



UNIVERSITI PUTRA MALAYSIA

***DISTRIBUTION AND MORPHOLOGICAL ASPECT OF
FRESHWATER GASTROPOD FROM NATURAL
ENVIRONMENTS***

SONIA SAMBAN BETTY

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SONIA SAMBAN ANAK BETTY

BACHELOR SCIENCE (HONORS) AQUACULTURE

2023

SONIA SAMBAN ANAK BETTY



**FACULTY OF AGRICULTURAL SCIENCES AND
FORESTRY**

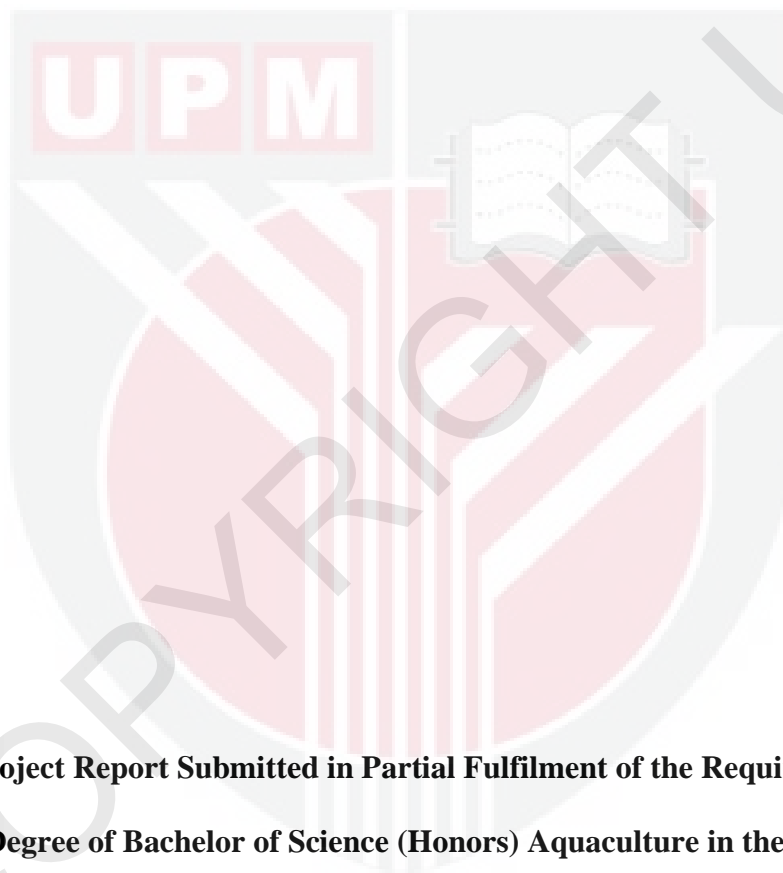
UNIVERSITI PUTRA MALAYSIA

2023

Distribution and morphological aspect of freshwater gastropod from natural environments

By

Sonia Samban Anak Betty



A Project Report Submitted in Partial Fulfilment of the Requirement for the Degree of Bachelor of Science (Honors) Aquaculture in the Faculty of Agriculture Sciences and Forestry University Putra Malaysia Bintulu Sarawak Campus.

2023

ABSTRACT

From March 2022 to January 2023, study on the diversity of freshwater gastropods in their natural environment were done at the University Putra Malaysia Bintulu Campus (UPMKB) at Bintulu and Sg, Tuah, Kanowit at Sibul. This study aims to determine the morphological variations between freshwater gastropod species through morphometric and geomorphometric and to study distribution pattern of freshwater gastropod in natural environment. In this study, 3 stations were chosen, and their coordinates was recorded at each station. The specimen will be identified based on the form and characteristics of the gastropod shell. The distribution of pattern and morphological variation measure through morphological descriptions, shell morphometric analysis and geomorphometric analysis. A total 120 individuals of freshwater gastropod have been recorded from three station. Seven species belong to three family such as Pachychilidae, Nassariidae and Ampullariidae was recorded. Based on result, 12 characteristics showed significantly different levels ($p < 0.05$) which were AL (0.001), WH3 (0.001), WH4 (0.001), WH5 (0.001), AW (0.001), WW7 (0.001), WW8 (0.001), BWW (0.001), AINL (0.001), LPM (0.011), LPU and AWW (0.001). Species of *Sulcospira* sp. and *Sulcospira collyra* having the same character of shell.

ABSTRAK

Dari Mac 2022 hingga Januari 2023, pembelajaran mengenai kepelbagaian gastropod air tawar dalam persekitaran semula jadinya telah dilakukan di Universiti Putra Malaysia Kampus Bintulu (UPMKB) di Bintulu dan Sg, Tuah, Kanowit di Sibul. Kajian ini bertujuan untuk menentukan variasi morfologi antara spesies gastropod air tawar melalui morfometrik dan geomorfometrik serta mengkaji corak taburan gastropod air tawar dalam persekitaran semula jadi. Dalam kajian ini, 3 stesen telah dipilih, dan koordinatnya direkodkan di setiap stesen. Spesimen akan dikenal pasti berdasarkan bentuk dan ciri-ciri cengkerang gastropod. Taburan ukuran corak dan variasi morfologi diukur melalui huraian morfologi, analisis morfometri cengkerang dan analisis geomorfometrik. Sebanyak 120 individu gastropod air tawar telah direkodkan dari tiga stesen. Tujuh spesies tergolong dalam tiga famili seperti Pachychilidae, Nassariidae dan Ampullariidae telah direkodkan. Dua belas ciri menunjukkan tahap berbeza yang signifikan ($p < 0.05$) iaitu AL (0.001), WH3 (0.001), WH4 (0.001), WH5 (0.001), AW (0.001), WW7 (0.001), WW8 (0.001), BWW (0.001), AINL (0.001), LPM (0.011), LPU and AWW (0.001). Spesies *Sulcospira* sp. dan *Sulcospira collyra* mempunyai perwatakan cengkerang yang sama.

ACKNOWLEDGEMENT

Prior to anything else, I would want to express my utmost appreciation and gratitude to my supervisor, Dr Hadi Bin Hamli, for his unwavering support of my degree, as well as for his patience, encouragement, excitement, and vast knowledge. His assistance was invaluable throughout my research and thesis writing. Working and studying under his direction is a great honour and joy.

I would also like to thank the Department of Science and Fisheries staff for their technical assistance. I would also like to convey my gratitude to my partner, Nelina Anak Edmond, for her assistance completing my thesis and sampling with me.

Aside from that, I am incredibly grateful to my parents for their love, prayers, care, and sacrifices for my education and future preparation. I am obliged to their inspiration and affection for the entirety of my academic career.

I would also like to show my appreciation to everyone around me who helped me directly or indirectly with the writing and research for my thesis.

APPROVAL SHEETS

I certify that this research project report entitled “Distribution and Morphological Aspect of Freshwater Gastropod from Natural Environments” has been examined and approved as a partial fulfilment of the requirement for the degree of Bachelor of Science (Honors) Aquaculture in the Faculty of Agricultural Science and Forestry, Universiti Putra Malaysia Bintulu Sarawak Campus.

