DEVELOPMENTAL BIOLOGY AND GENETIC DIVERSITY OF SELECTED NERITIDAE IN MALAYSIA WITH AN EMPHASIS ON *Nerita balteata*

by

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LIST OF ABBREVIATIONS

AMOVA – analysis of molecular variance

ATPS-α – Adenosine triphosphate synthase subunit alpha

bp – Base pair

BOLD – Barcode of Life Database

CBOL – Consortium for the Barcode of Life

COI – Cytochrome c oxidase subunit I

ML – Maximum likelihood

MP – Maximum parsimony

mtDNA – Mitochondrial deoxyribonucleic acid

NJ – Neighbor-joining

PCR – Polymerase chain reaction

SEM – Scanning electron microscope / microscopy

16S rRNA – 16S ribosomal ribonucleic acid

LIST OF SYMBOLS

F_{ST} – Fixation index

N – Sample size

H – Number of haplotypes

rg – Raggedness static

SSD – Sum of square deviations

t – Number of generations since the expansion

u – Mutation rate

θ_0 – $2uN_0$

N_0 – Population size before expansion

θ_1 – $2uN_1$

N_1 – Population size after expansion

π – Nucleotide diversity

h – Haplotype diversity

Nm – Gene flow estimates

BIOLOGI PERKEMBANGAN DAN KEPELBAGAIAN GENETIK

NERITIDAE TERPILIH DI MALAYSIA

DENGAN TUMPUAN KEPADA *Nerita balteata*

ABSTRAK

Gastropoda dalam kajian ini adalah dari genus *Nerita* yang dijumpai di kawasan berbatu dan berlumpur dalam zon pasang surut sepanjang pinggir pantai Malaysia. Kepelbagaian spesis dianalisis, ciri-ciri morfologi diterangkan, dan "barcode" sitokrom oksida I dihasilkan untuk setiap satu daripada 12 spesis yang dijumpai. Dua puluh satu populasi spesies yang difokus dalam kajian ini, iaitu *N. balteata*, dijujuk untuk sebahagian gen 16S rRNA untuk mengenalpasti struktur populasi nerite yang dapat dijumpai di kawasan ini. Walaupun wujud halangan geografik dan oseanografik dan sempadan habitat, nerita masih berupaya mengekalkan struktur homogen di antara Semenanjung Malaysia, Sarawak, dan Sabah. Jarak genetik di antara populasi-populasi adalah rendah, pohon filogenetik tidak mempunyai klad khusus yang menunjukkan populasi tunggal, dan gambarajah rangkaian menghasilkan dua haplotip utama yang merangkumi kebanyakan populasi yang dikaji.

Corak sebegini membawa kepada kajian terperinci ontogeni awal *N. balteata* untuk memahami dengan lebih mendalam keupayaan penyebaran spesies ini yang difahami memainkan peranan yang mustahak dalam keluasan taburan. Kaedah stereomikroskopi yang digunakan sebelum ini tidak dapat menjelaskan banyak ciri-ciri terperinci manakala mikroskopi pengimbasan elektron berjaya memberi imej

definasi tinggi untuk pengembangan dalam kapsul. *Nerita balteata* yang plantrotrofik dilihat mempunyai peringkat veliger dengan velum yang lebih terperinci dan tempoh larva yang lebih panjang jika dibandingkan dengan *Nerita japonica*—spesies yang berkembang terus, hanya mempunyai velum semasa pengembangan dalam kapsul dan tidak mengalami fasa veliger pelagik.

DEVELOPMENTAL BIOLOGY AND GENETIC DIVERSITY OF

SELECTED NERITIDAE IN MALAYSIA

WITH AN EMPHASIS ON *Nerita balteata*

ABSTRACT

The gastropods in this study are from the genus *Nerita* found in the rocky and muddy intertidal zones along the coasts of Malaysia. The species diversity was analyzed, morphological descriptions were made, and barcodes of cytochrome oxidase I were generated for each of the 12 species of nerites found. The nuclear gene, ATPS-α, successfully determined the phylogenetic status of each species. Twenty-one populations of the focal species of this study, *N. balteata*, were sequenced for 16S rRNA gene fragments to determine the population structure of nerites found in this region. Despite geographic and oceanographic barriers and habitat boundaries, the nerites managed to maintain a homogeneous structure between Peninsular Malaysia, Sarawak, and Sabah. Genetic distances between populations were low, phylogenetic trees did not exhibit distinct clades which denoted singular populations, and network diagrams produced two major haplotypes with most populations accounted for in each.

Such patterns led to the in-depth study of the early ontogeny of *N. balteata* in efforts to understand their dispersal ability thought to play a crucial role in their wide-spread distribution. While stereomicroscopy used previously was unable to capture many minute details of the egg and larvae structure, scanning electron microscopy was able to provide high-definition images of the intracapsular development. The

planktotrophic *N. balteata* was seen to possess a veliger stage with more elaborate velum and longer larval period compared to *Nerita japonica*—a direct developer in which velum only existed as long as larvae development was intracapsular, and pelagic veliger stage was absent.

