CHAPTER II

LITERATURE REVIEWS

2.1 Biology of spotted babylon

2.1.1 Taxonomy

Babylonia areolata Link, 1807 is belonged to Phylum Mollusca, Subphylum Conchifera, Class Gastropoda, Order Neogastropoda, Super family Muricacea and Family Buccinidae. The order Neogastropoda is very large, with about 5,000 species almost all members of this order are marine benthic and carnivorous. The family Buccinidae is one of the most diverse and dominant groups of predator prosobranch gastropods that distribute around area of the seabed. While revising the buccinid genus *Babylonia* of Gittenberger and Goud (2003) distinguished 11 extent species, two of which were polytypic with two subspecies each, and 12 fossil and extinct species. Six recent species are also known from the Mediterranean region. The oldest *Babylonia* species are from eocene deposits in Italy. The genus apparently originated in the Tethys Sea and became extinct in the Mediterranean region after the Miocene. Recently, the most common species are *Babylonia areolata, B. japonica* and *B. spirata.*

2.1.2 External morphology

Spotted babylon, *Babylonia areolata* commonly known as Hoy Wan in Thailand, is a carnivorous marine benthic gastropod, which the outer shape composes of a thick oval shell with a high, pointed apex. The shell is smooth and the body whorl is patterned, which round brownish patches on the white shell background array in 3 rows on body whorl. There is little furrow on body whorl which is spiral rib and has an oval palp to close aperture smoothly by using an elastic ligament. There are variations in shell color pattern of this marine snail. However, there is no report on that variation in *B. areolata* and a description of the snail. The differences are mainly on the color of patches and basal shell color; orange patches on white basal shell, white basal shell without patches reported on the website (http://www.image/austraoceasis.html). It has been proposed that it was a subspecies of *B. areolata* namely, *Babylonia arelata* (f) *austraceanensis* (Figure 2.1).

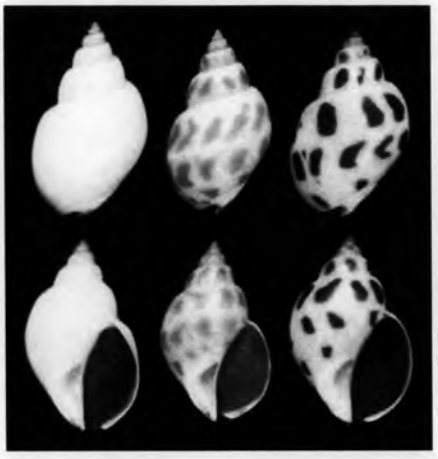

Figure 2.1 *Babylonia areolata* (right) snail varieties of color patterns found in the Gulf of Thailand (After: Chaitanawisuti et al., 2002).

2.1.3 Distribution in Thailand

Spotted babylon lives in sandy or muddy subtidal areas, usually less than 10-20 m in depth. They can be found throughout the littoral regions in the Gulf of Thailand. In Andaman Sea the distribution of spotted babylon is scattering and the number are very low. Mainly is *Babylonia spirata*, spotted babylon prefers the environment with salinity ranging from 28 to 35 ppt and water temperature of 25-30 $^{\circ}$C.

2.1.4. Life-history

Spotted babylon is dioecious. They can reproduce by internal fertilization all year with a maximum peak of the culture in February to August (Chaitanawisuti et al., 2002). The life cycle starts from eggs contained in capsule. The eggs are laid on muddy and substrata, which embryos develop inside the capsules. Larvae emerge as planktonic veligers about 7 days after the capsules are deposited; free living about 14-16 days until veligers settle and metamorphose after hatching then benthic life starts (Figure 2.1).

The duration of this planktonic period is dependent on a function of the developmental rate, which is influenced by environmental factors such as temperature, salinity, dissolved oxygen concentration, pH, pollutants, availability of food and suitable substrates (Pechenik, 1990; 1987). Key factors for larval survival are predation,

oceanographic conditions and starvation or limitation of food resources (Wehrtmann, 1991). Nutritional conditions experienced by planktonic larvae can affect a host of developmental processes and ecological constraints (Allison, 1994).

