and 4.14). This change shows that other factors such as geographical and ecological factors give influence in shaping the morphological variation across the studied population.

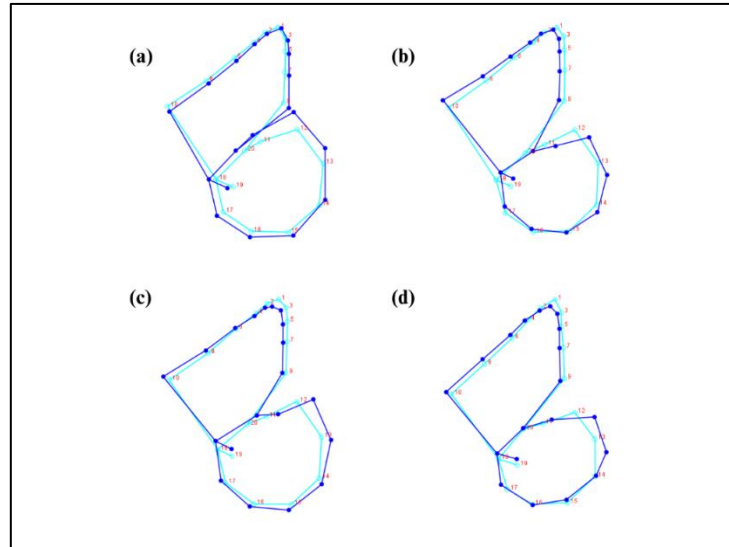


Figure 4.9: Shape changes along the PC1 based on landmark data for each locality: (a) Bau (b) Padawan (c) Siburan (d) Miri.

Table 4.13: The centroid size of shell of *Stomacosphthis hosei* using based on their locality using Procrustes ANOVA ($p < 0.0001$).

Limestone clusters	Effect	SS	MS	d.f	F	P
Overall	Individual	2.00×10^5	1.67×10^4	12	12.72	<.0001
	Residual	3.19×10^5	1.31×10^3	243		
Bau	Individual	2.70×10^4	9.01×10^3	3	6.53	0.0006
	Residual	9.25×10^4	1.38×10^3	67		
Padawan	Individual	8.85×10^4	1.77×10^4	5	12.56	<.0001
	Residual	1.82×10^5	1.41×10^3	129		
Siburan	Individual	4.26×10^4	2.13×10^4	2	18.90	<.0001
	Residual	6.65×10^4	1.13×10^3	59		
Miri	Individual	-	-	-	-	-
	Residual	-	-	-	-	-
Bau-Padawan	Individual	1.39×10^5	1.54×10^4	9	11.03	<.0001
	Residual	2.74×10^5	1.40×10^3	196		
Bau-Siburan	Individual	8.30×10^4	1.38×10^4	6	10.96	<.0001
	Residual	1.59×10^5	1.26×10^3	126		
Bau-Miri	Individual	3.51×10^4	8.77×10^3	4	6.02	0.0003
	Residual	1.03×10^5	1.46×10^3	71		
Padawan-Siburan	Individual	1.31×10^5	1.64×10^4	8	12.43	<.0001
	Residual	2.48×10^5	1.32×10^3	188		
Padawan-Miri	Individual	1.08×10^5	1.80×10^4	6	12.45	<.0001
	Residual	1.93×10^5	1.45×10^3	133		
Siburan-Miri	Individual	6.02×10^5	2.01×10^4	3	16.32	<.0001
	Residual	7.75×10^5	1.23×10^3	63		

Table 4.14: The shell shape of shell of *Stomacosphis hosei* using based on their locality using Procrustes ANOVA ($p < 0.0001$).

Limestone clusters	Effect	SS	MS	d.f	F	P
Overall	Individual	1.53×10^{-1}	3.54×10^{-4}	432	6.37	<.0001
	Residual	4.85×10^{-1}	5.55×10^{-5}	8748		
Bau	Individual	2.52×10^{-2}	2.33×10^{-4}	108	4.26	<.0001
	Residual	1.32×10^{-1}	5.47×10^{-5}	2412		
Padawan	Individual	4.20×10^{-2}	2.34×10^{-4}	180	4.39	<.0001
	Residual	2.47×10^{-1}	5.32×10^{-5}	4644		
Siburan	Individual	2.26×10^{-2}	3.14×10^{-4}	72	5.97	<.0001
	Residual	1.12×10^{-1}	5.26×10^{-5}	2124		
Miri	Individual	-	-	-	-	-
	Residual	-	-	-	-	-
Bau-Padawan	Individual	1.06×10^{-1}	3.29×10^{-4}	324	6.12	<.0001
	Residual	3.79×10^{-1}	5.37×10^{-5}	7056		
Bau-Siburan	Individual	7.26×10^{-2}	3.36×10^{-4}	216	6.26	<.0001
	Residual	2.44×10^{-1}	5.37×10^{-5}	4536		
Bau- Miri	Individual	4.02×10^{-2}	2.79×10^{-4}	144	5.01	<.0001
	Residual	1.42×10^{-1}	5.57×10^{-5}	2556		
Padawan-Siburan	Individual	7.43×10^{-2}	2.58×10^{-4}	288	4.87	<.0001
	Residual	3.59×10^{-1}	5.30×10^{-5}	6768		
Padawan-Miri	Individual	5.36×10^{-2}	2.48×10^{-4}	216	4.61	<.0001
	Residual	2.57×10^{-1}	5.38×10^{-5}	4788		
Siburan-Miri	Individual	3.57×10^{-2}	3.31×10^{-4}	108	6.13	<.0001
	Residual	1.22×10^{-1}	5.39×10^{-5}	2268		

In PCA, based on landmark data for all combined locality (Figure 4.10), the first three principal component (PC1, PC2 and PC3) accounted 52.78% of the total shape variation in *S. hosei*. PC1 described the highest amount of variation, 23.83% whereas PC2 is 19.95% respectively, while PC3 explained 9.00% of the variation, respectively (Table 4.16). Since the present analysis intended to further classify on specimen *S. hosei*, those landmarks with 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 4.10b) is used to visualize

the shape variation during shape transformation. Thus, PC1 and PC2 (Figure 4.10c) was highly affected by the median lip point (LM13) and the highest point of the lip (LM12).

Table 4.15: Eigenvalues of Principal Components for *S. hosei*.

Component	Eigenvalue	Component	Eigenvalue	Component	Eigenvalue
Comp 1	5.9822e-04	Comp 13	3.4283e-05	Comp 25	9.1278e-06
Comp 2	5.0093e-04	Comp 14	3.2201e-05	Comp 26	8.0231e-06
Comp 3	2.2596e-04	Comp 15	2.9511e-05	Comp 27	7.8067e-06
Comp 4	2.0733e-04	Comp 16	2.5982e-05	Comp 28	6.8834e-06
Comp 5	1.3826e-04	Comp 17	2.0663e-05	Comp 29	5.8778e-06
Comp 6	1.2716e-04	Comp 18	1.9032e-05	Comp 30	5.3840e-06
Comp 7	1.0485e-04	Comp 19	1.7089e-05	Comp 31	5.1515e-06
Comp 8	8.3060e-05	Comp 20	1.6443e-05	Comp 32	4.3090e-06
Comp 9	6.3351e-05	Comp 21	1.5319e-05	Comp 33	4.0817e-06
Comp 10	5.2690e-05	Comp 22	1.2584e-05	Comp 34	3.2900e-06
Comp 11	5.1128e-05	Comp 23	1.1578e-05	Comp 35	2.6786e-06
Comp 12	4.7947e-05	Comp 24	1.0350e-05	Comp 36	1.8544e-06

Table 4.16: Percentage of Total Variance Explained by Principal Components of *S. hosei*.