CHAPTER 1:

INTRODUCTION

1.1 Introduction

Neritidae, or more commonly known as nerites, is a family of gastropods which inhabit freshwater, brackish water, and marine environments. Nerites found in freshwater are from genera such as the *Neritina* and those found in seawater are from genera such as *Nerita.* The genera *Theodoxus* can be found in both freshwater and brackish water. The Neritidae became recognizable during the Cretaceous period (about 145-65 million years ago)—a period considered biologically significant as it plays an important role in the transition from early life-forms of the Paleozoic Era to the advanced diversity of the current Cenozoic Era. It was during this era that molluscs started to develop distinctively modern characteristics before the mass extinction which ended the period.

This family of gastropods have unique developmental modes. Besides hatching directly from their eggs into their adult forms like freshwater snails, some species of nerites also have a planktotrophic phase which involves a larval form called the veliger. The planktotrophic stages lasts for weeks and sometimes months enabling the nerites which possess this developmental mode to disperse large distances and, at times, across extensive boundaries. With the aid of genetic markers, patterns of dispersal, connectivity, or disjunctions can be explained and this, in turn, would provide insights on how behavioural and morphological adaptations propel the survival of nerites over such wide-spread geographical distances. Besides that, the understanding of palaeogeographic and oceanographic activities that significantly

influence the dispersal patterns of nerites would also help shed light on the population structures of these snails.

The early ontogeny of nerites merits attention for several reasons. Studying early ontogeny is essential in order to reveal important aspects of the structural basis of an organism as the structural biology of organisms can hardly be understood exclusively from their adult forms. The anatomical and functional needs of nerites contribute to their success in differentiation, growth, and survival. Description of the ontogenic transformations is essential for understanding the patterns behind the body plan formations and knowledge of intracapsular development is a necessity in understanding the functions of the different developmental stages. Such observations can be useful in the reconstruction of phylogenies and, together with genetic analyses, could provide useful information on evolutionary changes. Nevertheless, even with the advent of technology, detailed early ontogeny activity of nerites has seldom been reported.

In this project, one of the *Nerita* spp., *Nerita balteata,* was chosen based on its availability and ease to sample, to define the population structure of planktotrophic nerites in Malaysia using 16S rRNA gene fragments (Chapter 3). It was hypothesized that significant palaeogeological activity in the vicinity of the Isthmus of Kra would cause a distinct separation between populations from the two sides of the Malay Peninsula. This chapter will answer the question as to whether that scenario applies to *N. balteata*. Representative specimens from Thailand and Australia will also be included in this study for comparison. This project also focused on barcoding all the nerites which can be found in the intertidal zones of Malaysia. Two genetic markers

were chosen for this purpose. The mitochondrial DNA cytochrome oxidase I was chosen based on its barcoding properties whereas the nuclear marker ATPS-α was chosen for its paternal and maternal inheritance. The morphological characters and barcodes of sampled *Nerita* and *Neritina* species will be presented in Chapter 4.

The early ontogeny of *N. balteata*, *N. albicilla*, and *N. japonica* was studied for different developmental modes and morphological formations which aid the survival of nerites intracapsular and then, extracapsular (Chapter 5). Scanning electron microscopy was used to obtain high definition images of each developmental stage focusing on minute morphological changes in the eggs and veliger/larvae. This is novel as detailed images of stage by stage development have never been captured before.

1.2 Objectives

The objectives of this study were:

(i)   to characterize the population genetics and dispersal patterns of nerite populations using planktotrophic species *Nerita balteata*;

(ii)   to DNA barcode all Neritidae species found in the intertidal zones of Malaysia; and

(iii)   to study the early ontogeny of different intracapsular developmental modes which affect nerite dispersal using *Nerita balteata*, *Nerita albicilla*, and *Nerita japonica.*

CHAPTER 2:

LITERATURE REVIEW

2.1 Introduction to Neritidae

2.1.1 Classification

Nerites in this study are from the family Neritidae and genera *Nerita* and *Neritina*. The following is the scientific classification of this gastropod:

Kingdom: Animalia

Phylum: Mollusca

Class: Gastropoda

Clade: Neritomorpha

Superfamily: Neritoidea

Family: Neritidae

Genus: *Nerita / Neritina*

Nerites are small in size usually measuring less than one inch in diameter. Most species possess patterned, glossy shells that make them ornamental in the aquarium industry. These shells are used as initial identification traits in species recognition. However, to an untrained eye, some conspecifics may be classified as different species and some congenerics may be classified as the same species. Under most circumstances, an expert is unavailable and chances of erred identifications are high. Even though classical taxonomy has been used to identify species for two centuries now, the number of taxonomists is dwindling and the limited accuracy of this descriptive method has hampered conservation and management of morphologically similar species. Therefore, it is useful to have an alternative.

