CHAPTER V

DISCUSSION

5.1. Morphological Variation

Shells of gastropods contain a rich source of taxonomic information that can be used to interpret differentiation among taxa. Shell morphological characters are used as primary guidelines for species or subspecies identification in general handbooks and the taxonomic literatures (Chiu *et al.,* 2002). The present study of shell morphological variation shows the possibility in identifying species and subspecies in the genus *Mekongia* in Thailand. The results of discriminant analysis indicated that shell measurement character and shell measurement ratios differ significantly ($p < 0.05$) among populations based on Brandt's (1974) classification at the species or subspecies level, This results are congruent to Nei's genetic distances values (see Figure 4-20).

The canonical discriminant functions are presetting a good assistance tool to separate the different groups of snail combining with other methods. This will make an efficient decision in setting up taxonomic criteria before using snails in further sophisticated analyses.

5.2. Reproductive System Anatomy

Mekongia snails are dioecious, male the right tentacle is enlarged and use for sperm transfer (Fretter, 1953). The adult sex discrimination can be identified easily by the shape of the right tentacle: slender and pointed in female, thickened and rounded in male. *Mekongia* species brood offspring internally (ovoviviparous) as other viviparid members do.

The results of reproductive anatomical study revealed no significant differences in the shape or position of organ system among the sample of snail populations. This quite similar to various freshwater gastropods which reported in many species.

The reproductive system of male is simply composed of a single testis, vas deferens, prostate gland, and penial (the modified right tentacle). This modified right tentacle is used as sex determination and to identify members of the family Viviparidae (Brandt, 1974). The female reproductive organs composed of ovary, oviduct, albumen gland, seminal receptacle, and pallial duct. The pallial duct is modified to form a brood-pouch in which the embryos are retained until their development is complete. Both albumen gland and shell gland are developed in pallial oviduct of viviparid species, but capsule gland is replaced by a thin-walled brood pouch. Before the embryos enter it, each is provided with albumen and shell (Berry, 1974).

Berry (1974) studied the reproductive anatomy of two Malayan viviparid gastropods, *Siamopaludina martensi* and *Filopaludina sumatrensis*, which closely related to the Thai viviparids. The female reproductive organs of *Mekongia* snails in the present study were very similar to that of snails investigated by Fretter and Graham (1962) studied the genus *Viviparus* which revealed V-shaped of oviduct, albumen gland, and pallial oviduct quite similar to the Thai genus *Mekongia*. *Viviparus* belonging to subfamily Viviparinae distributed in Europe and North America, whereas all Thai viviparid snails belonging to subfamily Bellamyinae, are distributed in Africa, India, and Asia. The male in subfamily Viviparinae is characterized by having a single testis embedded in the coil of the digestive gland, but the male in subfamily Bellamyinae was distinguished by having a bean-shaped testis (Pace, 1973) which are in congruence with this study.

Vail (1977) investigated reproductive organs of 3 viviparid species, which results showed male and female reproductive organs of three species were no different among species. Finally, suggested that male and female reproductive system in all genera in subfamily Bellamyinae may be quite uniform.

5.3. Allozyme Variation

Allozyme analysis play an efficient part in the field of systematic biology because it can often detect taxonomic and phyletic diversity in group of organisms performing little morphological divergence, or in groups exhibiting complicated patterns of morphological variation (Toda *et al.*, 1998). Allozyme electrophoresis has provided many genetic markers for the analysis of problems in systematics.

In the present study, electrophoretic data of *Mekongia* showed that isozymes existed in different population for examples, GPI showed fixed allele in MH population. In addition, the enzyme activities were visible after staining and resolvable into one or more separated bands such as EST-α-1 revealed five alleles variation in only locus. The electrophoretic run has been revealed to be capable of distinguishing known genetic variations or variants (Richardson *et al.*, 1986).

Genetic Variation of *Mekongia*

Nei's genetic distance values were used to construct a UPGMA dendrogram. All samples were divided into four major clusters, one of which consisted of *M. swainsoni braueri* (BB), *M. swainsoni kmeriana* (KB), two populations of *M. swainsoni swainsoni* from ST and VB, and *M. sphaericula extensa* (MH); the second cluster consisted of three populations of *M. pongensis* from BK, NK, and NP, *M. sphaericula extensa* (SK), and *M. lamarcki* (TT); the third cluster consisted of three populations of *M. sphaericula sphaericula* from SR, UB, and VR; and the last cluster, *M. sphaericula spiralis* (TP) was only one sample in this cluster (Figure 4-20).

The 14 populations of the genus *Mekongia* in Thailand were groups into four major clusters by multiple gene substitutions and consequent large interspecific genetic distances (0.071 to 1.125), except *M. swainsoni* group have genetic distance among subspecies 0.010 to 0.017, Nei's genetic distance among species were very small. This is indicated that the *swainsoni* group is very close related among subspecies. However, shell morphology of *M. swainsoni* complex is significant difference between subspecies. This phenomenon may be explained by environmental differentiation. The possibility that environmental factors affect shell morphology has obvious implication for gastropod systematic (Vermeij, 1986). Several studies

reported the effect of environmental factors on growth rate (Palmer, 1990), shape (Gibbs, 1993), and color of shell (Neumann, 1959). Environmental differences could produce morphological variation without a genetic basis. This morphological variation of *M. swainsoni* group did not correspond with electrophoretic differentiation, suggest that the shell differ variation was environmentally induced.