Component	% Variance	Component	% Variance	Component	% Variance
Comp 1	23.83%	Comp 13	1.37%	Comp 25	0.36%
Comp 2	19.95%	Comp 14	1.28%	Comp 26	0.32%
Comp 3	9.00%	Comp 15	1.18%	Comp 27	0.31%
Comp 4	8.26%	Comp 16	1.03%	Comp 28	0.27%
Comp 5	5.51%	Comp 17	0.82%	Comp 29	0.23%
Comp 6	5.07%	Comp 18	0.76%	Comp 30	0.21%
Comp 7	4.18%	Comp 19	0.68%	Comp 31	0.21%
Comp 8	3.31%	Comp 20	0.65%	Comp 32	0.17%
Comp 9	2.52%	Comp 21	0.61%	Comp 33	0.16%
Comp 10	2.10%	Comp 22	0.50%	Comp 34	0.13%
Comp 11	2.04%	Comp 23	0.46%	Comp 35	0.11%
Comp 12	1.91%	Comp 24	0.41%	Comp 36	0.07%

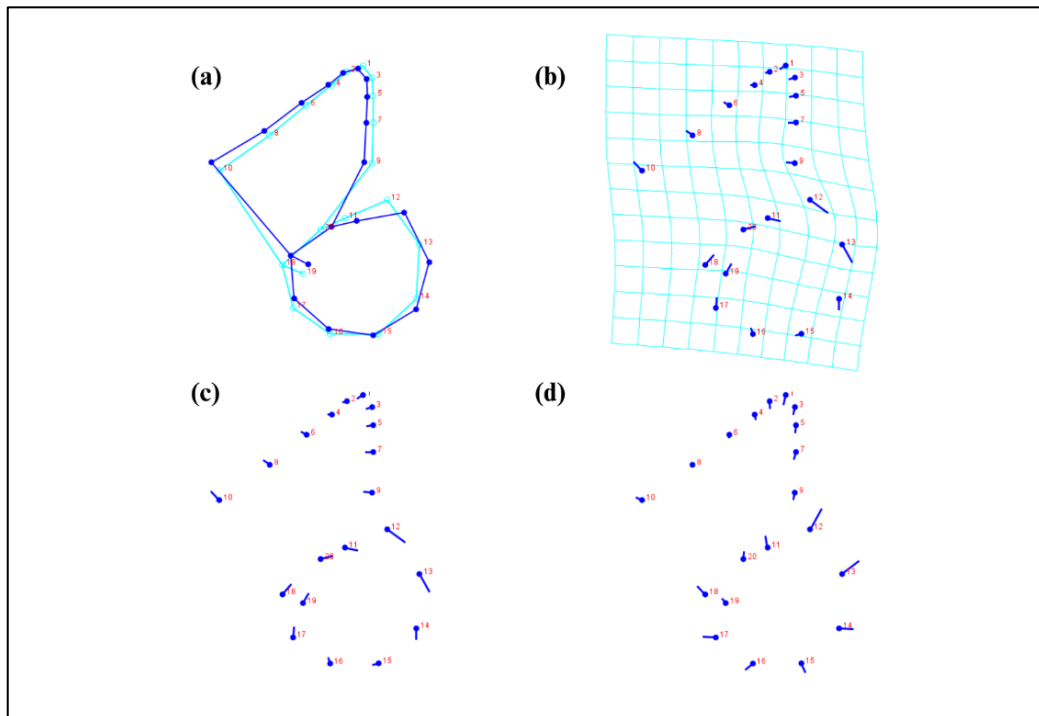


Figure 4.10: Shape changes of the *Stomacosphis hosei* based on landmark data for all combined locality. (a) The mean shape represented by the wireframe graph

that is averaged across all individual shapes. (b) The transformation grid dimensions of PC1 shows the number of grids used to construct the deformation grid. Lollipop graph based on Principal Component Analysis (PCA) illustrating the differences between PC1 (c) and PC2 (d) where they display the loadings of each variable.

4.2.3 Shell measurement analysis of *Stomacosphis hosei*

PCA on shell measurement shows 67.4% variation on the first principal axis with inertia coefficients indicating that populations with high factor score on PC1 have negative values for all measurements which means that all the original variable changes together in negative direction. Meanwhile, PC2 (12.5%) with inertia coefficients revealed shell diameter (D), apertural width (AW), and apertural height (AH) shows negative coefficients on shell (Table 4.17). In Figure 4.11, some specimens from Padawan (Temurang) are likely far from the other specimens is due to the effect of the other morphological characters. The PCA does not only interpreted for size but also captures variances across all measured traits such as shell shape or other structural traits. The specimens from Padawan (Temurang) might have different grouping of traits that push them to be clustered farther from others.

Table 4.17: Relative contribution of each variable to the inertia of first two principal component analysis component in PCA for *S. hosei*.

Measurements	PC1	PC2
Shell diameter (D)	- 0.2653218	- 0.02891755
Shell height (H)	- 0.2863573	0.01792944
Apertural width (AW)	- 0.2633938	- 0.05720317
Apertural height (AH)	- 0.2449398	- 0.06029372
Width of spire (SH)	- 0.2412182	0.06079979
Height of spire (SW)	- 0.2602745	0.01898319
Width of protoconch (PW)	- 0.2171839	0.05878273

Table 4.18: Eigenvalues and percentage of variance explained by principal components of *S. hosei*.