Nerites are hardy creatures. They cope with pounding waves, harsh sun, fluctuating tides, wind, salt, and rapid temperature changes. Those which live in higher parts of the rocky shore are out of water for a long time, and must deal with being dried out by the sun, wind, and salt, but are able to survive by using a combination of adaptations. Their strategy of grouping together helps to retain the little water left after the last high tide. The operculum acts as a trap door to the entrance of the shell and this aids retention of water inside the shell between high tides. Nerites found living in the lower reaches needs to be able to cope with pounding waves. They adapt to this by having a very strong muscular foot that helps them clamp firmly to the rocks. The egg capsules of the nerites which are sometimes enforced with spherulites also have part of their egg shells attached to the rocks to avoid loss of eggs due to strong wave action.

2.1.2 Morphology

2.1.2.1 Shell

The calcareous shell of a nerite (Figure 2.1) has a coating of conchiolin known as the periostracum or epidermis. This protects the shell from harmful solvents and chemicals commonly found in the habitat of nerites. The shell is usually thick and is covered by a layer of bristles in some species. Living specimens have simple to elaborate colours and patterns which aids in species identification. The pigments of colours are thought to have come from secretions of metabolic waste and play a part in natural selection (Kobluk & Mapes, 1989). The shell basically functions as a protective structure covering the visceral mass and providing a retreat for the foot and head which is extruded from the shell when active. In nerites,
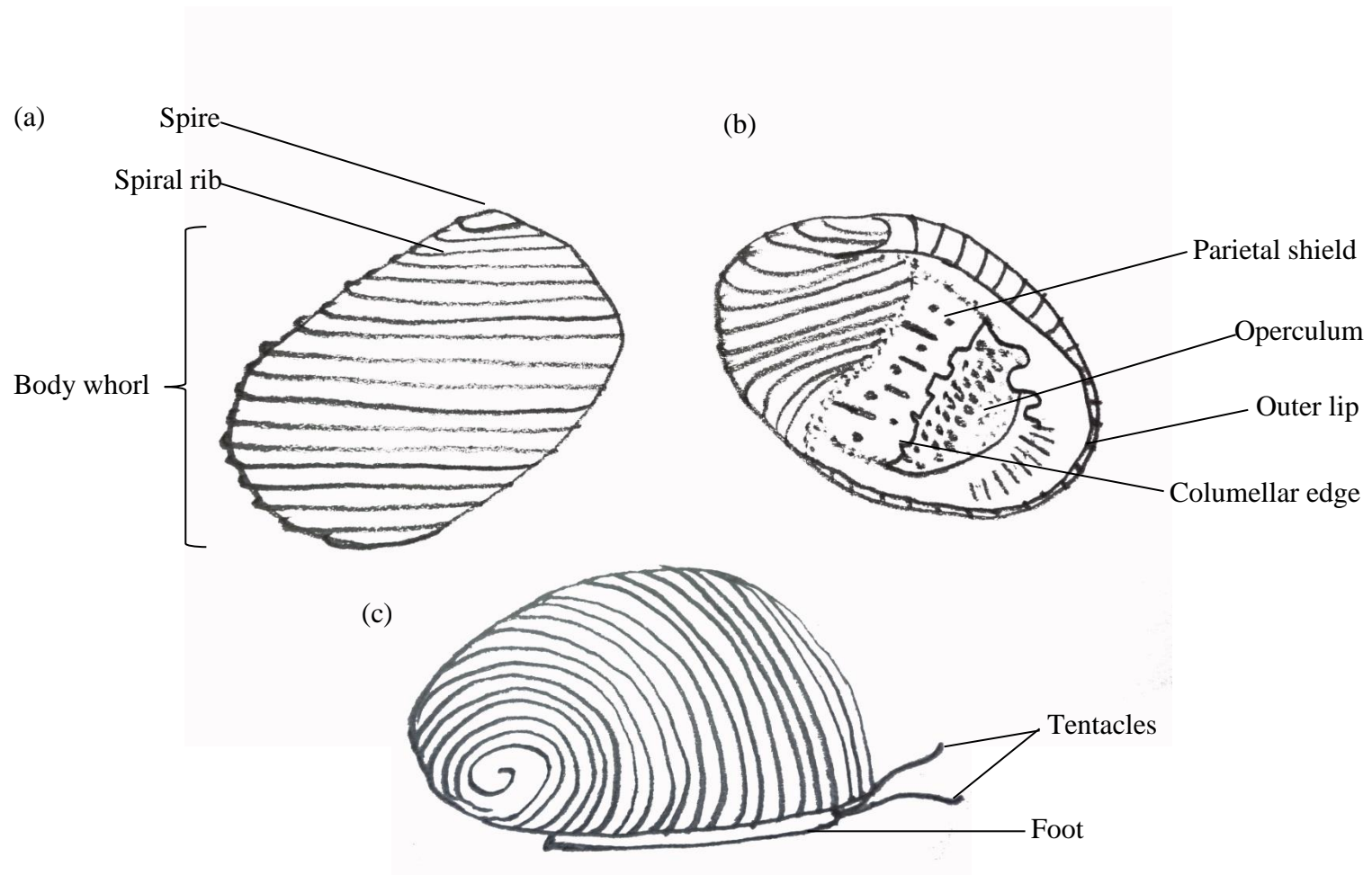
Figure 2.1: Nerite morphology. (a) Dorsal view of the shell. (b) Ventral view of the shell. (c) Lateral view with parts protrusible from the shell.

the shell is typically a globular tube which is closed at its apical end and opened at the other, where growth increments are added. The opened end is known as the aperture.