Nei's genetic distance (*D*) value obtained between *M. sphaericula extensa* for MH and *M. sphaericula extensa* for SK was 0.089. This value is smaller than those previously reported for many conspecific populations in various organisms ($D < 0.16$) (Thorpe, 1982). This might conclude that the two taxa are real conspecific. Genetic discrimination of *M. sphaericula extensa* populations could be due to some barrier among tributaries, inhibiting gene flow (such as a large, uninhabitable river).

The genetic distances values obtained between *M. sphaericula spiralis* and other subspecies of *M. sphaericula* ranged form 0.907 to 1.125, these values much greater than the conspecific level, and also their shell morphology is distinguish extremely (strong spiral ridge on shell surface), indicated that *M. sphaericula spiralis* should be deserved recognition as full species, *M. spilaris*. This pattern looks similar to a case reported by Woodruff *et al.* (1988) into two subspecies of *Oncomelania hupensis* from China. Their average genetic distance values were 0.62 ± 0.20, the authors suggested that these two taxa should be raised to species status.

Chi-square values calculated from the observed frequencies compared to the expected frequencies under Hardy-Weinberg equilibrium. Almost of observed frequencies within each population were not significantly different from Hardy-Weinberg expectation, expect *M. sphaericula extensa* from MH

population (p = 0.006). This is indicated that the subdivision within population may be occurred. Several explanations have been offered such as inbreeding (Singh and Green, 1984) to account for this phenomenon. Of this possible explanation, inbreeding is most plausible agents for the observed deficiencies of heterzygosity in natural population of prosobranch snails, because of their eggs are brooded by the female, so dispersal capabilities are even more restricted (Davis, 1982), suggested that prosobranch populations are often high differentiated genetically among populations. Inbreeding population with random mating and in the absence of selection, the frequencies of different alleles of a polymorphic gene can be predicted by Hardy-Weinberg equilibrium. However, if the sample from which allele frequencies are drawn comprises two or more genetically isolated populations, allele frequencies will usually depart from Hardy-Weinberg equilibrium.

Gene Flow among Populations

The slight different genetic distances among population of *M. pongensis*, M. *swainsoni swainsoni*, and *M. sphaericula sphaericula* is similar to that shown in the confamily *Sinotaia quadrata* (Chiu *et al.*, 2002), indicating high gene flow among population. *M. pongensis* (BK, NK, and NP) and *M. sphaericula sphaericula* groups, F_{ST} valve mean is 0.228 and 0.065. All of *M. pongensis* and *M. sphaericula sphaericula* populations have small genetic differentiation among populations, indicated that there are high gene flow and no subdivision among their populations. This may be explained that all collected populations were from the same Mekong drainage. The F_{ST} range from 1 to 0, 1 indicates and absolute subdivision among populations and 0 indicates no subdivision among populations (Chiu *et al.*, 2002). In contrast, *M. sphaericula extensa* snails have high levels of genetic differentiation among samples (MH and SK). High F_{ST} value mean

(0.544) of this groups, suggested that there is low gene flow among them, low allele frequency variance due to large effective population size, strong uniform selection, or combinations of these parameters (Katoh and Foltz, 1994).

The present study proposed the slightly changed classification especially the rearrangement of *M. sphaericula spiralis* to a separated species *M. spiralis*.

BRANDT (1974) CLASSIFICATION	THE PRESENT STUDY
Mekongia swainsoni swainsoni	*M. swainsoni swainsoni*
M. swainsoni kmeriana	*M. swainsoni kmeriana*
M. swainsoni braueri	*M. swainsoni braueri*
M. sphaericula sphaericula	*M. sphaericula sphaericula*
M. sphaericula extensa	*M. sphaericula extensa*
M. sphaericula spiralis	*M. spiralis*
M. pongensis	*M. pongensis*
M. lamarcki	*M. lamarcki*

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Consequently, a key for Thai viviparid snails, genus *Mekongia*, at species or subspecies levels should be revised according to the results of this morphology variation study as follows:

Key to species of *Mekongia*

1a. Adult shell greenish to brownish colored.....……..……..2

1b. Adult shell bright yellow colored, the umbilicus is opened...……..…….

……***M. pongensis***

2a. Apical whorl dark violet……..……...…..……..3

2b. Apical whorl not dark violet……..……...……***M. swainsoni***

3a. Shell ovate-conic, adult shell periderm bright green colored, the umbilicus is opened……..……...***M. lamarcki***

3b. Adult shell dark green colored, the umbilicus is closed.....***M. sphaericula***

Key to subspecies of *M. swainsoni*

1a. Adult shell larger than 25 mm ……..…….....2

1b. Adult shell smaller than 25 mm, shape like a hazel nut, apical whorl with a very low spire……...……***M. swainsoni kmeriana***

2a. Shell ovate-conic with elevated spire……..……...***M. swainsoni braueri***

2b. Shell subglobose with less spire ……..……***M. swainsoni swainsoni***

Key to the subspecies of *M. sphaericula*

1a. Shell surface is smooth…….....2

1b. Shell with obtuse spiral ridge..……...***M. sphaericula spiralis***

1a. Shell subglobose shaped……***M. sphaericula sphaericula***

1b. Shell elongate shaped...…….....***M. sphaericula extensa***