Principal Component	Eigenvalue	% Variance Explained
PC1	4.7211	67.44%
PC2	0.8737	12.48%
PC3	0.4871	6.96%
PC4	0.3315	4.74%
PC5	0.3023	4.32%
PC6	0.1706	2.44%
PC7	0.1138	1.63%

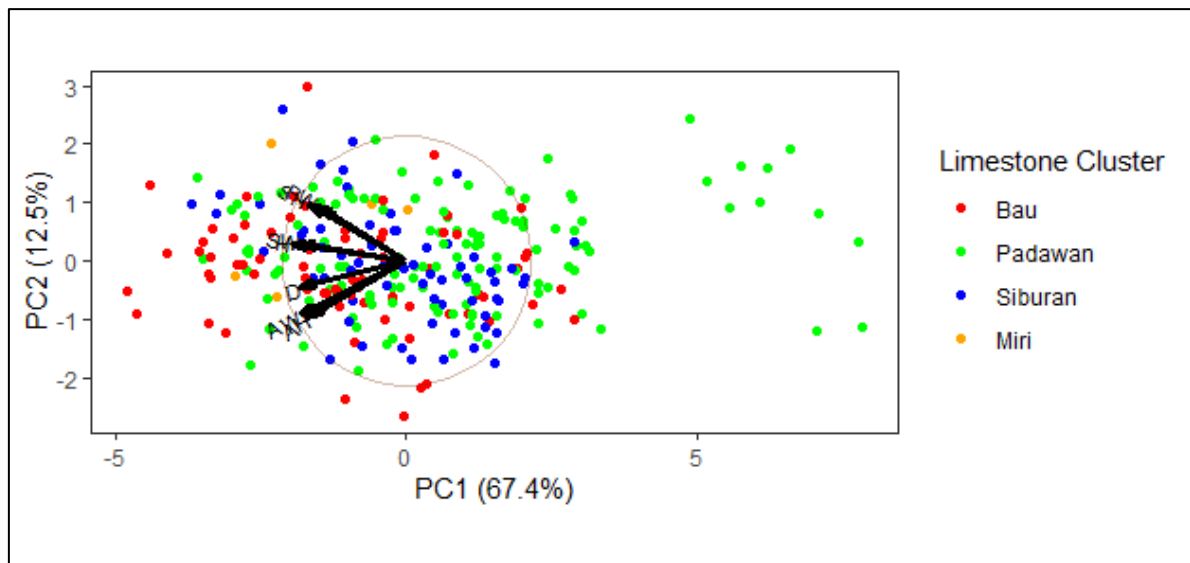


Figure 4.11: The Principal Component Analysis (PCA) of shell metrics demonstrated that the first principal axis, accounted for 67.4% of the variation in the first principal axis and 12.5% in the second principal axis.

In addition, from PCA it shows that shell height (H) is the most contributing variable on both dimensions, indicating its significant impact across all components, which suggested it could be used as proxy for overall shell size (Table 4.19). Thus, the shell height (H) was

used as covariate in regression analyses of all remaining linear metrics of shell size. All six shell measurements showed different relationships with the shell height (H) when comparing p-value for the difference in slope between region and the entire dataset. 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 shell height (H) strongly influences other shell characteristics (Figure 4.11).

Table 4.19: 'cos2' values for each variable across the first two principal components in the PCA for *S. hosei*.

Measurements	Dim 1	Dim 2	Total
Shell diameter (D)	0.5149941	0.382512775	0.8975068
Shell height (H)	0.8090831	0.101149990	0.9102331
Apertural width (AW)	0.8053802	0.074208775	0.8795889
Apertural height (AH)	0.7070578	0.002808992	0.7098668
Width of spire (SH)	0.5353337	0.313600217	0.7928540
Height of spire (SW)	0.6876505	0.105203464	0.8489339
Width of protoconch (PW)	0.5798815	0.081131772	0.6610133

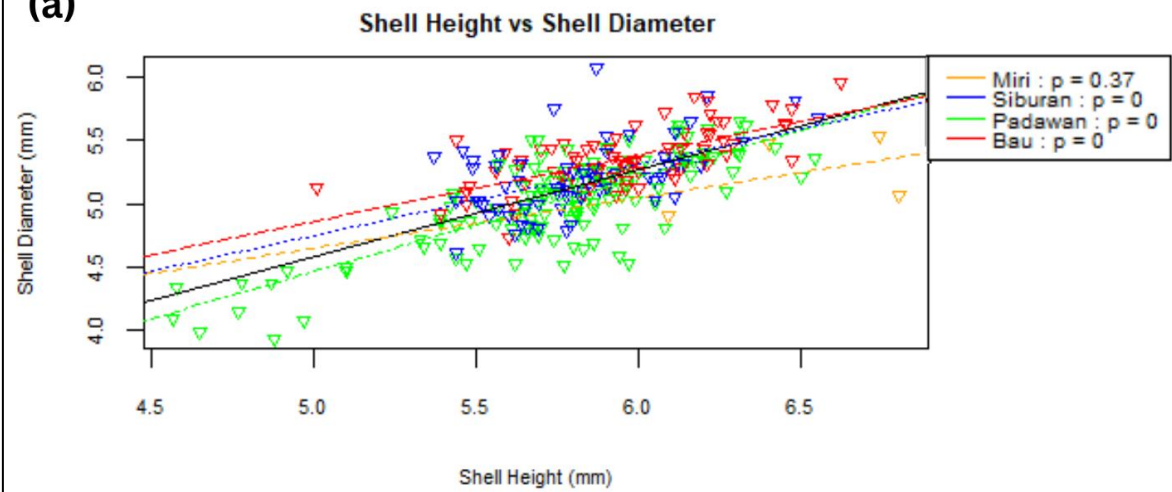
When region effects examined, specimens from all limestone cluster except for Siburan and Miri exhibited the most statistical differences ($p < 0.05$) where the result shows there are significant correlation between the shell height (H) and the other morphological measurements. In contrast, cluster Siburan, demonstrated more complex relationship between the shell height (H) and the other morphological measurements. It shows significant correlation between shell height (H) with all five measurements except for the relationship between shell height (H) and apertural height (AH) (Figure 4.12b). This insignificant correlated apertural height (AH) in cluster Siburan suggest that other factors that cause the aperture size change differently from the other clusters.

For cluster Miri, the result was more striking as there is no significant correlation were found between shell height (H) with all five morphological measurements except for the relationship between shell height (H) and height of spire (SH) (Figure 4.12d). This finding suggests that height of spire (SH) is the only morphological traits that strongly connected with shell height (H). On the other hand, when comparing shell height (H) and width of protoconch (PW) the regression line shows a downward trend, indicating that as the shell high increases, the width protoconch decreases (Figure 4.12f). This unusual pattern of correlation observed might be influenced by certain environment factors such as habitat or climate or ecological conditions, given that Miri is geographically distant from the other clusters.

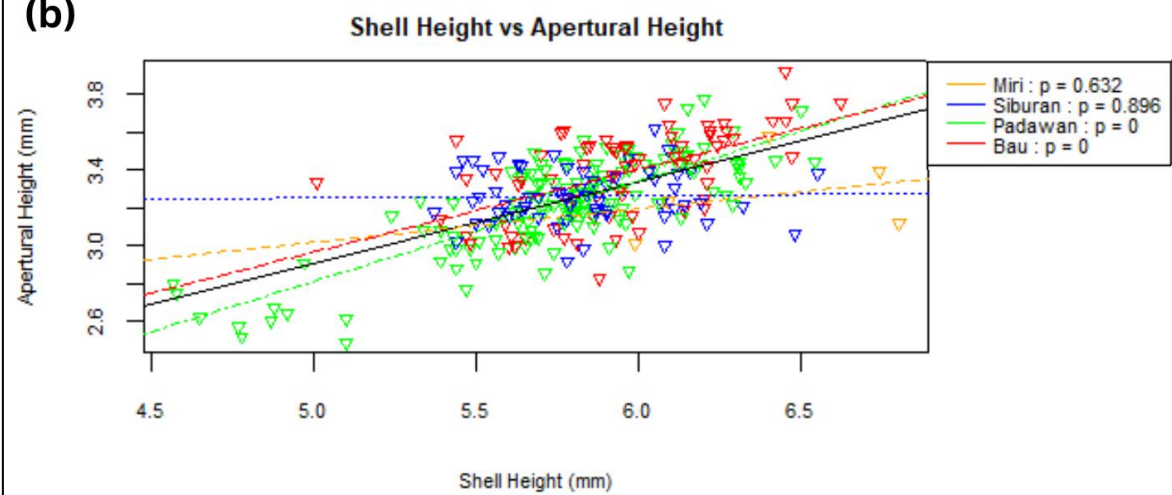
Table 4.20: R^2 and p values for linear regression of Shell Height (H) against other measurements across different regions.