Dr. Hadi bin Hamli

Faculty of Agricultural Science and Forestry

Universiti Putra Malaysia Bintulu Sarawak Campus

(Supervisor)

Dr. Zamri Bin Rosli

Faculty of Agricultural Science and Forestry

Universiti Putra Malaysia Bintulu Sarawak Campus

Dean

Date: 23/03/2023

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Sarawak is an archipelago in the Indo-West Pacific area of Indo-Malay-Philippines. Sarawak's coastline is approximately 1035 kilometres long (Long, 2014). Sarawak has also been designated a critical biodiversity hotspot in Southeast Asia (Hamli et al., 2013). Due to the area's diverse natural environment, it is claimed that the state of Sarawak is home to a vast number of various aquatic gastropod species. However, most studies indicate that the diversity of gastropods is determined by the properties of the habitat, particularly the sediment's moisture and the water's organic content. According to studies, physicochemical characteristics, climatic circumstances, and soil all affect the diversity of gastropods in different regions or habitats. (Hamli et al., 2013). Wetlands cover 1.24 million hectares in the state, accounting for 13% of the total land area. This marsh supports a rich range of floral and faunal species (Hamli et al., 2012; Idris et al., 2021). According to Hamli et al., 2012 Sarawak is home to a broad range of wetlands, including brackish water, coastal marine, mangrove habitats, seagrass beds, salt and freshwater marshes, freshwater ponds and lakes, and peat swamp forests.

Sarawak, an area with a diversified natural environment, is home to several aquatic gastropod species (Hamli et al., 2013). Sarawak's wetland biodiversity is dependent on the diversity of molluscs. The indigenous inhabitants of Sarawak consume a diverse

range of gastropod species gathered from wetlands. These molluscs supply a large amount of protein in locals' diets, and particular species contribute significantly to the fishing industry (Hamli et al., 2012). As a result, they are an essential component of the food chain (Baharuddin et al., 2017). Several gastropod research papers have been published in Sarawak (Wan Morni et al., 2017). According to Hamli et al., 2013 21 edible gastropod species are sold at wet markets and can be found on certain region such as Kuching, Sibü, Mukah, Bintulu, Limbang, and Lawas.

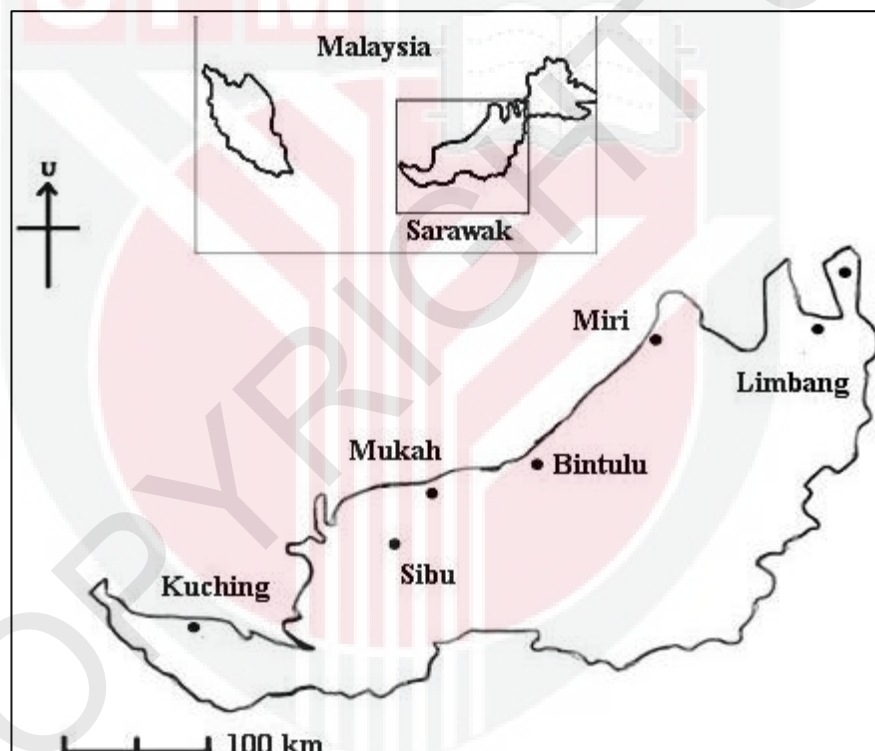


Figure 1.1 Location showing gastropod found at seven division at Sarawak, Malaysia (Hamli et al., 2013)

Mollusca is the second most varied phylum, with over 200,000 species found on land, mountains, rivers, seas, and oceans. Gastropoda is the phylum's largest molluscan class, with seven subclasses (Strong et al., 2008; Wan Morni et al., 2017). Gastropods with an operculum have a single shell. Some species, however, do not have any cover at all (Wan Morni et al., 2017). Gastropods are algal feeders, detritivores, and deposit feeders because they feed on algae that are alive and decomposing, organic detritus, and plant debris. Gastropods are also classified as either epifauna (those that live on solid surfaces) or arboreal (those that live on plants) (Baharuddin et al., 2017).

Except for Antarctica, freshwater gastropods can be found in almost any aquatic ecosystem, including rivers, lakes, streams, marshes, subterranean aquifers, springs, temporary ponds, and drainage ditches. Most freshwater gastropods are micro-herbivores or micro-omnivores that feed on bacterial films, algae, and diatoms (Strong et al., 2008).

1.2 Problem Statements

Although freshwater gastropods contribute significantly to the local economy throughout Sarawak, this wetland resource has yet to be adequately studied. To our knowledge, there are only a few published documents in Malaysia about freshwater gastropods. Indeed, presenting a precise figure of freshwater gastropods species in Sarawak is difficult, as local people may ingest the species of gastropod, they locate (Hamli et al., 2012). However, the knowledge distribution and morphological of natural gastropods is dwindling, particularly among the younger generation, who

prefer to buy rather than forage in natural habitats. To understand gastropod distribution pattern in the natural environment, this project was developed to collect data on freshwater gastropod diversity found in the natural environment. Thus, the results of this study will be used in the future to provide significant knowledge regarding freshwater gastropod species found in the natural habitat environment.

1.3 Objectives

The objective of the current study is:

1. To study distribution pattern of freshwater gastropod in natural environments.
2. To determine the morphological variation between freshwater gastropod species through morphometric and geomorphometric analysis.

CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomy

Hamli et al., (2020) claimed that the gastropods are the most diverse mollusk group in the phylum Mollusca. They account for around 75% of the 110,000 known species of mollusks. Over 50,000 of these species are Caenogastropoda, which primarily inhabit watery environments. Caenogastropoda and pulmonate heterobranchs were often the most prevalent forms of freshwater gastropods. It was stated that there are 49 categories and 364 species of caenogastropod snails.

According to Strong et al. (2007), out of the 409 recognised families of contemporary gastropods, 26 are made up totally or predominantly of freshwater-only taxa. Four families—Neritidae, Assimineidae, Hydrobiidae, and Stenothyridae—have a large taxonomic presence in freshwater biotopes. Caenogastropods are a part of the aquatic flora and fauna. They have numerous distinct colonisation events as well as several distinct, separate lineages. A number of caenogastropod groups, such as the Cerithioidea (Pachychilidae, Paludomidae, and Pleuroceridae) and the Rissooidea (Hydrobiidae and Pomatiopsidae), have demonstrated unusual endemic radiation. Some of these species are dioecious, which means they only have one male and one female for sexual reproduction (Hydrobiidae).