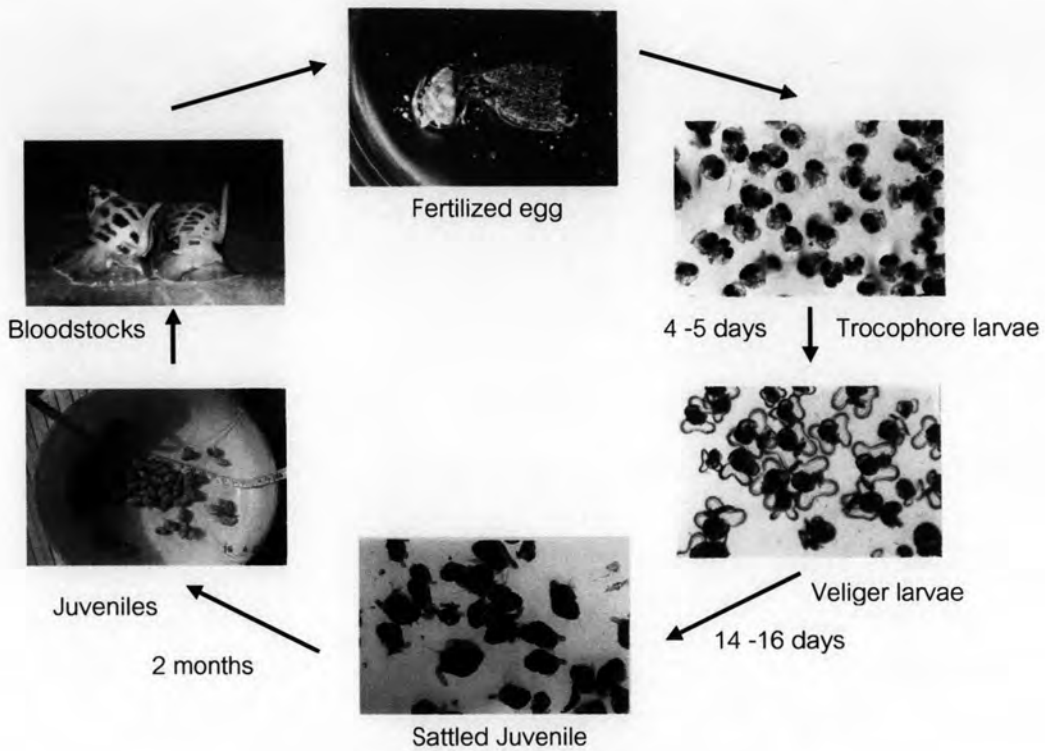

Figure 2.2 Life cycle of *Babylonia areolata* (Chaitanawisuti and Kritsanapuntu, 1999)

2.1.5 Food and feeding

Spotted babylon is carnivorous species. Feeding can be classified to two phases (veliger larvae and juvenile). Veliger larvae phase is the planktonic stage which moves following the water current. It is a filter feeder by using the velum and has cilia to blow the water with the plankton coming its mouth and filter to eat in which the plankton is a single cell of microalgae such as *Chaetoceros* sp., *Skeletonema* sp. and *Chlorella* sp. The second phase is settle juvenile stage to juvenile stage which feed on that detritus and fresh animals such as fish and shellfish. It use proboscis to suck food and then food pass through into the body in which the salivary gland produces the enzyme for digestion.

2.2 Shell color polymorphism

Shell color polymorphism is a distinctive feature of the populations of many marine and terrestrial gastropods. In gastropods, shell color may have three functions: communication, crypsis and thermoregulation. For these functions, it is the shell coloration itself which is selectively important. Several cases of so-called physiological selection on shell color have also been documented, where the shell color has been correlated with selectively valuable physiological traits including responses to temperature, salinity, metabolic rates and fecundity. It is suggested that in these cases, correlation between individual physiology and shell color polymorphism is a result of pleiotropic effects of genes responsible for the shell color or a linkage between them and genes determining certain physiological features (reviewed in Sokolova and Burger 2000).

