CHAPTER V

DISCUSSIONS AND CONCLUSIONS

In the classification system, organisms were grouped from species into genera, genera into families, etc. However, the most importance units for biodiversity were species. The species were natural evolutionarily significant units that could be defined in terms of various morphological, ecological, behavioral and genetic criteria (Tarbsripair, 1998). Organisms responded to their environments at different hierarchical levels ranging from the community level (species abundance patterns) to the cellular and genetic levels (Green et al., 1985). Variable environmental parameters were mirrored in populations with variable genomes and thus reflected a functional aspect of adaptability (Kepkay et al., 1980). The spatial and temporal patterns of morphological variation which might be reflected by complex genetic and plastic responses to several interdependent environmental factors as suggested in Trussell and Etter (2001). Therefore, the discussion on those results would be profoundly described in terms of morphological and genetic variation.

5.1 Morphological variation

The morphological variation in this study would focus only on the different of shell color patterns. *Babylonia areolata* samples collected from 8 sampling sites of 8 provinces were different in shell color and could be divided into 5 categories, brown, orange, white, rust and dark brown stripe. In the upper parts of the Gulf of Thailand, most of spotted babylon was brown and only low amount of rust samples were found in Trad. In the lower parts of the Gulf of Thailand, all five shell color patterns were found and dark brown stripe samples were found only in Prachuab Khiri Khan. The difference in shell color patterns might be the results of genetic variations or variety of environments or both. For example, the appearance of predator *Carcinus maenas* and the immediately change of salinity and temperature can play an important role on the difference in shell color of *Littorina saxatilis* (Ekendahl, 1998; Sokolova and Berger, 2000). In the desert land snail, *Trochoidea simmulata,* shell colors depend on

difference ability of $CaCO_3$ uptake and $CaCO_3$ content in its habitat (Ward and Slotow, 1997). Also, the variation in shell color can be related to environmental gradients such as climate, temperature, isolation (Burger et al., 1995), habitat (Ekendhal and Johannesson, 2007), wave exposure (Etter, 1988), salinity (Sokolova et al., 1995, 1997), metabolic rates (Steigen, 1979), predators (Allen, 1988) and fecundity (Wolda, 1967). From these studies the different of shell color of snails might happen although they were same species. In contrast, there were many reports on the effect of genetics on color polymorphisms showed in the mussels *Mytilus edulis* having blue shell and blown shell (Newkirk, 1980).

In conclusion, the results obtained from this study could be used to explain only the distribution of each shell color patterns of *B. areolata* and their sources, but could not conclude what was the real cause to influence the different of shell color patterns. There were others factors which might influence to morphological variation of *B. areolata*, such as available of food, pollutants, reproductive season, duration of planktonic period etc., and these factors might become an importance issue for further study.

5.2 Genetic variation

ISSR–PCR had been employed to reveal genetic variation in many animals such as silkworm (Pradeep et al., 2005), aphids (Abbot, 2001), Japanese flounder *Paralichthys olivaceus* (Liu et al., 2006) and shrimp *Fenneropenaeus chinensis* (Wang and Kong, 2002). In this study, the genetic variation of five different shell color patterns of *B. areolata* was investigated by ISSR technique and found that each of the 48 individuals was a unique ISSR genotype. It indicated extensive genetic variation in this study (polymorphism 66.67%). The similar result was found in *B. areolata* from Thailand (polymorphism 70.27%) and *B. areolata* from Hainan of Chaina (polymorphism 73.78%) investigated using RAPD (Yin et al., 2007). From the dendrogram obtained from ISSR data (Figure 4.5), there was no identified color group. None of the individuals was genetically identical base on the ISSR-PCR.

According to DNA sequencing technique, the genetic variation among five shell color patterns of *B. areolata* were very low (0.00% – 0.64 %), compared with the variation

of an out group *B. spirata* (12.72%- 13.56 %). This result showed that among five color patterns of *B. areolata* should be recognized as *B. areolata*. Unlike, the result of to *B. formosae formosae* and *B. formosae habie* indicated that the two subspecies deserve to be recognized as full species: *B. formosae* and *B. habai* (Liu and Chiu, 1998). In order to clarify the nucleotide sequence and genetic diversity of *B. areolata* and *B. formosae*, Su et al., (2007) analyzed the segments of mtDNA 16S rRNA and COI. The average number of nucleotide differences and nucleotide diversity of *B. areolata* population were 2.68 and 0.0042, while those of *B. formosae* populations were 5.62 and 0.0078, which demonstrated that *B. formosae* populations had more genetic polymorphic than *B. areolata*.

There was no clear relationship between five shell color patterns of *Babylonia* sp. The patterns of differentiation observed with mtDNA were also supported by results obtained from ISSR data. Therefore, it could be possible to concluded that the genetic marker (16s rRNA and COI genes) used in this study did not have sufficient variation to distinguish color genes in *Babylonia* sp. For ISSR marker, the genetic variation was very high, but there was no distinct ISSR profiles that could be used to differentiate among the five shell color patterns of *Babylonia* sp. The same resuit was reported by Piyapattanakorn (2008) as spotted babylon populations in the Gulf of Thailand showed clearly high levels of genetic variation, which meant that this species had ability to live in different environments better than lower level species. (Hamrick and Godt, 1996). This also indicates that this marine gastropod might exchange gene with each others (gene flow) within or among populations, to produce a new high efficiency generation in preserved the stability of genetic variation. Contradictory, in Andaman Sea *B. areolata* was rarely found. Therefore a natural process such as bottlenecks or random genetic drift might happen in this area.