Variables	Miri	Siburan	Padawan	Bau
H vs D	0.270 ($p = 0.37$)	0.266 ($p = 0$)	0.618 ($p = 0$)	0.439 ($p = 0$)
H vs AH	0.086 ($p = 0.362$)	0 ($p = 0.896$)	0.630 ($p = 0$)	0.320 ($p = 0$)
H vs AW	0.397 ($p = 0.255$)	0.172 ($p = 0.001$)	0.652 ($p = 0$)	0.391 ($p = 0$)
H vs SH	0.185 ($p = 0.027$)	0.553 ($p = 0$)	0.687 ($p = 0$)	0.579 ($p = 0$)
H vs SW	0.845 ($p = 0.469$)	0.674 ($p = 0$)	0.604 ($p = 0$)	0.430 ($p = 0$)
H vs PW	0.335 ($p = 0.307$)	0.326 ($p = 0$)	0.509 ($p = 0$)	0.575 ($p = 0$)

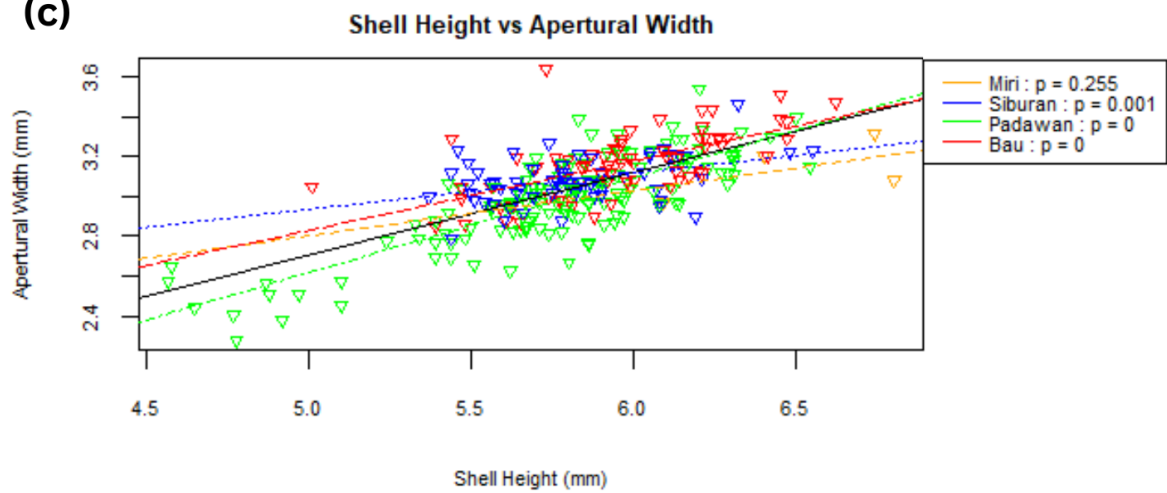
(a)



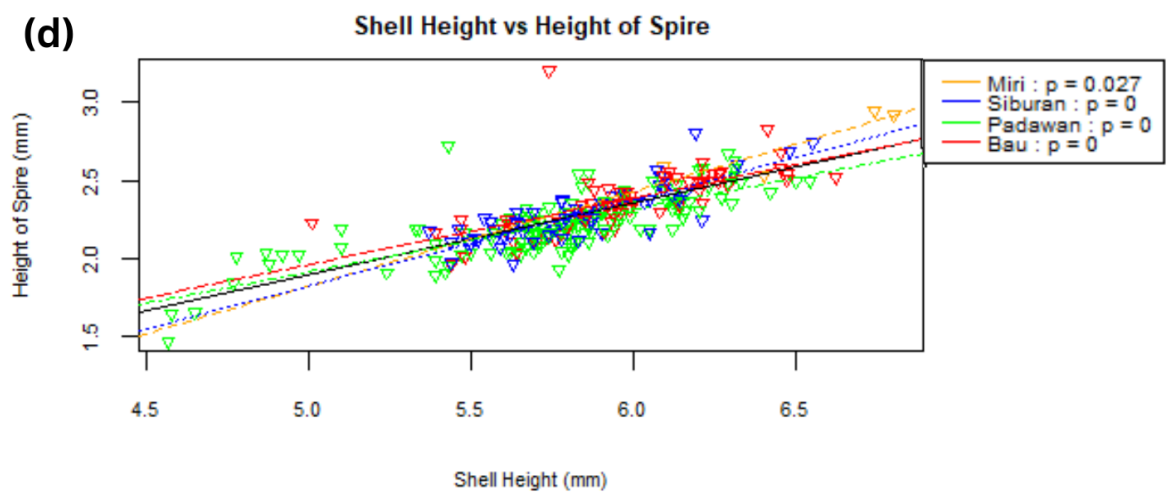
(b)



(c)



(d)