The variation in shell color is related to environmental gradients such as climate, isolation, wave exposure and salinity (reviewed in Sokolova and Burger, 2000). Such variation has proved to be stable and repetitive through time and space. The effects of environmental factors on color polymorphism have been most thoroughly investigated. For example, visual predation has been assumed to contribute to the maintenance of prey polymorphisms. Mimicry and niche selection by each morph may enhance polymorphism (Endler, 1988). If morphs select visually compatible backgrounds, polymorphism persists if visual predators exert frequency-dependent selection by preying on color morphs when they are common relative to their compatible background. Not only do biotic factors but also physical factors of the environment such as temperature affect color patterns (Cook, 1986). This suggests an adaptive value to shell color in gastropods and has stimulated numerous experimental works on the adaptive significance of shell coloration.

The gastropod mollusks (snails and slugs) can be found from the abyssal depths of the oceans to the tree tops of tropical forests; they are the largest class in the Phylum Mollusca and are one of the most significant groups contributing to the biodiversity in shallow-water marine environments. Among the largest, most well-known gastropods in such marine environments are the neogastropods, a megadiverse group of mostly carnivorous snails that originated in the Mesozoic, and underwent extensive

radiations in the Tertiary (Bouchet and Rocroi, 2005; Ponder and Lindberg, 1997). In gastropod species studied so far in this respect, a direct genetic control of shell coloration has been demonstrated (Ekendahl and Johannesson, 2007). The evolution and maintenance of morphology especially shell-color variation is often described to natural selection, under genetic control (Hughes and Mather, 1986). However, phenotypic expression (morphology) may be a consequence of ontogenetic circumstances (Raup, 1966). Many molecular genetic methods were studies. As the studies of Robinson and Davinson (1996) suggested that color polymorphism is a characteristic that can be defined by the coexistence, within a population, of two or more color morphs genetically determined and segregated, where the frequency of the rarest morph is not maintained only by mutation In the phylum mollusca there is often an association between the shell color and the dominant background color (Cain, 1977). Differences in the diversity of color morphs within populations are also caused by differences in the level of gene flow among genetically divergent populations (Davison and Clarke, 2000). Genetic drift and inbreeding are other important factors that affect the levels of polymorphisms. Loss of variation as a result of strong founder effects have been observed in land snail populations introduced from Europe to North America (Brussard, 1975). This suggests an adaptive value to shell color in gastropods has stimulated numerous experimental works on the adaptive significance of shell coloration. In addition, polymorphisms can be maintained by gene flow between different niches when organisms occupy more than one environmental niche. Thus, color polymorphisms appear to be maintained by environmental heterogeneity.

2.3 Method of determining genetic variation

Since the mid 1980s, genome identification and selection has progressed rapidly with the help of PCR technology. A large number of marker protocols that are rapid and require only small quantities of DNA have been developed. Three widely-used PCR-based markers are Restriction fragment length polymorphism: RAPDs (Williams et al., 1990), SSRs or microsatellites (Tautz, 1989), and amplified fragment length polymorphism: AFLPs (Vos et al., 1995). Each marker technique has its own advantages and disadvantages. RAPD markers are very quick and easy to develop (because of

the arbitrary sequence of the primers) but lack reproducibility (Karp et al., 1997; Virk et al., 2000). AFLP has medium reproducibility but is labour intensive and has high operational and development costs. Microsatellites are specific and highly polymorphous (Karp et al., 1997), but they require knowledge of the genomic sequence to design specific primers and, thus, are limited primarily to economically important species.