The variations of shell color patterns of *B. areolata* were higher in the lower parts of the Gulf of Thailand (Nakorn Sri Thammarat, Songkhla and Pattani) than the upper parts (Trad, Chanthaburi, Rayong, Phetchaburi and Prachuap Khiri Khan). In contrast, the investigation on genetic variation of this marine snail populations showed that the populations of the lower part of the Gulf have lower genetic diversity than the upper part. However only spotted babylon, which has brown color were used in that

study (Piyapattanakorn, 2008). In this study, genetic markers that can be used to differentiate among five shell color patterns of *B. areolata* could not be discovered. Therefore it would be better if there were further study of *B. areolata* by using others techniques. The technique would be highly sensitive in detecting genetic differences between individuals such as AFLP (Vos et al., 1995) RNA arbitrarily primed polymerase chain reaction; RAP PCR (Welsh et al., 1992) and RAPD-PCR (Williams et al., 1990).

5.3 Cross breeding

The preliminary cross breeding of spotted babylon broodstock was conducted with nine breeding trials, among 3 shell color patterns of broodstocks under hatchery conditions. The broodstock showed no sign of stress with normal feeding of fresh trash fish, no mortality and moved actively in the spawning tanks. The spawning tanks were equipped with flow-through seawater system with the flowing rate more than 200% per day, seawater monitoring also showed best results of normal seawater quality for all breeding trials. Nevertheless the result showed no spawning of egg occurred throughout the experimental period for all breeding treatments Chaitanawisuti and Kritsanapuntu (2002) reported that spotted babylon *Babylonia areolata* (brown-spotted pattern) spawned all year round with maximum peak during January to May that might be the reason why there was no spawning took place for all breeding experiment. I would like to push my suspect into the affect of sex ratio one male to one female in the spawning tank might make the important roles on breeding behavior of the broodstock. Although, Chaitanawisuti and Kritsanapuntu (2002) stated that the sex ratio of male: female of 1:1 was used for the hatchery production but the spawning tanks contained more than fifty pairs of broodstocks. In addition, Chaitanawisuti (personnel communication) mentioned that successful breeding and spawning of spotted babylon broodstock (brown-spotted and orange-spotted patterns) with sex ratio of 3 female to 1 male or 1 female to 3 male could take place in plastic spawning tanks with the similar bottom area of 0.25 m^2 (Figure 3.6). During high spawning season (January to March), the experiment of sex ratio 1:1 still had no spawning taking place but the experiment of sex ratio 2:6 spawned continuously. The high hatching rate was found and the larvae could develop into settled juveniles within twelve days after hatching. Nevertheless,

the crossbreeding by using sex ratio 2:6 showed that the pairwise of orange pattern (male) vs. white pattern (female). F1 were orange and white shell color pattern, the pairwise of white pattern (male) vs. brown pattern (female) F1 were all brown shell color pattern and the pairwise of brown pattern (male) vs. white pattern (female) F1 were brown shell color pattern more than white color pattern. It was noticeable that genetic such as dominance or recessive gene might effect in these cases. The study showed that there was no difference in genetic patterns among the three morphological patterns of spotted babylon broodstocks. However, cross breeding among the broodstocks of brown basal shell and orange patches on white basal shell should be concentrated on their application use for breeding program or genetic improvement providing better growth and survival of larvae and juveniles. Moreover, the further study should be elucidated that, what were the factors influence to the morphological shell pattern of spotted babylon because the sale price of this species was determined by shell pattern and color.

5.4 Conclusion

From the analysis of genetic variations of *B. areolata* by using molecular technique, ISSR-PCR and DNA sequencing technique, as mentioned before, the results might conclude as these:

1. The samples of the spotted Babylon which collected from the Gulf of Thailand were found that, there was more variation of shell color patterns of ones which habituated in the lower parts than the upper parts of the Gulf of Thailand.

2. The results of ISSR-PCR technique, by using ISSR 4 primers showed high genetic variations in these sample groups (polymorphism 66.67%) but none of characteristic DNA bands to specific with color banding patterns. The dendrogram which showed genetic relatives among sample groups found that there was not clear differentiation among the shell color patterns sample groups of *B. areolata*.

3. The results of DNA-Sequencing techniques, which obtained from the percent differences of cytochrome oxidase I sequences, to compare genetic variations in each pair of shell color patterns, showed that the percent differences of *B. areolata* were so

low (0.00% - 0.64%). Thereafter comparing with an out group of *B. spirata* (using to be a control), the percent differences were higher number (13.56%).

4. The results of experiment from 2 molecular techniques implied that spotted babylon *B. areolata* which were 5 different of shell color patterns, could probably be the same kinds, same species, but not with *B. spirata*.

5. Spotted babylon with difference shell color pattern are the same species.

6. The cross breeding of spotted babylon was not successful enough because of many reasons such as seasons for cultivation, temperature of seawater and limit of time.

7. This study might useful to produce the specific color shell pattern of *B. areolata* for commercial markets.

5.5 Suggestions

This study did not found any genetic remarks which specific to 5 shell color patterns of *B. areolata*. Therefore the different shell color patterns might cause from genetic variations or environment factors or both of them, rendered insufficiently details and inadequate information. Nevertheless there were some suggestions for further study.

1.The cross breeding of this marine gastropod to investigate of shell color patterns should be between January–May, because in this period the broodstock gave a maximum peak of laying egg under hatchery conditions .

2. Sex ratio 1:1 was not suitable for cross breeding of spotted babylon, should select another sex ratio for more efficient reproduction.

3. To classify the different of marine snails, especially the variety of shell color patterns, many branch of science which were highly relative such as biology, morphology, ecology, evolution, molecular techniques etc. should be carefully integrated together to find a definite answer.