A complete coil on the shell is known as a whorl (Figure 2.1a). Each whorl connects with the one preceding it at a line called the suture. The parietal is found on the ventral side of the shell, mostly flattened (Figure 2.1b). This is one of the most useful identification features of nerites as it may be smooth, pustulose, or grained. Spire refers to the adapical visible part of all the whorls except the last. The outer lip refers to the termination of the outer side of the shell at the aperture or the abaxial part of the peristome. Opposite this side is the inner lip consisting of two parts (i) the columellar lip (which is formed by the columellar) and (ii) the parietal lip (extending from the columellar to the suture). The columellar edge is useful in species identification because of the unique projections of teeth each species of nerite possesses.

2.1.2.2 Parts protrusible from the shell

In nerites, part of its body remains permanently in the shell while part of it is protruded when the nerite is active. The protruded part which consists of the head and foot can be retracted into the shell (Figure 2.1c). Retraction happens by means of the columellar or retractor muscles—the only muscles attached to the shell of the nerite. When this happens, an operculum partly or completely closes the aperture of the shell. The operculum is attached to the metapodium—the posterior part of the foot. The anterior part of the foot is known as the propodium whereas the middle part is known as the mesopodium. The foot is broad, flattened dorso-ventrally, tough, and

contains mucous glands. The head of the nerite has sensory organs. Nerites have a pair of cephalic tentacles which point obliquely forward. They also have two eyes at the base of these tentacles. The mouth, which is a simple opening, could be a blunt snout or a long retractable proboscis in the head. In some cases, the length of the proboscis exceeds the length of the head and foot.

2.1.2.3 Solid structure associated with the shell

Usually, the operculum is the only solid accessory to the shell (Figure 1b). This structure is present in most prosobranch families including Neritidae. The primary purpose of the operculum is to close the aperture when the head-foot structure retreats into the shell. Majority of opercula are made of light and horny material, can sometimes be calcareous, and conform to the shape of the aperture. Opercula can be categorized into three classes depending on whether their structure is spiral, lamellar, or circular. The colour, pattern, and projections on the operculum are important clues to a species' identity.

2.1.3 Life cycle

2.1.3.1 Egg capsules of Neritidae

The egg capsules are lens-shaped, oval or circular, and generally less than 2 mm in diameter. The egg capsules are often arranged in rows or in patches, which may be produced by a single or several female(s). Many species tend to select depressions in the substratum for depositing their egg capsules. This gives them protection against desiccation. Some species deposit their egg capsules on the shell of the congeners. Majority of nerite egg capsules comprise a shallow, thin-walled tambour (Andrews, 1935), covered by a thick-walled cap, enclosing the embryos within. The lower

tambour is mostly a thin membrane that adheres to the substratum, but has a distinct thickened, raised rim. The upper cap, which also has a thickened rim, is a more robust structure with a substantially thicker wall (8–40 μm) throughout. The external surface of the cap is structurally complex, with reinforcement material comprising of either calcium carbonate spherulites manufactured internally or material derived from consumed items such as grains of sand, diatoms, and foraminifera. Cap and tambour are firmly attached to each other along their rims. They separate partially or completely when the larvae are ready to hatch. A thin membrane surrounds the insides of the capsule. The eggs and larvae are surrounded by a fluid which is, in most cases, albumen.

2.1.3.2 Planktotrophic larvae

Most gastropods have planktonic larvae that are potentially dispersive (Thorson, 1950). These larvae are termed planktotrophic and feed on smaller organisms than themselves to survive. The nutrition is essential to build complex structures of the gastropod which will later function in locomotion and feeding while the larvae remain planktic. In some species, the swimming veliger larvae stage can persist for weeks or sometimes, months. This is an important factor in the dispersal of gastropod species because many benthic marine animals move very little in adulthood. These complex structures are normally resorbed during metamorphosis before the larvae settles and continues with a sessile mode on land. There is little expenditure per egg parents as eggs are laid with little yolk content but many of them are produced at one time—up to 85000 eggs per spawning in the gastropod *Littorina irrorata* (Bingham, 1972).

According to Scheltema (1976), the planktic stage of planktotrophic species is divided into two-phases. The (i) growth and development phase is followed by a (ii) delay phase in which development is essentially completed. It is the delay period that gives dispersal flexibility and determines how long each species is able to remain planktic. Because planktotrophic species can survive on planktic food, it does not need the supply of yolk for its survival during the delay phase. Therefore, they can remain planktic for a long period of time and disperse further. Long-distance marine dispersal is considered an important biogeographical process which prevents the tendency of populations to become genetically isolated (Myers *et al*., 2000). According to Wright (1931), the dispersal of one individual per generation between populations is enough to offset genetic disjunction that could happen because of genetic drift and localized selection.