However, dating back to the 1990s, studies with simple sequence repeat stretches indicated that are ubiquitous constituents of eukaryotic genomes and highly variable showing extensive length polymorphisms (Tautz and Renz, 1984 and Tautz, 1989). These DNA stretches having 1–5 bp core repeat unit lengths named as microsatellites (Litt and Luty, 1989), short tandem repeats (STR) Edwards et al. (1991), simple sequence repeats (SSR) (Jacob et al., 1991), or simple sequence length polymorphisms (SSLP) (Tautz, 1989), were shown to be valuable genetic markers with codominant Mendelian inheritance and presumed even distribution. Variation in microsatellite containing loci could be detected using PCR, and these loci can be polymorphic enough to genotype individuals (Akkaya et al., 1992 and Rafalski et al., 1996). However, PCR based detection of variation at SSR loci is technically demanding and relatively expensive requiring construction of genomic libraries, selection and sequencing repeat containing clones and synthesizing primers flanking the core repeat units to survey allelic variation in the loci. A variant approach circumventing these disadvantages, based on amplification of genomic segments flanked by the inversely orientated and closely spaced SSR loci using microsatellite core unit bearing oligonucleotide primers which could be either nonanchored or anchored to 5' or 3' end of the repeats with 1–3 arbitrary nucleotides, has been described (Zietkiewicz et al., 1994; Rafalski et al., 1996). This technique call named is inter-SSR (ISA or ISSR). ISSRs are semiarbitrary markers amplified by PCR in the presence of one primer complementary to a target microsatellite. Initially this marker was used in higher plants and animals (Zietkiewicz et al., 1994) and has been used to determine genetic variation of animal population; such as Japanese flounder (Liu et al., 2006), surf clam (Hou et al., 2006), sea cucumber (Bing et al., 2005), bivalve *Gemma gemma* (Casua et al., 2005) Mediterranean cyprinodontiform fish (Maltagliati et al., 2006).

Molecular techniques provide a new type of data that can be used to reconstruct or verify previously established classifications that were based on morphological or physiological characters (De Bruin et al., 2003). The choice of a molecular marker technique depends on its reproducibility and simplicity. The best markers for genome mapping, marker assisted selection, phylogenic studies, and crop conservation have low cost and labor requirements and high reliability.

2.3.1 ISSR technique

The molecular marker inter-simple sequence repeats (ISSR) was chosen to examine genetic variations and shell color patterns of *Babylonia areolata* in the Gulf of Thailand because ISSR analysis requires no prior knowledge of the genome, leads to multilocus and highly polymorphous patterns (Zietkiewicz et al., 1994) and entails PCR-amplification of regions between adjacent (within 4000 bp), inversely oriented microsatellites using a single SSR containing primer (Zietkiewicz et al., 1994; Karp and Edwards, 1997; Wolfe, 2005). Gel electrophoresis of the PCR product generates bands of a particular size for that locus representing intervening stretches of DNA between microsatellites (Nagaoka and Ogihara, 1997, Larsen and Medlin, 1997). Usually several paired microsatellites exist, resulting in multiple band generation (Vis, 1999). Bands are then scored as present or absent. ISSRs have been reported as highly reproducible, polymorphic, and informative (Zietkiewicz et al., 1994) because they identify alleles simultaneously at multiple, interspersed loci throughout the genome (Sunnucks, 2000). Each band corresponds to a DNA sequence delimited by two inverted microsatellites. Like RAPDs, ISSRs markers are quick and easy to handle, but they seem to have the reproducibility of SSR markers because of the longer length of their primers thus, they are reproducible and highly polymorphic DNA markers, polymorphisms should be easier to detect because variable regions in the genome are targeted (Rafalski et al., 1996; Bornet and Branchard, 2001) and the technique is quicker and more straightforward than AFLPs and does not require the high development cost of conventional SSRs.

2.3.2. mtDNA sequencing technique

Mitochondria, the organelles responsible for aerobic metabolism in eukaryotic cells, possess their own small circular DNA molecules. Each cell may contain one to hundreds of mitochondria. Thus the useful properties of mtDNA are its high copy number, while any nuclear gene may be present in just two copies per cell (one from each parent in diploid organisms) (Smith, 1991). Some metabolically active tissues, such as eggs or insect flight muscle, are particularly rich in mitochondria and mtDNA. High copy number makes it easy to analyze PCR amplified directly from total DNA of animal tissues, without the necessity of isolating and cloning individual genes.

Another useful characteristic of mtDNA is a single small, circular molecule, double stranded DNA molecule of between 16,000 to 20,000 base pairs. Most animal mt-genomes consist of a single circular molecule containing 37 genes: 13 encoding polypeptides involved in oxidative phosphorylation, two encoding ribosomal RNAs and 22 encoding tRNAs of the mitochondrion's own protein synthesizing system. Among invertebrate mtDNAs, extensive gene rearrangements have occurred, resulting in considerable differences in gene organization between taxa (Boore, 1999; Wolstenholme, 1992). Because it is unlikely that identical gene orders would arise independently, it has been argued that similar gene arrangements are indicative of common ancestry. Thus, comparisons of mtDNA gene order may be of great value in resolving phylogenetic histories, particularly those involving deep branching events that prove difficult to resolve by nucleotide or amino acid sequence analyses (Boore and Brown, 1994; Boore, 1999).

