



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 *Stomacosmethis* 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 *Stomacosmethis* based on their shell characters. In this study, only one selected *Pincerna* species (*P. globosa*) and three species of *Stomacosmethis* (*S. hosei*, *S. sadongensis* and *S. galbanus*) can be found in Sarawak. A total of 283 adults *P. globosa* and 355 adults *Stomacosmethis* (*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 *Stomacosmethis* 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*, *Stomacosmethis*, morphological variation, geometric morphometric, Principal component analysis (PCA)

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

ABSTRAK

Genera Pincerna dan Stomacosmethis 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 Stomacosmethis berdasarkan ciri-ciri cangkerangnya. Dalam kajian ini, hanya satu spesies Pincerna yang dipilih iaitu P. globosa dan tiga spesies Stomacosmethis iaitu S. hosei, S. sadongensis, dan S. galbanus yang ditemui di Sarawak. Sebanyak 283 individu dewasa P. globosa dan 355 individu dewasa Stomacosmethis (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 pengesanan 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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LIST OF ABBREVIATIONS

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 *Stomacosmethis* 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 *Stomacosmethis*, 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, *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.

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.