2.1.3.3 Direct developers

Besides planktotrophic larvae, there are direct developing or crawl-away larvae. The gastropods with this developing mode produce eggs which later hatch into larvae which crawls away from the egg masses. In this developmental mode, supplementary food source in the form of nurse eggs is laid together with viable eggs. The ratio of nurse eggs to viable eggs may vary considerably within a species. For example, the gastropod *Buccinum undatum* lays up to thousands of eggs per capsule but only tens of these develop into juveniles (Portmann, 1925).

The larvae of direct developers are generally believed to have the lowest dispersal potential compared to the planktotrophic larvae but contradictions exist. A study conducted by Martel and Chia (1991), showed that the common belief that larvae

which go through direct development have poor dispersal abilities is unlikely for their species of study. On the contrary, the invertebrates in their study such as the small gastropod, *Barleeia* sp., have alternative dispersal mechanisms that were equally effective as the mechanisms of free-swimming larvae. According to Martel and Chia (1991), frequent drifting excursions in the gastropod may enhance rafting opportunities and this may favour long distance dispersal. Their project also proved that post-metamorphic drifting does occur in marine bivalves and gastropods and the mode of development does not always affect dispersal abilities.

2.1.3.4 Early ontogeny studies of Neritidae

Several studies have been conducted regarding the description of the larvae of Neritidae even though they are limited. Some descriptions are brief like those of Lewis (1960) on the size and length of the veliger larvae of *Nerita peloronta*, *N. tessellata*, and *N. versicolor*. Other descriptions are more detailed as in the study of the egg masses and larval development of some prosobranchs conducted by Natarajan (1957). This study described that the newly-hatched veliger of *N. albicilla* has a shell of one whorl which is slightly pitted in appearance and measured 0.150–0.167 mm across the shell. The velum of the veliger larvae is bilobed, colourless and is bordered with long cilia. The eyes are black and prominent and tentacles were not observable. The foot of the veliger is ciliated and greenish in colour. It also has reddish brown pigments on either side at the base of the foot. Operculum is present but the otocysts are not clear. Similar characteristics of the *N. albicilla* larvae were also described in great detail by Risbec (1932).

Risbec (1932) documented significant information of *N. reticulata* larvae. The number of larvae contained in an egg capsule is highly variant but generally reduced and much smaller when compared to the larvae of *N. albicilla.* The development which takes place inside the eggs demands a long period of time. Many larvae fill the egg capsules and therefore, restrict their movement. They have small eyes. The velum is reduced while the foot is notably large. This latter organ is flattened dorso-ventrally and the anterior region has many small transparent ballonets fused together but easily disappear when burst.

Hulings (1986) reported on the early development of *N. forskali* and *N. polita*. In *N. forskali*, various stages of embryonic development from uncleaved ova, averaging 0.15 mm in diameter, to veligers with eyes, averaging 0.20 mm long, were found enclosed within the membrane that lines the capsule. The average number of ova-veligers per capsule was 117. Hatched veligers have eyes and operculum that averaged 0.21 mm in length. In *N. polita*, uncleaved ova are about 0.19 mm in diameter and veligers 0.25 mm in length. Hatched veligers averaged 0.25 mm long.

2.1.3.5 Larval dispersal

The movement of larvae is affected by various factors such as oceanic currents and water temperatures. At a finer geographic scale, regions of upwelling and coastal heterogeneity are long recognized as influencing the transport and settlement of larvae (Wing *et al*., 1995), and proven as notable barriers to population connectivity (Banks *et al*., 2007; Nicastro *et al*., 2008). The velocity of the oceanic current also affects the movement of nerite larvae (Crandall *et al*., 2010). The current velocity, in turn, is affected by climatic fluctuations which makes the currents move faster. This

would shorten the time larvae take to move across oceans. On the other hand, occurrences of mesoscale eddies would entrain larvae and restrict their movement and net transport (Kinder *et al*., 1985; Lessios *et al*., 1984).

Dispersal or lack thereof, in marine species is also affected by body size. Many small-bodied marine species make up a large part of mangrove fauna. Small body size is considered an asset to population mixing when regarding motion due to water movement and potential rafting (Donald *et al*., 2005). Extreme tides, surf, or wind may contribute to large-scale dispersal events and facilitate population connection. The ability to cling to drifting debris is an important part of dispersal. There is evidence that dispersal of marine species can and does take place by rafting. Rafting on macroalgae has been reported for the pelagic larvae of *Cellana strigilis* limpet compex (Reisser, 2012), sea horses (Teske, 2005), trochid gastropods (Donald *et al*., 2005), and ascidians (Jackson, 1986). Rafting also serves as a primary means of initial colonization to new land masses (Benzie, 1999; Palumbi, 2004).

Direct methods of studying dispersal involve tracing the movement of individuals either through observation or by tagging and recapture, and subsequent estimates of reproductive success. While these methods are applied to some species, it cannot be applied to planktonic larvae of marine organisms. Indirect methods such as the use geographic patterns of genetic variation to infer the amount of migration that must have occurred to produce the existing pattern are better. Indirect methods also assess the cumulative effect of gene flow among populations and return higher estimates of gene flow than direct methods.