The transcribed regions are highly conserved. The non-coding region A+T rich region of mtDNA evolves rapidly, while the genes such as those coding for the large and small subunits rRNA, or Cytochrome Oxidase subunits I and II (CO I and CO II) evolve at lower rates. Mutations in mtDNA are acquired faster than in the nucleus, and thus provide a scale for measuring even recent population data. Most importantly, mtDNA is maternally inherited thus can be used to define monophyletic groups of individual. Without of recombination makes the history of its inheritance easier to trace and provides an important tool for analysis recent population history (Singh et al., 1995). In addition, there has been significant study on the mtDNA genome and much

comparative information is available in the databases. Beside mtDNA is not sensitive to environmental selection pressure (Franck et al., 2000).

2.4 The previous studied of *Babylonia* sp.

The study of spotted babylon have been confined to investigations into breeding behaviour (Kannapiran, 1994), general biology (Thirumavalavan ,1987), fishery status (Ayyakkannu, 1994), food preference, environmental tolerance (Patterson et al., 1995, 1994) and the impact of starvation on larvae (Zheng et al., 2005), While Chiu and Liu (1994) observed on the copulation and egg-laying behaviors and the influence of varying larval concentrations on the developmental performance (Shieh and Liu, 1999). Most of the studies usually concentrated or related to aquaculture were hatchery and culture technique (Singhagraiwan 1996; Chaitanawisuti and Kritsanapuntu, 1997a, b, 1999, 2000; Chaitanawisuti el al., 2001a,b, 2002). Beside there are also studied on the acute and subacute toxicities of lead and cadmium in spotted babylon (Supanopas, 2003 and Tanhan, 2003).

Liu and Chiu (1998) has been studied allozyme and morphological of *Babylonia formosae formosae* and *B. formosae habei*. The result indicated that the two subspecies deserve to recognize as full species *B. formosae* and *B. habei*. In recently Yin et al. (2007) was applied RAPD technique to study the genetic diversity of two cultured populations of *B. areolata* from Thailand and Hainan in China, it were good condition. However, the level of genetic diversity of Hainan population was higher than that of Thailand population the mtDNA molecule of *Babylonia areolata* has been reported; the sequence from cytochrom oxidase subunits I in *Babylonia* and *Zemiropsis* (Gastropoda, Caenogastropoda, Babyloniidae), anatomy, shell morphology, distribution and DNA (Gittenberger and Uit de Weerd, 2005). The comparison of DNA sequence has been used in mtDNA analysis phylogenetic the genus *Babylonia* is more closely related to the volutoideans than buccinoideans (Harasewych and Kantor, 2002). Ten species of the family Buccinidae from the Chinese coast based on 28Sr RNA gene (Dong et al., 2008 unpublished). Molecular systematics of family Buccinidae (Mollusca) from China by using 18S ribosomal RNA gene (Dong et al., 2007 unpublished). Su et al. (2007) studied genetic polymorphism of mitochondrial DNA sequences in *Babylonia areolata* and

Babylonia formosae by used the segments sequence of 16S rRNA and COI gene of 22 individuals from west Guangdong waters and east Guangdong water length 506 bp for 16S rRNA gene and 640 bp for CO I gene were obtained, respectively. For *Babylonia areolata* in Thailand, mtDNA have used to study genetic variation of 4 populations from Rayong, Pechaburi, Songkhla and Pattani (sequence lengths 542 bp, 542 bp, 533 bp and 542 bp respectively; Phongdara, et al., 2007 unpublished) and Phongdara, et al., 2008 unpublished reported sequence in database of GeneBank by using internal transcribed spacer (ITSs) containing patairl sequence of both 18S rRNA gene and 5.8S rRNA gene of this species.