2.2 Freshwater Gastropod

Prosobranchia and Pulmonata are the two groups that make up the freshwater gastropods. More than 500 families of freshwater Prosobranchia and 150 families of Pulmonate have reportedly been identified and are found all over the world. 2020 (Hamli et al.). According to Brown and Lydeard (2010), gastropods have a univalve shell with a radula that resembles a file for feeding on periphyton coatings on rocks and plants. With an estimated 80–100,000 recognised species and a potential total diversity of up to 200,000, mollusca is also second only to arthropods in terms of species diversity. The most prominent molluscan class, Gastropoda, has successfully and repeatedly infiltrated continental seas (Strong et al., 2007). Around the world, freshwater gastropods are an essential and diverse component of aquatic ecosystems (Bohm et al., 2021).

According to Brown and Lyread (2010), another noteworthy trait of freshwater gastropods, is intraspecific variation in life histories, productivity, morphology, and feeding preferences, which enables them to flourish in rocky freshwater environments. For instance, many groups of freshwater gastropods in North America have endangered species. Freshwater gastropods make up 5% of the world's species of gastropods, according to Bae and Park (2020), however they are under disproportionately high threat. Freshwater gastropods, often known as gastropod snails, are mollusks that live in freshwater. Over 4000 species of freshwater gastropods and about 5000 species of gastropods overall are thought to exist (Gladstone et al., 2022).

2.3 Morphology

According to Brown and Lydeard (2010), the development of gastropod shells is a crucial aspect of systematics. A gastropod's family determines the type of its shell. Gastropod shapes can be categorised as either external or internal. The three different forms of shells are spiral, conical, and planit spiral. A caenogastropod's shell thickness can also vary, from thin and easily broken to thick and challenging to break. A portion of the opening is frequently reflected over the body of the whorl to create the inner lip. They may have spines, little hammerings called malleations, coloured bands, or ridges called carina along the edge of the whorl on their surface. The periostracum is the shell's outermost organic layer made of protein. It might stop calcium carbonate from dissolving causing shell degradation. According to Brown and Lydeard (2010), the soft component, has a head, a foot, a mass of organs, and a mantle.

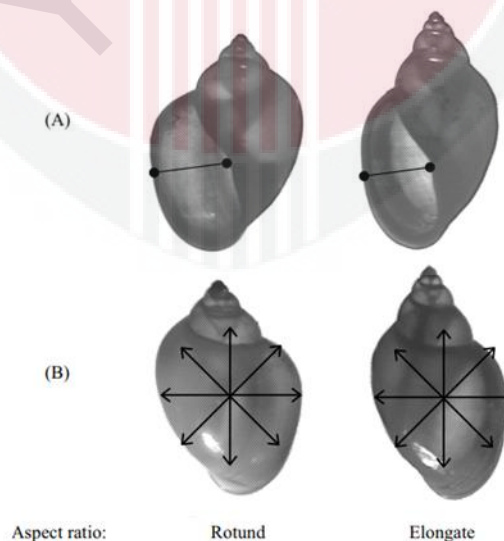


Figure 2.1 Shell morphology. (A) Ventral view showing shell apertures. (B) Dorsal view DeWitt et al., (2000)

2.4 Feeding

Lombardo (2002) asserts that because freshwater gastropods may be essential components of aquatic systems, the characteristic of gastropod feeding ecology may have broad implications. Additionally, they think that only epiphytes are consumed by freshwater gastropods. Freshwater gastropods are either herbivorous or detritivores; they occasionally eat carrion or tiny invertebrates connected to periphyton. Freshwater gastropods prefer eating epiphytes over living macrophytes since the former offers a higher energy return; nevertheless, if epiphytes become scarce, herbivory on plants may occur. Diet specialists are more prone to engage in directed foraging than generalist feeders. High populations of gastropods can deplete periphyton before consuming macrophytes, lowering the variety of species of macrophytes (Pyron and Brown 2015). The nutrition and habitat of thirteen groups of freshwater gastropods, including the physidae, planorbidae, Biomphalaria, Bulinus, lymnaeids, and limpets, have been summarised. Dillon (2014) asserts that for instance, the sympatric *Physa Vernalis* consumes less algae than the omnivorous lymnaeid *P. columella*.

2.5 Distribution

According to Hamli et al., (2013) prior study, the tropical region of Thailand has edible gastropods. However, estimating how many distinct species of edible gastropods exist in Sarawak, Malaysia, is difficult. In addition, in the seven-division state of Sarawak, a total of 21 edible species from 11 families of gastropods have been identified and given names. In the Bintulu division, 13 species of gastropods have been discovered, more than in any of the other six divisions.

Idris et al., 2021 argue that compared to previous investigations, this one contained fewer gastropod species. Other investigations in Thailand have uncovered 47, 43, and 56 species. Bintulu and Kuching possessed a vast assortment of edible gastropods. This location features a restricted variety of edible mollusks. On Phuket Island, Thailand, gastropods were considered the most valuable mollusks group. The Corbiculidae family was the oldest and best-known of all genera (Hamli et al., 2021). 551 individuals of five species and two groups were detected at sampling sites in Bintulu (Hamli et al., 2020). *Melanooides tuberculata* was the most prevalent freshwater gastropod found at all sampling sites in Bintulu. Also identified were the gastropods *Sulcospira testudinaria*, *Brotia siamensis*, *Sulcospira schmidtii*, and *Tylomelania* sp.

2.6 Habitat

Lakes, ponds, rivers, streams, and marshes are many and diverse freshwater habitats in the circumpolar region (Wrona and Reist 2013). According to Kumar (2016), the study of organisms in water is called freshwater organism ecology. Limnology is the study of the physical, chemical, geological, and biological properties of freshwater, he added. Freshwater habitat only makes up a small percentage of the earth's surface compared to marine and terrestrial ecosystems. Freshwater ecosystems exist in the lentic and lotic waters. While lotic water is in motion, lentic water is still. The region's varied environments are home to a wide variety of species.

There are many gastropods in lakes and rivers (Pyron and Brown 2015). On every continent, freshwater gastropods can be found in almost body of water, including

rivers, lakes, streams, marshes, underground aquifers, and springs. Aquatic plants, stones, boulders, timber, solid surfaces, and soft mud all support a variety of organisms. While some are aquatic and able to endure dry seasons, others can hibernate in the soil (Strong et al., 2007).

2.6.1 Abiotic Factor

Bae and Park (2020) state that various environmental factors, such as temperature and precipitation, as well as physiographical factors, have an impact on the distribution and structure of gastropod assemblages. These factors also have a significant impact on the structure of freshwater gastropod assemblages. It is challenging for freshwater to recover from its variability after being disturbed because sessile gastropod species cannot adapt to harsh environments. Most caenogastropods are more tolerant to temperature extremes and fluctuations in dissolved oxygen than pulmonates. For instance, while tropical pulmonates may be able to survive temperatures as high as 40°C for extended periods of time, most pulmonates can tolerate cold around 0°C for several months. Freshwater gastropods grow more quickly and begin to reproduce early at ordinary temperatures. Freshwater gastropods are restricted to water with a calcium concentration of at least 25 mg/L in about 45 percent of cases (Brown and Lydeard 2010). Olkeba et al. (2020) found favourable correlations between freshwater gastropod abundance and physical-chemical water quality indicators such water temperature and chemical oxygen demand, but negative correlations with turbidity and dissolved oxygen.