Indirect methods usually use frequency or sequence markers. Frequency markers derive their power from frequency arguments: alleles that are relatively rare but common in a few populations suggest these populations are connected by gene flow (Hellberg, 2009). Microsatellites are the primary codominant frequency markers commonly used. On the other hand, sequence markers derive their power from the ability to infer relationship between alleles (Hellberg, 2009). MtDNA sequences usually serve as sequence markers to date whereas single-copy nuclear sequences are emerging as another form of these markers. These markers produce enough variation or divergence to lend power, but not so much that every individual is unique or that sequence alignments become ambiguous (Hellberg, 2009).

2.1.4 Habitat and distribution

Nerites can be found attached to crevices of rocks, inside rotten branches, and on top the roots of mangrove trees. Juveniles are usually found in the high intertidal zones further from the reaches of marine predators and high-energy wave action. As they grow and reach adulthood they move into the mid intertidal zone where they will encounter predation and wave pressure but food abounds.

Nerites can be found all over the world along the equator where temperatures are warm enough for their survival (Frey & Vermeij, 2008). In Malaysia, intertidal nerite species inhabit rocky shores whilst others inhabit mangrove areas (particularly with *Rhizophora* and *Bruguiera* trees). They attach themselves in crevices of rocks and walls along waterfronts, in tyre rings at jetties, and in nutrient-rich areas. Occasionally, an individual species can be found having its own microhabitat along

the tropical rocky shores. At other times, two or three species can be found occupying the same microhabitat. For example, in a study which involved *N. tessellata*, *N. versicolor*, and *N. peloranta* of St. Ann's Bay, Jamaica, it was discovered that there was strong correlation between nerite distribution and the microhabitats found within the grey and black zones of rocky shores (Cairns & Wagner, 2000). *Nerita peloranta* was found in areas with major physical stress due to water loss by extreme heat and sun exposure. *Nerita tesselata* existed in areas subjected to frequent submersion and consequently, lower oxygen levels while *N. versicolor* was found in intermediate areas. It was concluded that the separation of these species was due to physical adaptations to their environment rather than interspecific competition.

2.1.5 Diversity of the family Neritidae

In the family Neritidae, 110 were estimated to be freshwater species (Strong *et al.*, 2008), while some are brackish water species and others are fully marine species. Yeung (2004) previously estimated more than 100 species in the genus *Nerita* but later, Reisser *et al.* (2012) estimated a modest 70 species. Apparently, diversity evaluation for these taxa is problematic because of the absence of global revisions at the family level and unstandardized generic concepts applied locally and between regions. Only some of these fauna are well-researched and have gone through systematic revision using the latest molecular and morphological methods while others seem to be neglected. This gives rise to older and broader concepts of tropical genera which are more likely to be polyphyletic when counterparts in temperate areas tend to be narrowly defined (Strong *et al.*, 2008). At times, the number of species is overestimated but this is presumably made up for by fauna yet to be

inventoried and thousands waiting to be discovered either from cryptic or entirely new taxa (Lydeard *et al*., 2004).

2.1.6 Importance of nerites

Nerites play an important role in the food web (Alfaro *et al*., 2006; Abrantes & Sheaves, 2009). They are gregarious herbivores that graze on green or brown algae commonly found on their substrates. In wetland food webs, they are the primary consumers of algae, water plants, and some insects. Nerites help reduce the abundance of foliose algae and young stages of other sessile invertebrates, and alter the relative abundance of encrusting algae. They are important in the conversion of plant matter into animal material besides themselves being food for carnivorous and omnivorous animals.

Their natural predators are crustaceans such as crabs and swamp-inhabiting mammals such as otters, fish, and birds. They are even consumed by other nerites from the genera *Clithon* and *Vittina* which feed facultatively but extensively on the eggs of various confamilial species after breaking the capsule wall by means of intensive radular rasping (Kano & Fukumori, 2010). Freshwater slugs of the genus *Strubellia* (Heterobranchia: Acochlidia) also feed on nerite eggs in Melanesian streams. Predation on marine nerite eggs has been reported for some muricid species in the Indian Ocean and southwestern Pacific (Taylor, 1976; Fairweather, 1987; Fairweather & Underwood, 1991).

Besides playing their role in the food web, nerites are used by fishermen and fishing enthusiasts as bait for catching fish. Humans consume these snails as delicacies or as

traditional cures for several ailments. For East Malaysians, nerites are a common dish in everyday meals and can be bought off the stalls set up on roadsides at RM2.00 per packet (Figure 2.2). In Vietnam, several species of nerites can be found served in restaurants. These nerites are a source of protein, vitamins and minerals (Aminoz, 2012).

Nerites have also been used as biomonitors of heavy metal pollution in many studies (Cubadda *et al.*, 2001; Gay & Maher, 2003; Conti & Cecchetti, 2003; Foster & Cravo, 2003; Liang *et al.*, 2004; Hamed & Emara, 2006; Devagi & Arfiziah, 2009). This is because these gastropods are sedentary, abundant, of relative longevity, and are easily collected and weighed (Yap & Cheng, 2009). Yap and Cheng (2009) demonstrated how levels of heavy metal such as plumbum, ferum and zinc can be quantified from areas of land reclamation, urbanization, shipping, and other industrial activities, using nerites. This study inferred the safety levels of the aquatic environment in those areas and its potential threats to human health.