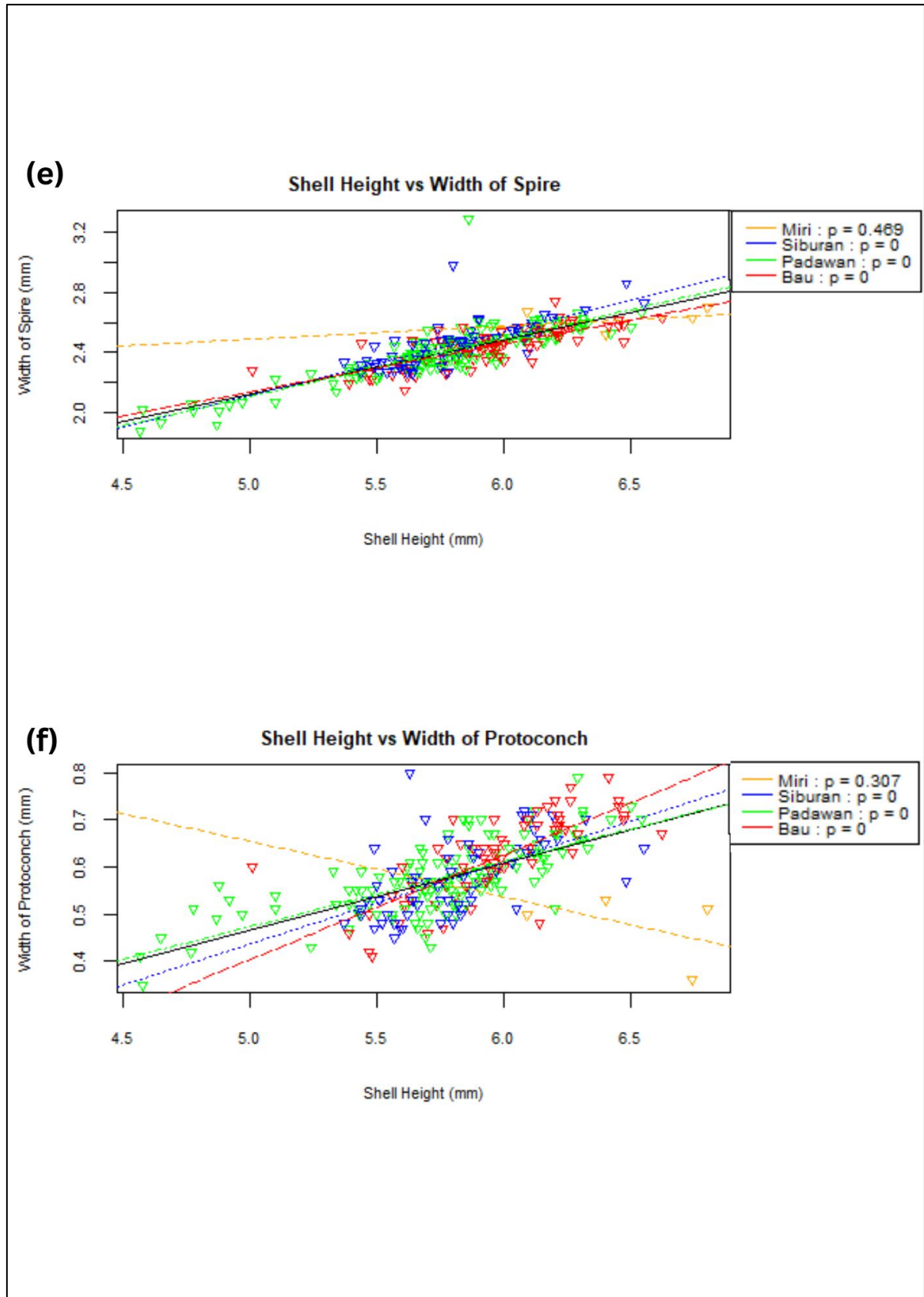


Figure 4.12: Regressions analyses of shell characters of *Stomacosmethis hosei* from different regions with the dependent variable being apertural width. (a) Shell

Height vs Shell Diameter. (b) Shell Height vs Apertural Height. (c) Shell Height vs Apertural Width (d) Shell Height vs Height of Spire. (e) Shell Height vs Width of Spire. (f) Shell Height vs Protoconch Width.

CHAPTER 5

DISCUSSION

5.1 Morphological variation of the genus *Pincerna*

Shell size of *Pincerna* can differ significantly among species or populations due to environmental factors such as habitat preferences (Chiba, 2002; Hausdorf, 2003) or predator pressure (Baur, 1988; Paul, 1991; Perry & Arthur, 1991). Variation in shell shape and shell colouration are reflecting of adaptations to microhabitats and their environments such as soil type or humidity (Martin & Sommer, 2004).

5.1.1 The distribution of the genus *Pincerna* in Sarawak

Since *Pincerna globosa* is the only known species of *Pincerna* found in Sarawak and considered as an endemic species to Borneo. Interestingly, despite being widely reported in Sarawak by Marzuki et al. (2021), *P. globosa* was not observed in limestone cluster Miri or Niah during this study. This absence could be attributed to environment changes, geographical factors or sampling limitations.

Pincerna globosa only can be found in specific ecological niches, favouring areas with dense vegetation and shades areas. These habitats provide the humidity and protection for the snail's survival. Vegetation and soil are also the factors to explain the land snail distribution and abundance (Wehner et al., 2019). In some cases, as the plant biomass increase (denser vegetation), it creates physical barriers that make it more difficult for the land snails to move through. However, it also provides them with an advantage by making it harder for the predators to locate them (Wehner et al., 2019). In dry soils, the snail density and species richness are low. Nevertheless, in soils with high moisture, both density and richness species increase as pH rises (Martin & Sommer, 2004). In addition, some

populations of *P. globosa* can be observed at the base of the limestone cliffs where the combination of moisture and mineral composition supports their habitat. The observation revealed that *P. globosa* that was collected from limestone rocks is very little. This could be due to the presence of little-disturbed limestone habitats on some localities as undisturbed limestone habitats support more land snails since they were known for their high diversity (Boonmachai et al., 2023).

Based on the Shapiro-Wilk normality test, the data on *P. globosa* distribution were normally distributed ($p > 0.05$), suggesting reliable insights into their geographic spread. Strikingly, they are dominantly widespread in the western part of Sarawak with no *P. globosa* observed or identified in the limestone cluster Miri and Niah (Table 4.2). This variation that spotted in *P. globosa* distribution within western part of Sarawak highlights the potential for similar patterns of variability to happen in the other population across Southeast Asia, which influenced by several factors such as geological factors, environmental condition and ecological pressures.

For instance, the genus *Mandarina* from Bonin Islands, Japan shows significant variation both morphologically and ecologically (Chiba, 2002). In some populations with fewer *Mandarina* adapt by expanding their habitat range and trait diversity which leading to high ecological and morphologically variation. This variation is influenced by ecological interaction and the interspecific competition of species in specific environment. The viviparid snail *Cipangopaludina chinensis* is a widely distributed freshwater gastropod in Taiwan also shows variation in shell morphology (Chiu et al., 2002). This variation is affected by ontogenetic allometric growth and environmental influences and it showed spire height is the most significant trait driving variation among populations. These examples highlight some

the role of ecological interactions and environmental factors that influence the shaping of shell morphology within the genus of *Pincerna*.

5.1.2 Shell shape variation of *Pincerna globosa*

The geometric morphometric analyses show variations among populations of *P. globosa*, with landmark analysis revealing significant variation across regions including Bau, Padawan, and Siburan (see Figure 4.2). The obtained results demonstrate a clear size and shape differences between shell specifically from the Padawan where they tend to have taller aperture whereas, Siburan and Bau have rounded to wider apertural opening (see Figure 4.1). The PCA analysis from landmark analysis also show 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 suggesting a correlation with the width of the aperture. The aperture index is significant for size (Appendix C) which support the importance of LM10 and LM15 for shape.

These differences are similar with a study by Madec et al. (2003) where they examined different population of *Cornu aspersum*, a type of land snails and they show significant variation based on shell shape and size. Other than geographical pattern, environmental factors including temperature and rainfall may contribute the populations to vary in size and shell shape where it could be related to niche traits (Sherratt et al., 2017; Khalik et al., 2019). The weather report from Malaysian Meteorological Department between 2017 and 2021 reported that Bau, Padawan and Siburan show average of 3400-4600 mm rainfall and temperature ranging from 21 to 37 °C which indicates moist and humid environment during this period. However, the data for the years 2022 and 2023 are currently unavailable. This condition is common in tropical regions where they have high levels of rainfall and humidity. This environment is well-suited for land snail since they tend to be located in microhabitats

that have low humidity and/or high temperature to maintain the moisture levels (Moreno-Rueda et al., 2009).