Freshwater gastropod abundance rose with water temperature and alkalinity but fell with higher dissolved oxygen concentrations. Different environmental factors have an impact on the abundances of distinct freshwater snail species. When water temperature, water depth, nitrate concentration, alkalinity concentration, and predator abundance increased, for example, *Bulinus globosus* abundance increased; however, it decreased as chloride concentration, ammonia concentration, and canopy cover increased.

2.6.2 Biotic Factor

Olkeba et al. (2020) assert that the occurrence and abundance of freshwater gastropods are significantly influenced by biotic factors. Freshwater gastropod distribution and abundance were mostly influenced by biological factors, such as the presence of numerous predators and rivals. According to Hoverman et al. (2011), biotic variables have been widely suggested as crucial elements in structuring snail colonies. One of the biotic elements that might adversely affect gastropod development and fertility is competition. It was proposed that habitat disruption and predation reduce the density of gastropod species to a point where interactions between species are not adversely impacted by competition.

Molluscivorous fish, which have a significant impact on the number of freshwater gastropods, are the greatest threat to thin-shelled gastropod species. Neubauer et al. (2022) claim that variation in freshwater gastropod speciation and extinction rates can be explained by biotic factors such as diversity-dependence, where greater species slow down diversification, as well as predatory, trophic, or mutualistic interaction. Olkeba

et al. (2020) found that the number of freshwater gastropods decreases as the number of predators increases, but that the presence of macrophytes may reduce the amount of predation and interspecies competition.

2.7 Morphological Study

According to Chiu et al. (2002), the characteristics of the shell and operculum, were particularly useful in identification. The significance of each shell trait may be immediately reflected in the morphometric analyses. Morphometrics is the quantitative study of biological form variation and how it relates to other variables. Biology's use of morphometrics has provided novel insights into evolution and changes. According to Trusch (1998), size and shape have been particularly valuable for studying the shells of some genera of gastropods.

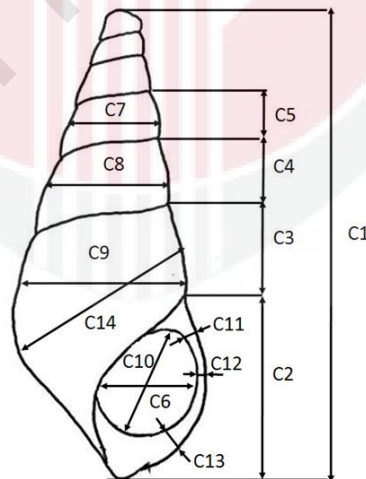


Figure 2.2 Measurement of gastropod shell characteristic study based on Sherley (1996) and Hamli et al., (2020).

Table 2.1. Detail of morphometric shell based on Sherley, (1996) and Hamli et al., (2020).

Measurement number	Abbreviation	Descriptions
C1	SL	Shell length: Maximum shell length
C2	AL	Aperture length: Aperture's largest exterior dimension as measured along a line offset from the shell's long axis.
C3	WH1	Whorl height: measured from the suture between neighbouring whorls to the point where the edge of the periostracum on the first whorl meets the outside margin of the apertural lip.
C4	WH2	Whorl height: involving the top and bottom sutures of nearby whorls
C5	WH3	Whorl height: the space between the top and bottom sutures of nearby whorls
C6	AW	Aperture width: Maximum aperture width defined as the distance between the outer border of the apertural lip and the line separating the periostracum on the columella
C7	WW1	Whorl width: dimensions were obtained from the midpoints of arcs produced by

		the outer edges of subsequent whorls along lines parallel to the sutures.
C8	WW2	Whorl width: dimensions were obtained from the midpoints of arcs produced by the outer edges of subsequent whorls along lines parallel to the sutures.
C9	BWW	Body Whorl width: dimensions were taken along lines parallel to the sutures from the midpoints of arcs formed by the outer edges of successive whorls
C10	AINL	Interior aperture length: the aperture's maximum internal length as measured from the inside borders of the lip
C11	LPL	Lip thickness: Measured from the inside to the exterior margins are the lower elevated regions of the apertural lip.
C12	LPM	Lip thickness: Measured from the inside to the exterior edges are the middle-elevated portions of the apertural lip.
C13	LPU	Lip thickness: Measured from the inside to the exterior margins are the upper elevated regions of the apertural lip.

C14

AWW

Aperture whorl width: the whorl's widest dimension above the aperture when it is parallel to the columella

2.8 Geomorphometric Analysis

Cruz et al. (2011) state that landmark analysis, which involves data collected as two or three-dimensional coordinates of biologically significant landmarks on specimens, has been used in many studies concerning geometric morphometrics techniques. This method does not directly quantify and analyse variations in biological shape. Clabaut et al. (2007) stated that geometric morphometric has shown relevant in evolutionary studies of a variety of species, including the cichlids of Lake Tanganyika in Eastern Africa, and that this method is more effective than standard morphometrics at differentiating between populations. Cruz and Vallejo (2009) defined geometric morphometrics can be used to assess interspecific variations in for, particularly for taxa systematic has proven difficult. According to Johnson et al. (1991), there have been challenges in determining appropriate landmarks and the unique growth of shells, but it is still a reliable tool for determining shell shape variations. As a result, geometric morphometrics has not been widely applied to gastropod shell (Carvajal - Rodriguez et al., 2005).

CHAPTER 3

METHODOLOGY

3.1 Study Area

The freshwater environment study area was located Universiti Putra Malaysia Bintulu Campus, Bintulu (UPMKB) that approximately at Latitude ($3^{\circ}12'41.3''\text{N}$) and Longitude ($113^{\circ}05'34.5''\text{E}$) and also located at Sg.Tuah,Kanowit. This study was carried out starting from April 2022 until January 2023. This study area covers about 714.717 hectares of land. A total of 3 stations was selected in this research and the coordinates of each station was recorded (Table 1.1). Figure 3.1 shows location of sampling station at Universiti Putra Malaysia Bintulu Campus and Sg.Tuah,Kanowit.

Table 3.1 Coordinates of 3 stations

No.	Stations	Coordinates
1	S1	Latitude ($3^{\circ}12'27.50''\text{N}$) Longitude ($113^{\circ}4'29.71''\text{E}$)
2	S2	Latitude ($3^{\circ}12'29.18''\text{N}$) Longitude ($113^{\circ}4'30.50''\text{E}$)
3	S3	Latitude ($3^{\circ}12'22.73''\text{N}$) Longitude ($113^{\circ}5'3.40''\text{E}$)



Figure 3.1 Google maps of sampling location at UPMKB



Figure 3.2 Google maps of sampling location at Sg. Tuah, Kanowit.