2.1.7 Molecular markers in phylogenetics and population studies of gastropods

Molecular methods have been used in phylogenetics and species recognition for more than fifty years. In the beginning, allozymes were used (Avise, 1975). This was followed by the introduction of mtDNA examination—a method widely used today to identify species (Avise, 1994). Its maternally-inherited, non-recombinant, and rapidly-evolving traits make mtDNA a valuable marker in phylogeographic and population studies. Commonly used mtDNA genes for species identification are cytochrome *b* (Parson *et al*, 2000; Hsieh *et al*., 2001) and cytochrome oxidase I

Figure 2.2: *Nerita balteata* sold at a roadside stall in Sabah.

(COI) (Hebert *et al*., 2004; Ward & Holmes, 2007; Dawnay *et al*., 2007). Besides these, markers such as ATPS (Jarman *et al.,* 2002; Frey & Vermeij, 2008), ITS1 (Nilsson *et al*., 2008; Li & Dao, 2011; Schoch *et al.,* 2011), 28S (Park & Chung., 2003; Cepeda *et al.,* 2012), and microsatellites (Routtu *et al*., 2007; Vanhaecke *et al.*, 2012) have also been used.

In population studies, there are two schools of thought in the genetic structure of marine species with planktonic larval stages. A review of literature has indicated that most species with long larval life tend to have no genetic differentiation over long distances as demonstrated by gastropods *Littorina scutulata* (Kyle & Boulding, 2000) and *Morula marginalba* (Hoskin, 1997), sea anemone *Anthopleura elegantissima* (Edmands & Potts, 1997), and sea urchin *Echinothrix diadema* (Lessios *et al*., 1998). In contrast, other species with long larval life history show significant genetic differentiation over their geographical range, such as the gastropod *Littorina plena* (Kyle & Boulding, 2000), the pearl oyster *Pinctada margaritifera* (Benzie & Ballment, 1994), and starfishes *Acanthaster planci* (Benzie, 2000) and *Linckia laevigata* (Williams & Benzie, 1997).

A previous study on California black abalone, *Haliotis cracherodii* Leach, 1814, indicated a restriction in gene flow and inferred lack of interpopulation dispersal. They used allozyme loci which detected significant genetic differentiation but their mtDNA cytochrome oxidase I (COI) sequence analysis did not reveal the same results. A study on the same species conducted later by Gruenthal and Burton (2008) using COI, AFLP, and microsatellite methods, indicated genetic structure of natural populations. Their microsatellite analyses detected significant pairwise population

divergence scattered throughout their sampled range. AFLP results provided further support for isolation-by-distance among samples. Even though the COI sequences analyses showed little evidence of restricted gene flow among natural populations, they concluded that the populations of *H. cracherodii* along the California coast are not panmictic and that larval dispersal is not sufficient to genetically homogenize the species.

Diaz-Ferguson *et al*. (2010) tested for genetic structure of rocky shore trochid gastropod, *Cittarium pica*, using DNA sequence variation at the mitochondrial COI and 16S loci. They found substantial differentiation among the Caribbean sites which they sampled from. This genetic differentiation was contradictory to a previous assessment of Caribbean connectivity carried out based on larval dispersal from hydrodynamic models which saw one of the populations in the Bahamas exhibiting strong relationships with Eastern Caribbean sites in this study.

Phylogeographical disjunction was also detected in abundant, high-dispersal, littoral gastropods (Waters *et al*., 2005). Analyses of mitochondrial DNA sequences obtained from intertidal gastropods, *Nerita atramentosa*, in Southern Australia revealed a split between the east and west populations. The two clades resulting from the analyses were highly divergent with little overlap in between. This biogeographical incongruence is not characteristic of species with a planktotrophic veliger phase of 5-6 months. Waters *et al*. (2005) explained this disjunction was due to a paleogeographical barrier called Wilsons Promontory which helped maintain discontinuity between the two parts of Southern Australia. But besides the isthmus,

oceanic currents (East Australian Current and Leeuwin Current) also played a part the differentiation of the Australian *N. atramentosa*.

Other than palaeogeographical barriers and oceanic currents, pre- or post-zygotic barriers or Allee effects may also cause population divergence (Crandall *et al*., 2008). This was concluded when Indian and Pacific Ocean populations of *N. albicilla* produced reciprocally monophyletic clades. This pattern has previously been reported in other studies (Duke *et al.,* 1998; Williams & Benzie, 1998; Benzie, 1999; Reid *et al*., 2006). Low numbers of effective females per population size pose as barriers to reproduction as migrants from one clade are unable to reproduce successfully with members of the other clade even though oceans are crossed, simply due to the Allee effect.