The variations in shell size and shape among populations could also be due to different temperature and ecological condition (Anderson et al., 2007; Sherratt et al., 2017) and the light intensity that could affect the pigmentation of the shell (Khalik et al., 2019). Morphological variation for gastropod shells also may change from genetic differences 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 are influenced by moderate temperature around $18 \pm 1^\circ \text{C}$ and moist environment (Stringer et al., 2014). The observed morphological differences between among populations of a species are probably due to random ecological variations or differences in the environments in which these populations exist. Similarly, it is suspected that variation in *P. globosa* in different population of among studied population could be due to random ecological variation or cryptic diversity of the genus *Pincerna*. The different limestone habitats have characterised by the dense vegetation and limited sunlight exposure which could create condition that influence the shell morphology and ecological adaptation within this genus.

5.1.3 Shell size variation of *Pincerna globosa*

The PCA results based on measurement of *P. globosa* show all the variables are positively correlated with each other along the principal component which indicate there are larger in shell size with globose shell and wide aperture. Globose shell has been interpreted as accommodate for them to climb vertical vegetation (Heller, 1987) since they mostly can be found at vegetation area. As a result, they tend to have large foot and large-mouthed shell.

While from the graph (Figure 4.3) also shows that the individual from all regions displays overlapped based on their shell measurement which indicates that shell size alone may not enough to differentiate between populations. The specimens that were far from the others could have high or low values for certain shell metrics compared to the rest of dataset causing them to appear distance from others.

The pattern of shell size and shape within specific geographic regions expose the correlation or similarities between the physical characteristic of the shells and geographic locations to identify the process that effect the shell morphology variation. For instance, Johnson and Black (1998) and Chiba (1999) demonstrate that environment factors and geographic isolation can influence the morphological variation in land snails which supporting the idea that specific regions can affect the shell characteristic. 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 where there are no significant different in the Padawan and 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 examined in different geographical or across regions.

In addition, the morphological shell size parameters shows that *P. globosa* in Padawan have relative largest shell size compared to the *P. globosa* in Bau and Siburan since Padawan population characterized by a higher mean shell size indicates they possess larger shells (Appendix E). Additionally, snails in higher population densities may exhibit smaller adult sizes (Goodfriend, 1986; Baur, 1988), and this effect is believed to be influenced by the impact of pheromones on their growth rates which also observed in *P. globosa*. Padawan had the least number of specimens compared to Bau and Siburan, meaning the higher is the value of the population, the more likely that the size of the individual is to reduced. This can

be observed in study conducted by Anderson et al. (2007), where the density also affects adult *Oreohelix cooperi* by measured number of shells found 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 and Koene, 2015) and average rainfall (Hausdorf, 2006; Rangarajan et al., 2013) can also affect the shell size of *Pincerna*.

5.2 Morphological variation of the genus *Stomacosphthis*

Morphological variation is often reflecting on adaptation of species or populations to specific habitats such as limestone karst environments, shaded areas or humid surroundings (Schilthuizen et al., 2003; Boonmachai et al., 2023). *Stomacosphthis* species usually can be found covered with dirt as seen in *S. hosei*, act as one of the defences mechanisms from predators or ecological adaptations (Schilthuizen, 2003; Marzuki et al., 2021).

5.2.1 The distribution of the genus *Stomacosphthis* in Sarawak

The genus *Stomacosphthis*, represented by species *S. hosei*, *S. galbanus* and *S. sadongensis* are known to be found in Kuching, Sadong and Serian divisions of Sarawak and is endemic to Borneo too (Páll-Gergely et al., 2020; Marzuki et al., 2021). This species has been spotted entirely within this clusters, highlighting its geographical range. Furthermore, according to Páll-Gergely et al. (2020), *S. galbanus* has been recorded in Niah National Park where they are known to be highly abundant. Consistent with these findings, our study also identified these species in the same clusters, which make Niah NP plays a crucial role in this species that may be influenced by the specific ecological factors within the area. These finding highlight the ecological importance and unique distribution of the species within the Borneo landscape.

All species under the genus *Stomacosmethis* has been consistently observed on limestone rocks in shaded areas, where it is not directly exposed to the sunlight. In addition, the shell of *Stomacosmethis* is often covered with dirt which is difficult to remove. This coating likely functions as a form of camouflage or defence (Schilthuizen et al., 2003), helping to protect the species from predators by blending them into their rocky surroundings. In addition, *Stomacosmethis* is usually foraging on the wet limestone rock surfaces that covered with lichens. These snails often observed foraging also on wet vertical limestone rock surfaces covered with mosses and lichens (Marzuki et al., 2021). The limestone areas have high calcium carbonate content, as Boonmachai et al. (2023) highlight, these areas provide the essential nutrients needed for the land snail's survival and reproduction. Limestone has a high pH, which provide snails to preserve their shells for a longer period of time, so they are not easily break down (Schilthuizen et al., 2003).

Based on the Shapiro-Wilk normality test, the data on the distribution of *Stomacosmethis* species were normally distributed ($p > 0.05$), and indicating reliable insights into their geographic spread. In limestone cluster Miri and Niah could not be identified because they only have one locality in one cluster which resulting in NA (not available) for the p-values (Table 4.12). This variation in the distribution of *Stomacosmethis* within Sarawak suggests that alike patterns of variability possibly will be present in the other population across Southeast Asia, potentially influenced by several factors such as geological factors, environmental condition and ecological pressures.

For instant, there is morphological variation on *Bradybaena similaris* in China, there were several subspecies have been identified (Nurinsiyah & Hausdorf, 2018). This shows that China has the highest species richness in in this species but these subspecies are geographically overlapped which they cannot be considered separate geographical subspecies.

The study on *Pomacea canaliculate*, a freshwater apple snail in Maninjau Lake, West Sumatra, found that shell size (length and width) varies based on land use and water quality (Takarina et al., 2023). They spotted the larger shell sizes tend to be found in forest and paddy field where the shell length and width are influenced by environmental factor like temperature.

5.2.2 Shell shape variation of *Stomacosmethis hosei*

For *Stomacosmethis hosei*, the geometric morphometric analyses indicate variations among population when comparing specimens across different limestone clusters including Bau, Padawan, Siburan and Miri (see Figure 4.8). The landmark analysis revealing significant variation in shell morphology, showing how populations from different clusters displayed unique size and shape features. The findings prove that morphological variation between population from different clusters emphasizing how geographical and environmental factors contributes to this variation within the species.