3.2 Sampling technique

At each sampling, triplicates of each station will be conducted. Specimens will be counted and randomly selected from each sampling point and stored in the sample zip bag for the identification process. All the collected specimens will be preserved using 10% formalin and specimens brought to the laboratory for the identification process.

3.3 Species Identification

Identified specimens will then be photographed using a digital camera for identification characters on the shell. Specimens' taxonomy characteristics will be identified based on (Perez *et al.*, 2004), (Köhler and Glaubrecht 2006.), and (Glöer 2019.).

3.4 Morphology Measurement

14 measurements were used to measure the shell morphology of freshwater gastropod. 120 individuals of freshwater gastropod were measured and analysed based on shell characters.

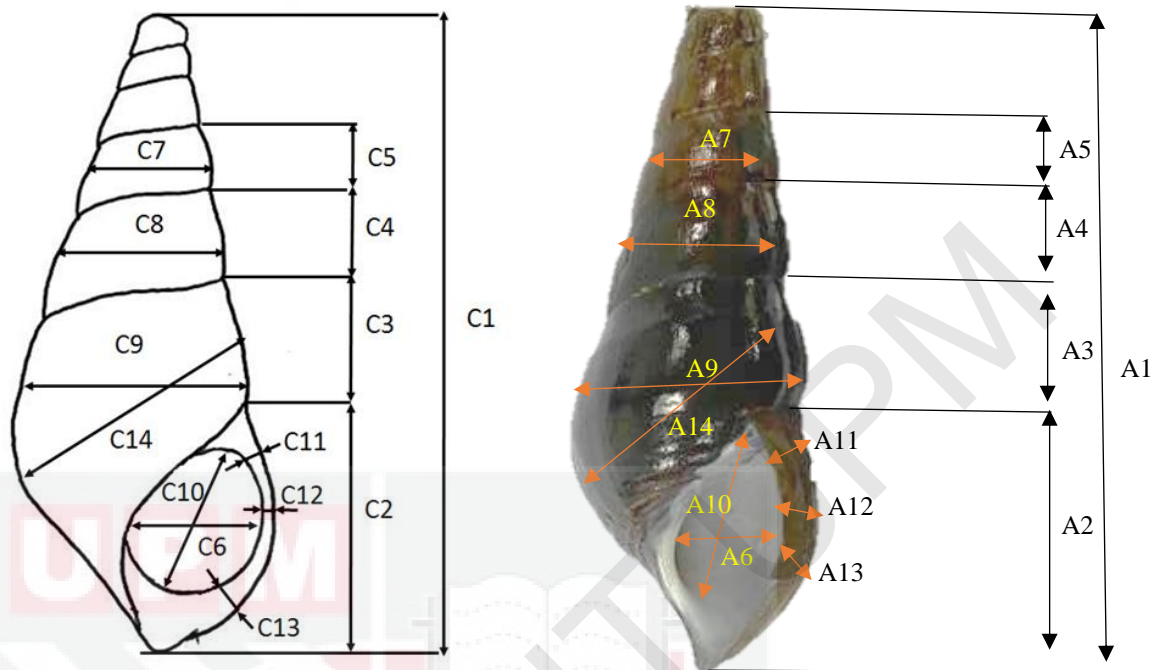


Figure 3.3 Shell measurements of freshwater gastropods.

Table 3.2. Detail of morphometric shell based on Sherley, (1996) and Hamli et al., (2020).

Measur ement number	Abbrevi ation	Descriptions
A1	SL	Shell length: maximum shell length
A2	AL	Aperture length: Maximum aperture outside dimension measured along an offset line to the right of the shell's long axis
A3	WH3	Whorl height: measured from the suture between adjacent whorls to the intersection of the outside margin of the apertural lip and the edge of the periostracum on the first whorl that meets the apertural lip
A4	WH4	Whorl height: between adjacent whorls' top and bottom sutures
A5	WH5	Whorl height: between adjacent whorls' top and bottom sutures
A6	AW	Aperture width: maximum aperture width measured from the edge of the periostracum on the columella to the outer edge of the apertural lip
A7	WW7	Whorl width:

A8	WW8	Whorl width: Dimensions were taken from the midpoints of arcs formed by the outer edges of successive whorls along lines parallel to the sutures.
A9	BWW	Body Whorl width: Dimensions were taken along lines parallel to the sutures from the midpoints of arcs formed by the outer edges of successive whorls.
A10	AINL	Interior aperture length: maximum interior length of the aperture measured from the apertural lip's interior edges
A11	LPL	Lip thickness: Lower raised apertural lip areas measured from the inside to the outside edges
A12	LPM	Lip thickness: The apertural lip's middle raised areas, measured from the inside to the outside edges
A13	LPU	Lip thickness: upper raised apertural lip areas measured from the inside to the outside edges
A14	AWW	Aperture whorl width: upper raised apertural lip areas measured from the inside to the outside edges

3.5 Geomorphometric Analysis



Figure 3.4 Coordinates of 14 landmarks for geomorphometric analysis.

Landmarks on the specimens were digitised using the software Image J, as shown in Figure 3.4. The significance level of the One-way ANOVA resulted in the selection of landmarks. Palaeontological Statistics (PAST) 4.03 was used to analyse the landmark data. The Procrustes transformation of coordinates XY eliminates isometric size variation and orientation as specimen factors in a common coordinate system. Adams et al. (2004) performed a principal components analysis (PCA) of shape to determine the linear combinations of variables that account for most of the data variations.

3.6 Data Analysis

Using Paleontological Statistics (PAST) version 4.03, analysis of variance (ANOVA) and Tukey's mean comparison test were used to determine the proportion of all measured morphometrics of the freshwater gastropod sample. Principal Component Analysis (PCA) was used to further assess whether there are variations between species that are significant for all shell morphometrics. Using the programme Image J, the landmarks of the coordinates for each sample were examined.



CHAPTER 4

RESULTS

A total of 120 freshwater gastropods has been recorded from three sampling locations: Station 1 (Sg, Tuah Kanowit), Station 2 (Air Terjun, UPMKB) and Station 3 (Borneo Base, UPMKB).

4.1 Species Identification and Morphological Description

Recorded gastropods comprise seven species which belong to three family such as Pachychilidae, Nassariidae and Ampullariidae. A total of 82 individuals under Pachychilidae was collected, including 17 individuals of *Sulcospira collyra*, 18 individuals of *Sulcospira testudinaria*, 21 individuals of *Sulcospira schmidtii*, 14 individuals of *Brotia Castula* and 12 individuals of *Sulcospira* sp. The Nassariidae family was represented by *Clea* sp. with a total of 14 individuals recorded. Meanwhile, the Ampullariidae family was denoted by *Pila* sp. with a total of 24 individuals documented.