A study on geographical subdivision, demographic history, and gene flow of the intertidal snail, *N. scabricosta*, from the tropical eastern Pacific also showed significant differences between its Panama and Gulf of California/Baja populations (Hurtado *et al*., 2007). Apparently, this is due to the longevity of the larvae and/or the vertical strata at which the larvae are transported. *Nerita scabricosta* are thought to have a shorter larvae life-span and these larvae are carried at the vertical range in which surface equatorial currents and counter-currents deflect from the coast, preventing connectivity between populations. Several other marine taxa also show genetic divergence across the Gulf of California or between the Gulf and the Pacific Baja peninsula. For the rocky reef blennioid fishes *Axoclinus nigricaudus* and *Malacoctenus hubbsi* (Riginos & Nachman, 2001; Riginos & Victor, 2001), and penaeid shrimps *Penaues stylirostris* and *Penaeus californiensis* (Aubert & Lightner,

2000; de la Rosa-Velez *et al*., 2000), isolation by distance and pelagic larval duration seem to be the explanations.

On the contrary, many cases have reported genetic congruence in populations of organisms isolated by distance or geographic boundaries. This includes the study conducted by Myers *et al*. (2000) who sequenced the COI gene of populations of nerites *Clithon spinosus*. The *C. spinosus* showed no evidence of genetic isolation at any of the scales tested. This indicates that the larvae of *C. spinosus* are most probably long-lived planktotrophs and can survive a 140-km trip between islands. It was concluded that all individuals of *C. spinosus* were part of a panmictic population.

Nerita plicata sampled from the Indo-Pacific also displayed panmictic patterns for its populations (Crandall *et al*., 2008). This species managed to remain panmictic over a distance of 22000 km. This is because there were no major changes in the geostrophic flow of the South Equatorial Current during the most recent glacial maximum (Thunnel *et al*., 1994) which could have separated or reunited lineages spanning such a barrier. The absence of a clear physical barrier to dispersal combined with relatively high coalescent estimates of gene flow in the region argues against allopatric divergence in the Central Pacific.

Indeed, population genetic analyses can provide an indirect measure of connectivity among populations (Bossart & Prowell, 1998; Waples, 1998; Hellberg *et al*., 2002; Thorrold *et al*., 2002). The more popular ways are to measure gene flow and values of F_{ST} and its analogues (such as Φ_{ST}). Gene flow, defined as the movement of

gametes or individuals from one place to another and incorporation of the genetic material into the recipient population, influences both the population structure and geographic distribution of a species, as well as the adaptation of populations to their local environments (Slatkin, 1987). Gene flow is usually seen as a homogenizing force, preventing the differentiation of populations that exchange gametes or individuals (Mayr, 1963; 1970).

High gene flow due to pelagic larval dispersal was detected among South Pacific archipelagos in amphidromous gastropods *Neritina canalis* and *Neripteron dilatatus* (Neritomorpha: Neritidae) (Crandall *et al*., 2009). Although the adults of amphidromous species live and reproduce in streams, rivers, or estuaries, their planktonic larvae are released downstream to the ocean, where marine salinities are required for their successful development (Anger *et al*., 1990; Diesel & Schuh, 1998; Crandall, 1999; Diele & Simith, 2006). After metamorphosis and recruitment to river mouths, juveniles migrate upstream to freshwater habitats (Schneider & Frost, 1986; Blanco & Scatena, 2005; Torres *et al*., 2006). Their population ecology is more similar to that of a marine species because of their pelagically dispersing larvae. They colonize Central Pacific archipelagos that lie 2000 km away from the nearest freshwater habitat by having relatively long pelagic larval durations extending 5-6 months (Waters *et al*., 2007) or even a year (Ford, 1979). These dispersal capabilities ensures for their widespread distribution (Scheltema, 1971).

Similarly, no evidence was found for restricted gene flow between Mexican and Panamanian populations of *N. funiculata*. The populations maintained panmictic across the coastal areas of the tropical eastern Pacific. The larvae of this species are

thought to have a long life-span and move with currents at deeper strata to maintain connectivity between the Mexican and Panamanian sites. Long-distance dispersal across thousands of kilometers like this are common and have been observed in several species that remain genetically uniform even at whole ocean and inter-ocean scales (Palumbi, 1994; Lessios *et al*., 2003; Hurtado *et al*., 2004).

2.2 Biogeography and physical oceanography in Malaysia

2.2.1 Geological History

The geological history and biogeography of Malaysia encourages population segregation rather than their homogeneity. This country comprises of five major geological terrains: (1) the Western Belt of Peninsular Malaysia (the Isthmus of Kra, a narrow land bridge located approximately in the middle of the Thai-Malay Peninsula, is situated here); (2) the Core region (also known as part of Sundaland and consisting of the rest of Peninsular Malaysia); (3) the Central region (northern Sarawak and western Sabah); (4) the Kinabalu zone (a geological suture zone here is considered to mark the remnant of a once-open ocean basin that became closed some 25 million years ago), and (5) Eastern Sabah (Metcalfe, 2011) (Figure 2.3).

While the Core region is considered to have been in its present position since distant geological past, the other terrains have more dynamic histories. Fossils suggest that the Western Belt was once attached to Gondwana at the northwest of Australia and, Eastern Sabah may have been attached to the Asian continent near Hong Kong. Central-northern Sarawak and western Sabah came into existence by growth of the Southeast Asian landmass (Metcalfe, 2011). The South China Sea (SCS), which