The results demonstrate a clear size and shape differences between shell specifically from different limestone clusters. Specifically, the Bau cluster is characterised by *S. hosei* with a higher and wider aperture. In contrast, *S. hosei* from limestone cluster Padawan and Miri display apertures that rounded to wider in shell (see Figure 4.8). The different in the aperture morphology may be a response to the environmental conditions such as humidity, mineral composition of the limestone, moister or competition. The PCA analysis result from landmark analysis of *S. hosei* also provide valuable insight into morphological variation within this species. Specifically, the PC1 is significantly influenced the median lip point (LM13) and the highest point of the lip (LM12). This finding indicates the PC1 is strongly connected with the aperture of the shell as these two landmarks play a crucial role in defining

the aperture shape and structure. The aperture index is significant for size (Appendix F) which support the importance of LM13 and LM12 for shape.

The aperture of some land snails is different based on their function like accommodation, where for this species they tend to have planar aperture (McNair et al., 1981), means flat and they align closely to the hard substrate such as rocks. The snail's strong foot evolved to be well adapted to this flat shell opening and can make them hanging or cling to the rock tightly. The small aperture usually can be found in the drier environment, so they can retain moisture for survive in dry condition (Goodfriend, 1986). While for bigger opening aperture, they provide protection where the snails extend their body and allow more movement but can easily expose to predator as they move slower (Paul, 1991).

In gastropods, the aperture shape is one of the morphological traits that serve to defend against predators (Wada & Chiba, 2013). They explained that the modification of aperture shape of land snails helps them escape from eaten by predators such as snakes which makes them harder to extract from the shells. Some micro snails also developed another survival skill as they can survive in the predator's digestive system with a calcareous epiphragm (Wada & Chiba, 2013). The observed morphological differences between among populations of *S. hosei* are likely influenced by random ecological variations or differences in the environmental pressure in which these populations exist. The diverse limestone habitats have characterized by the abundant of limestone karsts, which create condition that influence the shell morphology and ecological adaptation within this genus.

5.2.3 Shell size variation of *Stomacosmethis hosei*

The PCA results for *Stomacosmethis hosei* reveals interesting insights into the morphological variation of this species. Specimens from all limestone clusters display overlapped pattern in the PC1 based on their shell measurement which indicates that shell size

alone may not sufficient to fully differentiate shell variation among populations. However, the observation on *S. hosei* from Padawan (Temurang) is slightly separated from the others and does not overlap with the other species in the PCA plot suggest that this population has distinct morphology compared to the other. This separation suggests that the Padawan clusters possesses a different morphology that make it apart from the other clusters, possibly driven by environmental or ecological conditions. Body size measurement of land snail also can be influence by geographical factors such as latitude and altitude. For instance, Hausdorf (2003) studied that the north-west European land snail, is significant different in body size where they decrease in shell size when the latitude is higher and vice versa. Latitude influences only a small amount of body size variation (Hausdorf, 2003) which suggesting other factors such as environmental condition or habitat characteristic likely to play crucial role in driving morphological differences.

However, PCA result (Figure 4.8) based on measurement of *S. hosei* shows all the variables are negatively correlated with each other along the principal component, suggests that the measured traits indicated inversed relationship as one variable increases, the others tend to decrease. Our findings of the regression correlation between the shell height (H) with all six morphological measurements from *S. hosei* were revealed, showing that the relationship between shell height and shell size is consistent across the population studied. Specifically, the analysis reveals that there are no significant different among population in limestone cluster of Siburan and Miri (see Figure 4.9). However, there could be differences in how strong the specific characteristics of this correlation such as aperture size and shape may vary when examined shell measurement in different geographical regions. This could suggest that ecological or environmental conditions are affecting the aperture size in these populations.

This phenomenon can be caused by phenotypic plasticity concept (Chiba, 1999) on *Mandarina* snails that shows significant different on shell shape and size depends on their population and trade-offs (DeWitt et al., 2000) where the parameter increases either shape or size then decrease in other traits which reflecting underlying environmental constraints affecting the species' morphology. For instance, Goodfriend (1986) observed that *Pleurodonte lucerne* have larger shells are relatively thinner shell where this explain that a negative allometric relationship in shell size thickness especially in drier areas.

5.3 The morphological variation on the genera *Pincerna* and *Stomacosmethis*

The geometric morphometric analyses of both *Pincerna globosa* and *Stomacosmethis hosei* reveal variations in shell size and shape across different population in Sarawak. The comparison between these two species highlights how specific characters in landmarks and their corresponding in PCA results reflect the morphological variation in their shells. The geometric morphometric analyses shows that *P. globosa* and *S. hosei* from different limestone clusters shares the same variation which focusing the part of the aperture where they have higher and to wider apertural openings. This supported by the landmark analysis where the PCA result suggest the PC1 is primarily influenced by the highest point of the lip (LM10) and the external point of the lip at the left profile of the shell (LM15) for *P. globosa* and the highest point of the lip (LM12) for *S. hosei*.

The PCA results for both *P. globosa* and *S. hosei* also shows that the individual from all limestone clusters display overlapped based on their shell measurement which indicates that shell size alone may not sufficient to differentiate shell variation among populations. This observation also shows that *P. globosa* and *S. hosei* species with smaller number of populations tends to have larger or taller shell sizes. Additionally, snails in higher population densities may exhibit smaller adult sizes (Goodfriend, 1986; Baur, 1988), and this effect is

believed to be influenced by the impact of pheromones on their growth rates which also observed in *P. globosa* and *Stomacosmethis*. Limestone cluster in Padawan (*P. globosa*) and Miri (*S. hosei*) has the least number of specimens compared to other regions, which indicate the higher is the value of the population, the more likely that the size of the individual to reduced (see Appendix H & G). This can be observed in study conducted by Anderson et al. (2007), where the density also affects adult *Oreohelix cooperi* by measured number of shells found 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 and Koene, 2015) and average rainfall (Hausdorf, 2006; Rangarajan et al., 2013) can also affect the shell size of *P. globosa* and *S. hosei*.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study has shown that the genus *Pincerna* and *Stomacosphthis* display a normal distribution in their particular habitats. Interestingly, for *S. galbanus* stands out as the only species of *Stomacosphthis* uniquely found in Niah National Park, consistent with its reported distribution. Our study also has shown that shell shape and size of *P. globosa* and *S. hosei* varies among populations. The finding has shown that there are significant differences between the morphological characters (shape and size) of these five-limestone cluster (Bau, Padawan, Siburan, Miri and Niah) of different localities of the species *P. globosa* and *S. hosei*. For *P. globosa*, there is significant shell shape differences of LM10, the highest point of the lip and LM15, the external point of the lip at the left profile of the shell, among three difference limestone cluster (Bau, Padawan and Siburan). As for shell measurement, the most variable characters are the aperture height (AH) and aperture width (AW). While, *S. hosei* shows significant difference of the median lip point (LM13) and the highest point of the lip (LM12) across four difference limestone (Bau, Padawan, Siburan, and Miri). For shell measurement, the most variable traits are shell height (H) and shell diameter (D).

In environment where resource is abundant and competition is low; the body size would directly influence by resource availability. Although this study demonstrates variations in both geometric and morphometric but the factors that lead to the variation among population still remain untested. In term of shell size measurement, it seems that population density does not affect the size of the shell. For example, when comparing the high-density population with low-density population of *P. globosa* and *S. hosei* from both areas possesses

similar shell size although they have different population density. Therefore, this study cannot conclude that population density affects shell size at least for these two species.