Family Pachychilidae



Figure 4.1. *Sulcospira collyra* Köhler, Holford, Do & Ho, 2009

Origin name: *Sulcospira collyra* Köhler, Holford, Do & Ho, 2009

Descriptions: Medium-sized, elongate turreted, slender shape, slightly eroded tip, usually with seven whorls remaining. Suture is shallow, whorls are flattened in diameter, and there is no discernible sub-sutural depression. The aperture is small, ovate, and slightly angularly produced below: the outer lip is well rounded and sharp, while the inner lip is inconspicuous. Brown colour that is occasionally marbled (Fig.4.1)

Habitat: Freshwater

Distributions: Hoa bong, Ninh Binh Province (Vietnam), Brunei Darussalam



Figure 4.2. *Sulcospira schmidti* (E. von Martens, 1908)

Origin name: *Melania schmidti* E. von Martens, 1908

Descriptions: The shell has globular, conical, turreted, and elongated shapes. Shells with rounded or pointed basal lips and spiral striae at the bottom final body whorls. Axial sculpture is missing, but spiral sculpture is present, and the operculum is oval. The color is dark black. (Fig.4.2).

Habitat: Forest swamps, rivers, streams along paddy fields

Distribution: Borneo, Kota Marudu, Kota Kinabalu, Tenom, Keningau



Figure 4.3 *Sulcospira testudinaria* (von dem Busch, 1842)

Origin name: *Melania testudinaria* von dem Busch, 1842

Local name: Tekoyong sungai

Descriptions: Elongate conic, turreted, or shell viable. dark brown to black shell solid, huge to extra-large (39-40 mm). Only 7-9 flattened whorls remain due to apex erosion. Only a few noticeable spirals—three near the suture and two at the base of the body whorls—make up the sculpture. oval aperture, straight outer lip. oval-shaped opacity with 3–4 whorls that increase (Fig.4.3).

Habitat: Freshwater rivers, creeks

Distributions: Localities in West, Central and East Java.



Figure 4.4. *Sulcospira* sp.

Genus: *Sulcospira* Troschel, 1858

Description: Conical shell with spiral lirae but no axial line. 4-6 whorls in the operculum. Smooth and inflated protoconch. Black as a shell. Oval and somewhat produced at the base, the aperture (Fig.4.4)

Habitat: Freshwater, running water, river

Distribution: West Java, Indonesia

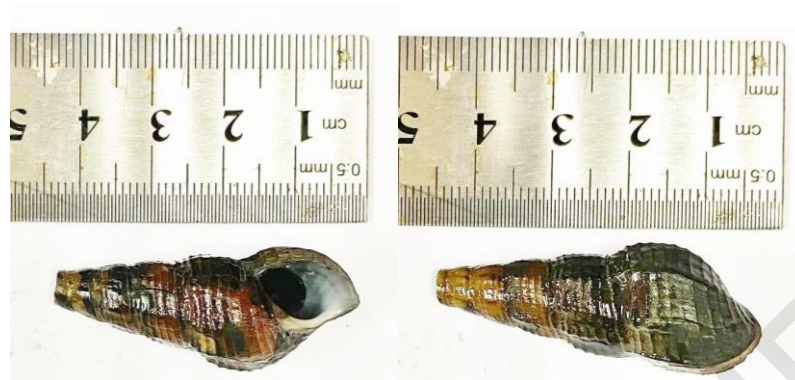


Figure 4.5. *Brotia costula* (Rafinesque, 1833)

Genus: *Brotia*

Origin name: *Melania costula* Rafinesque, 1833

Local name: Tekoyong ulu

Descriptions: Thick, conical, with spores and an axial cord, and a rounded periphery in dark brown. The solid shells have 6 to 11 whorls and a pyramidal, turreted, and eroded spore that ranges in color from light brown to olive brown. The sculpture is composed of axial ribs with nodules and basal spiral ridges. The whorls of the body are relatively large, and the aperture is oval, well rounded, or angled below and pointed above (Fig 4.5).

Habitat: living in habitat ranging from clear, narrowly tangled jungle creeks with sandy bottoms, large muddy rivers

Distributions: Northeast India, Bangladesh, Burma, Thailand, the Malaysia Peninsula, Sumatra, Cambodia, and Vietnam.

Family Nassariidae



Figure 4.6. *Clea* sp.

Genus: *Clea* H. Adams & A. Adams, 1855

Descriptions: Shell moderate in size, moderate elongate with smooth aspects, comprising about 6 inflated whorls. Outer lips smooth, slightly sinuous. Dark brown in color (Fig 4.6).

Habitat: freshwater and mangrove swamps, ponds, and rivers

Distributions: Kota Kinabalu, Kiansom forest, Philippines, Brunei

Family Ampullariidae



Figure 4.7 *Pila* sp.

Genus: *Pila* Röding, 1798

Descriptions: Small, six whorls, ochre to dark brown, highly solid, present longitudinal striations, globose body, imprinted suture, and angular body whorl describe this shell. Operculum corneous, with a somewhat concave and polished exterior surface (Fig.4.7).

Habitat: waterfalls, rivers

Distributions: India, Myanmar

4.2 Freshwater Gastropod Distribution

Sulcospira collyra, *Sulcospira testudinaria* and *Sulcospira schmidti* only present at Station 2 and Station 3 (Table 4.1). Meanwhile, *Sulcospira* sp., *Clea* sp. and *Pila* sp. were only available at Station 1. *Brotia castula* was present at both Station 1 and

Station 3. Furthermore, *Sulcospira* sp., *Brotia castula*, *Clea* sp., and *Pila* sp. were absent on Station 2. Therefore, Station 1 and Station 3 consist of the highest recorded species in the present study.

Table 4.1: Freshwater gastropod distribution on selected areas of natural environments.

Species	Station 1 (Sg.Tuah, Kanowit)	Station 2 (Air Terjun UPMKB)	Station 3 (Borneo Base UPMKB)
<i>Sulcospira collyra</i>	-	+	+
<i>Sulcospira testudinaria</i>	-	+	+
<i>Sulcospira schmidtii</i>	-	+	+
<i>Sulcospira</i> sp.	+	-	-
<i>Brotia castula</i>	+	-	+
<i>Clea</i> sp.	+	-	-
<i>Pila</i> sp.	+	-	-

Present = +; Absent = -

4.3 Shell Morphometric Analysis

Morphometric analysis showed twelve measurements of shell morphometric used to differentiate among species that have been found in this research (Table 4.2). Based on result, 13 characteristics showed significantly different levels ($p < 0.05$) which were AL (0.001), WH3 (0.001), WH4 (0.001), WH5 (0.001), AW (0.001), WW7 (0.001), WW8 (0.001), BWW (0.001), AINL (0.001), LPM (0.011), LPU and AWW (0.001). The mean aperture length ranges from 0.3650 to 0.5350 with the range of standard length from 0.003 to 0.050. The whorl height 3 mean shell range 0.1441 to 0.2702 with standard length range from 0.001 to 0.027, whorl height 4 mean ranges from 0.0977 to 0.1545 with standard length ranges from 0.001 to 0.006 and whorl height 5 mean shell range from 0.0551 to 0.1380 with standard deviation range from 0.000 to 0.009. The mean of aperture width ranges from 0.1733 to 0.3655 with standard deviation ranges from 0.000 to 0.030. The mean shell for whorl width 7 and 8 range from 0.1072 to 0.3444 with standard deviation ranges from 0.000 to 0.008. The mean shell body whorl width ranges from 0.2650 to 0.5728 with standard deviation ranges from 0.003 to 0.077. The mean interior aperture length ranges from 0.2264 to 0.5066 with standard deviation ranges from 0.001 to 0.021. The mean of lip thickness upper and middle ranges from 0.0281 to 0.0066 with standard deviation ranges from 0.001 to 0.035. Lastly, the mean aperture whorl width ranges from 0.0337 to 0.0154 with standard deviation ranges from 0.000 to 0.004.

Based on result aperture length *Sulcospira sp.* and *Brotia castula* showed similar shell characters with *Sulcospira collyra*. but on result whorl height 3 *S.schmidti*, *B.castula* and *Clea sp.* showed identical shell characters with *S.testudinaria*. On aperture width

result, it showed that *S.schmidti* showed similar shell character with *S.testudinaria*. meanwhile on whorl width 7, it showed that species *Clea* sp. showed same shell character with *B.castula*. For body whorl width, the result showing that species *S.schmidti* showed similar character with *S.testudinaria* meanwhile *B.castula* showed identical shell characters with *Sulcospira* sp. On shell character of interior aperture length, the result showing that *B.castula* have identical shell character with *Sulcospira* sp. meanwhile shell character on lip thickness upper showed no significant level for seven species and each species have a different shell characters.

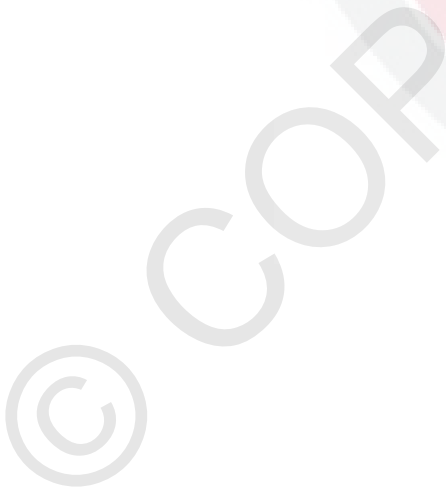


Table 4.2. Analysis of one-way ANOVA for 12 morphometric characteristics proportions with standard length.

Shell characters	<i>S. collyra</i>	<i>S. testudinaria</i>	<i>S. schmidtii</i>	<i>Sulcospira</i> sp.	<i>B. castula</i>	<i>Clea</i> sp.	<i>Pila</i> sp.	<i>p</i> value
AL/SL	0.380±0.006 ^a	0.3073±0.003 ^b	0.3650±0.005 ^a	0.4964±0.020 ^c	0.3868±0.005 ^a	0.4828±0.018 ^c	0.5350±0.050 ^d	0.001
WH3/SL	0.2285±0.003 ^a	0.1441±0.001 ^{ba}	0.2011±0.003 ^{db}	0.2702±0.002 ^c	0.2183±0.002 ^{ab}	0.2139±0.027 ^{abd}	0.1968±0.018 ^d	0.001
WH4/SL	0.1421±0.006 ^a	0.1381±0.036 ^a	0.1097±0.003 ^b	0.1545±0.004 ^a	0.1452±0.001 ^a	0.1391±0.001 ^a	0.0977±0.001 ^b	0.001
WH5/SL	0.1380±0.006 ^a	0.0982±0.00 ^b	0.0616±0.004 ^c	0.1347±0.004 ^a	0.1124±0.009 ^b	0.0724±0.005 ^d	0.0551±0.007 ^c	0.001
AW/SL	0.3300±0.004 ^a	0.1733±0.030 ^b	0.1867±0.000 ^b	0.3227±0.002 ^a	0.2590±0.002 ^c	0.2216±0.015 ^d	0.3655±0.016 ^e	0.001
WW7/SL	0.2281±0.003 ^a	0.2045±0.001 ^b	0.1440±0.000 ^c	0.2455±0.002 ^d	0.1857±0.001 ^e	0.1860±0.010 ^e	0.1072±0.007 ^f	0.001
WW8/SL	0.3340±0.006 ^a	0.2227±0.001 ^b	0.2402±0.002 ^c	0.2512±0.003 ^d	0.2335±0.003 ^c	0.3444±0.008 ^e	0.2168±0.007 ^b	0.001
BWW/SL	0.3855±0.007 ^a	0.2650±0.002 ^b	0.2745±0.003 ^b	0.5281±0.006 ^c	0.4161±0.077 ^a	0.4941±0.015 ^c	0.5738±0.022 ^d	0.001
AINL/SL	0.3104±0.006 ^a	0.2264±0.001 ^b	0.2787±0.003 ^c	0.3870±0.001 ^d	0.2909±0.003 ^d	0.3587±0.020 ^f	0.5066±0.021 ^e	0.001
LPM/SL	0.0141±0.002 ^{ab}	0.0251±0.035 ^{ab}	0.0226±0.003 ^{ab}	0.0181±0.001 ^{ab}	0.0066±0.002 ^a	0.0281±0.004 ^b	0.0158±0.002 ^{ab}	0.011
LPU/SL	0.0141±0.002 ^a	0.0177±0.003 ^a	0.0266±0.003 ^a	0.0178±0.002 ^a	0.0126±0.002 ^a	0.0265±0.002 ^a	0.0169±0.002 ^a	0.063
AWW	0.0202±0.001 ^a	0.0139±0.000 ^b	0.0337±0.002 ^d	0.0217±0.002 ^a	0.0154±0.001 ^b	0.0027±0.002 ^c	0.0231±0.004 ^{ac}	0.001

*Note: Different alphabetical order between column indicates significant different $p < 0.05$

4.4 Geomorphometric Analysis

The PCA of 40 coordinates with 560 landmarks of 40 individual of freshwater gastropods from genus *Sulcospira* showed a clear separation amongst the group of genera. Based on figure 4.8, it showed that species of *Sulcospira* sp. and *S.collyra* having the same character of shell and this two species have the same species because based on result, the coordinates overlaps with each other. It also showed that *S. schmidti* showing more differences shell characters with the other species as from the result it is showing that the plot was distributes further other than species.