6.2 Recommendations

From this study, it shows that these species offer valuable ecological insights as they can adapt to diverse environment condition, serve as bioindicators and species identification which highlighting their importance in both ecological and conservation. It is suggested that a broader range of environmental factors needs to be considered when interpreting morphological variation within *Pincerna* and *Stomacosphthis* between populations. The observed differences in aperture size in both genera need for further research to explore more detail on ecological and microhabitat behind this variation to aim better understanding on the factors influencing the morphological variation across geographical regions. These finding highlight the potential of these land snails to offer valuable ecological insights as they can adapt to diverse environment condition, serve as bioindicators and assist in species identification underscore their importance in both ecological and conservation efforts. Future research could also incorporate molecular assessment of *P. globosa* and *S. hosei* of different populations to explain the possible morphological divergent and cryptic diversity. It is also suggested to explore the possibility of microclimatic parameters as this could be important in shaping the shell and size of *P. globosa* and *S. hosei*.

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APPENDICES

Appendix A: Analyses of Variance (ANOVA) for seven shell measurement for *Pincerna globosa*.

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		

Appendix B: Analyses of Variance (ANOVA) for seven shell measurement for *Stomacosphthis hosei*.

Measurements		Df	Sum Sq	Mean Sq	F value	Pr(>F)
Shell diameter (D)	Region	3	5.298	1.7660	17.77	1.46e-10
	Residuals	271	26.926	0.0994		
Shell height (H)	Region	3	3.233	1.0775	9.565	5.03e-06
	Residuals	271	30.528	0.1126		
Apertural width (AW)	Region	3	1.647	0.5489	15.49	2.48e-09
	Residuals	271	9.600	0.0354		
Apertural height (AH)	Region	3	1.339	0.4462	8.774	1.42e-05
	Residuals	271	13.781	0.0509		
Width of spire (SH)	Region	3	1.795	0.5984	16.00	1.32e-09
	Residuals	271	10.137	0.0374		
Height of spire (SW)	Region	3	0.369	0.12287	4.952	0.00231
	Residuals	271	6.723	0.02481		
Width of protoconch (PW)	Region	3	0.1162	0.03873	6.174	0.00045
	Residuals	271	1.6998	0.00627		

Appendix C: t-Test: Paired Two Sample for Means (AW/AH) for *Pincerna globosa*.

	Variable 1	Variable 2
Mean	4.559505	4.51894
Variance	0.226291	0.22224
Observations	283	283
Pearson Correlation	0.918125	
Hypothesized 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	

Appendix D: t-Test: Paired Two Sample for Means (AW/AH) for *Stomacosmethis hosei*.

	Variable 1	Variable 2
Mean	3.053164	3.270109
Variance	0.041047	0.055182
Observations	275	275
Pearson Correlation	0.81071	
Hypothesized Mean Difference	0	
df	274	
t Stat	-26.0579	
P(T<=t) one-tail	1.95E-76	
t Critical one-tail	1.650434	
P(T<=t) two-tail	3.9E-76	
t Critical two-tail	1.96866	

Appendix E: Range and mean for sample group of *Pincterna globosa* based on different regions. 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).

Lime-stone cluster	Number of individuals (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

Appendix F: Range and mean for species *Stomacosphthis* based on localities. Measurements of the shell of *Stomacosphthis*: 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).

Limestone clusters	Number of individuals (N)	D	H	AW	AH	SW	SH	PW
<i>S. hosei</i>	275	6.08-3.93	6.80-4.57	3.64-2.28	3.92-0.33	3.29-1.88	3.21-1.47	0.80-0.35
Range		2.15	2.23	1.36	3.59	1.41	1.74	0.45
Mean		5.160	5.834	3.053	3.259	2.473	2.281	0.585
SD		0.342	0.351	0.202	0.294	0.160	0.208	0.081
SE		0.021	0.021	0.012	0.017	0.009	0.012	0.005
<i>S. sadongensis</i>	47	5.48-4.33	5.67-4.49	2.95-2.16	2.69-1.93	2.56-1.96	2.34-1.62	0.69-0.32
Range		1.15	1.18	0.79	0.76	0.6	0.72	0.37
Mean		4.882	4.992	2.508	2.294	2.228	1.960	0.486
SD		0.261	0.267	0.159	0.172	0.138	0.143	0.091
SE			0.038	0.039	0.020	0.021	0.013	
<i>S. galbanus</i>	33	6.73-5.86	6.41-4.92	3.75-3.08	3.72-2.52	3.88-2.57	2.48-1.71	0.81-0.41
Range		0.87	1.49	0.67	1.20	1.31	0.77	0.40
Mean		6.29	5.592	3.388	3.280	2.765	2.064	0.591
SD		0.241	0.318	0.195	0.245	0.225	0.145	0.069
SE		0.042	0.055	0.034	0.042	0.039	0.025	0.012

Appendix G: Range and mean for species *Stomacosphis hosei* based on localities. Measurements of the shell of *S. hosei*: 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).

Limestone clusters	Number of individuals (N)	D	H	AW	AH	SW	SH	PW
Bau	72	5.96-4.73	6.62-5.01	3.64-2.85	3.92-2.38	2.74-2.15	3.21-1.96	0.79-0.41
Range		1.23	1.61	0.79	1.09	0.59	1.25	0.38
Mean		5.357	5.943	3.161	3.382	2.441	2.366	0.614
SD		0.237	0.299	0.167	0.231	0.125	0.196	0.088
SE		0.027	0.035	0.019	0.027	0.015	0.023	0.010
Padawan	135	5.65-3.93	6.54-4.57	3.54-2.28	3.77-2.49	3.29-1.88	2.72-1.47	0.79-0.35
Range		1.72	1.97	1.26	1.28	1.41	1.25	0.44
Mean		5.029	5.760	2.979	3.214	2.398	2.221	0.577
SD		0.362	0.381	0.224	0.253	0.177	0.198	0.074
SE		0.031	0.032	0.019	0.021	0.015	0.017	0.006
Siburan	63	6.08-4.61	6.55-5.37	3.46-2.79	3.62-2.92	2.98-2.26	2.80-1.96	0.8-0.45
Range		1.47	1.18	0.67	0.70	0.72	0.84	0.35
Mean		5.212	5.822	3.082	3.262	2.459	2.281	0.578
SD		0.285	0.261	0.113	0.138	0.148	0.175	0.079
SE		0.035	0.032	0.014	0.017	0.018	0.022	0.010
Miri	5	5.53-4.91	6.8-5.99	3.31-2.97	3.58-3.01	2.70-2.52	2.95-2.4	0.55-0.36
Range		0.62	0.81	0.34	0.57	0.18	0.55	0.19
Mean		5.202	6.404	3.122	3.268	2.614	2.678	0.49
SD		0.283	0.367	0.134	0.224	0.077	0.245	0.075
SE		0.126	0.164	0.059	0.100	0.034	0.109	0.034