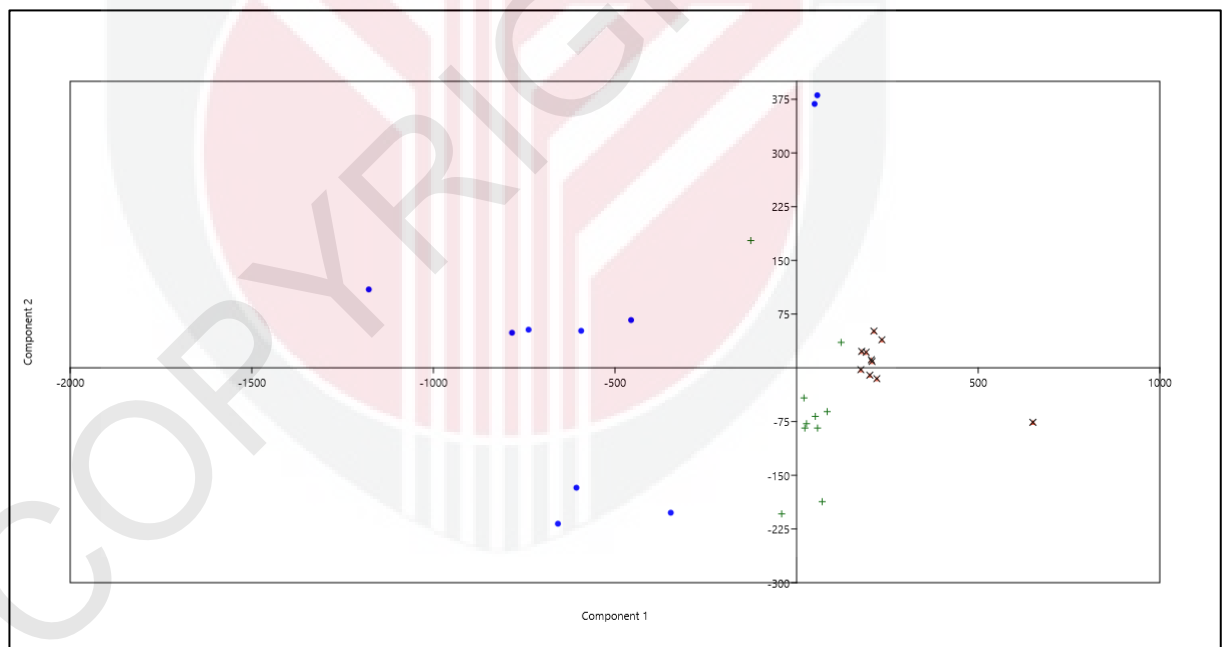


Figure 4.8. Plot result of principal components analysis of 40 individual with landmarks of 560 shell landmarks genus *Sulcospira* (*S.collyra* ▲, *S.testudinaria* ●, *S.schmidti* +, *Sulcospira* sp. X).

CHAPTER 5

DISCUSSION

Shell character of 14 measurement was used to measure every sample of freshwater gastropod. According to Hansen et al. (2003), the physical characteristics of the freshwater ecosystem, such as the amount and quality of the water flowing through it, as well as other elements that affect the occurrence of freshwater gastropods, are very important. According to Chiu et al. (2002), the gastropods' shells are a valuable source of taxonomic data that may be utilised to understand the evolutionary relationships between different taxa. In general handbooks and the taxonomic literature, shell morphological characteristics serve as the main criteria for identifying species. The three most crucial taxonomic traits are shell width, shell shape, and spire height. Despite the use of shell morphology in taxonomy and systematic studies of gastropods, these morphological characteristics might be confused by intrinsic and extrinsic factors.

The freshwater gastropods currently living have shown that the family Pachychilidae is primarily found in freshwater habitats. In mainland southeast Asia, two genera were said to represent the Pachychilidae: *Brotia* H.Adam, 1866, and *Sulcospira* Troschel, 1858. From northeast India to Sumatra and Borneo, this family of freshwater gastropods can be found. According to Köhler and Glaubrecht (2006), it is currently uncertain how far the genera extend into Southern China to the east and how much of their range overlaps with that of Borneo, eastern Thailand, Laos, Vietnam, and Cambodia. The key to characteristics showed that aspects of shell

measurement characteristics are necessary to identify these genera, however many of the species' identities are still unknown. According to Köhler et al. (2009), *Brotia* was first recognized based on the types of species' round and multispiral opercula. The widespread *Brotia* is characterized by circular opercula with around eight whorls that are members of two distinct clades. According to Francis et al. (2011), it is widely dispersed in Singapore, Indonesia, Sri Lanka, both sides of the Thai and Malay Peninsula, and India. Fast-moving water, such rivers or rocky streams, is where *Sulcospira testudinaria* typically exist. According to Marwoto and Isnaningsih (2012), this species originated linked to stones in a river with sand and gravel.

14 shell morphometric measures can be utilized to recognize between species. Six shell morphometric parameters, however, that were correlated with shell length and able to confirm variance between seven species were established. According to Köhler and Glaubrecht (2006), The minimal measurement of a shell morphometric analysis, employed at least five shell features to confirm variation between gastropod species. Shell length, aperture length, whorl height, whorl width, aperture whorl width, and shell length are the most frequent shell characteristics that are utilised to confirm species variation.

According to Dillon and Jacquemim (2015), there are two types of shell morphometrics: classical morphometrics and geometric morphometrics. While geometric morphometrics is an analysis method based on digitised shell landmarks, traditional measuring is focused on linear measurement. The results of PCA reflect apparent shape differences and similarity among several *Sulcospira* species and serve to quantify these differences and similarities. The shell features of *Sulcospira* sp. and *Sulcospira collyra* are identical. Similar to *Sulcospira collyra*, *Sulcospira* sp. have a shell with a medium-sized shell feature. Both of these species feature whorls that are

flattened in diameter and have relatively small apertures (Kohler, 2009). Although there were some variations in shell morphology among *Sulcospira*, Sherly (1996) noted that it was impossible to separate populations or subgroup populations that were distinct from one another in terms of size and form based solely on shell morphology. According to Ismail and Elkarmi (2006), the relationship between shell length and width, as well as between aperture length and width, is linear.

Gastropods from the Pachychilidae family made up the majority of the species that was founded on freshwater environments. According to Hamli et al. (2013), this species has been observed in freshwater habitat in Kuching, Bintulu, Miri, and Limbang. Additionally, *Brotia* sp. a freshwater species has been found in Borneo, Sumatra, Java, and the Malaysian Peninsula (Kohler and Glaubrecht, 2003). According to Ng et al. (2014), Ampullariidae species can be found throughout the tropics and subtropics of Africa, Asia, and the Americas. They are primarily associated with freshwater habitat bodies. Hamli et al (2020) assert that the freshwater gastropoda was influenced by area and number of macrophytes because the higher the number of macrophytes on environments the higher the survival rate of gastropod.

CHAPTER 6

CONCLUSION

As a conclusion, a total of 120 freshwater gastropoda has been recorded from location of three station which are Station 1 (Sg, Tuah Kanowit), Station 2 (Air Terjun, UPMKB) and Station 3 (Borneo Base, UPMKB). The recorded of freshwater gastropoda were comprises with seven species that belong to the three families which are from family Pachychilidae, Nassariidae and Ampullariidae. A total of 82 individuals from family Pachychilidae, 14 individuals from family Nassariidae and 24 individuals from family Ampullariidae was documented on this research.

Identification of species was identified using a traditional method of morphometric and geometric morphometric that traditional method based on linear measurement meanwhile geometric morphometric was analysed based on landmark of shell. 12 characteristics showed significantly different levels ($p < 0.05$) on shell morphometric analysis which were AL (0.001), WH3 (0.001), WH4 (0.001), WH5 (0.001), AW (0.001), WW7 (0.001), WW8 (0.001), BWW (0.001), AINL (0.001), LPM (0.011), LPU and AWW (0.001). Geometric morphometric of 40 individuals of freshwater gastropoda showed species of *Sulcospira* sp. and *S.collyra* having the same character of shell. The existing freshwater gastropod revealed that family Pachychilidae mostly found on freshwater habitat. Family Pachychilidae represented by two species which are *Brotia* H.Adam, 1866 and *Sulcospira* Troschel 1858. 14 measurements of shell morphometric used to differentiated among species.

To enhance differentiated identification of gastropoda species, it is important to stress the application of more tools in morphometric research. More research is required to assess the shell traits of freshwater gastropods with similar traits, like the species *Sulcospira*.

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APPENDIX

