CHAPTER II

LITERATURE REVIEW

2.1. Morphological Variation Study

Mekongia Crosse & Fischer, 1876 was classified in the family Viviparidae, subfamily Bellamyinae. Their shell surface has no color bands, subglobose or ovoidal. There are the important characteristics for separating them from other genera. Shell external morphology *i.e.* the color of apex and shell, shell shape, and shell size differentiation are the main characters for *Mekongia* classification. Taxonomic identifications of species and subspecies levels were based on morphological quality (Brandt, 1974).

The subspecies level is defined as an aggregate of phenotypically similar populations of a species inhabiting a geographic subdivision of the range of that species and taxonomic difference from other populations of that species. The species that contains two or more subspecies is called polytypic. The species that were distinguished subspecies level is called monotypic species (Mayr and Ashlock, 1991). There are a lot of problem in identifying viviparids including *Mekongia*. These unresolved problems are still exhibited in Brandt's classification of some species complex such as *M. sphaericula* (Figure 2-1).

The species complex is highly variable in shell morphology (Clench and Turner, 1956; Clench, 1962; Clench and Fuller, 1965; Thompson, 1984), but the genetic basis of this variation is uncertain. The high degrees of morphological inter- and intraspecific variations have led to some taxonomic confusion (Katoh and Foltz, 1994).

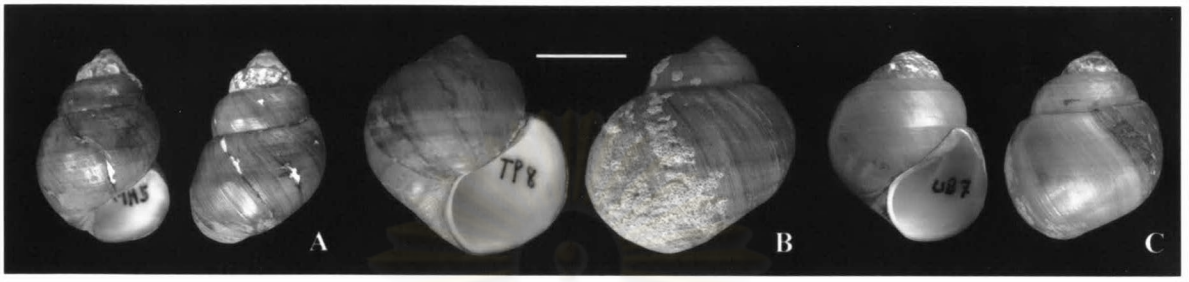

Figure. 2-1. Shell morphological variation of *Mekongia sphaericula* complex. A = *M. sph. extensa*, B = *M. sph. spiralis*, and *M. sph. sphaericula*. Scale bar = 1 cm.

Interspecific variation in shell shape and size are frequently observed due to allometric growth between young (juvenile) and adult snails, or different environmental conditions experienced by habitat fragmentation which can result in isolated populations. Allomeric growth is defined as different proportions correlated with changes in the absolute magnitude of an organism or a specific part under consideration (Gould, 1966). Analysis of the shell size of *Viviparus georgianus* species revealed intraspecific differences possibly due to environmental factors (Katoh and Foltz, 1994).

Many malacologists investigated both inter- and intraspecific variation by used morphometric statistical analyse such as Valovirta and Vaisanen (1986), Klinhom (1989), Henriquez *et al.* (1993), and Chiu *et al.* (2002). The discriminant analysis is a statistical technique which most used to distinguish several mutually exclusive groups. The concept concerns with the linear combination of the independent variable are formed and served as the basis for classifying cases into one of the groups. This technique has been applied in several biological fields (Sokal and Rohlf, 1973).

The descriptive statistic, mean and standard deviation (SD), and the coefficient of variation (CV) are commonly used to discriminate the variation. The CV (percentage of the mean = SD/mean X 100) is an indicator of the homogeneity of samples. The calculation of CV is particularly useful when comparable samples of the same species from different localities are investigated or when the variability of different varieties of the same sample is compared (Vail, 1977). Moreover, some character indices were still used to describe the shell shape differentiation (Henriquez *et al.*, 1993). The shell characters, *i.e.* shell height, shell width, body whorl height, body whorl width, aperture height, and aperture width are the major criteria. The statistical analysis on the computer software SPSS version 11.0. It was used as tool in this study.

Many investigators use statistical method to find the suitable characters and relationships between snail populations Davis (1969), Carpenter *et al.* (1978), and Harasewch (1987). However, only study in shell characters do not provide satisfactory base for distinguishing between species. The shells of gastropod do not represent good taxonomic criteria since they exhibit individual variation due to the age of snail, the type of habitat, and in case of aquatic snails the quality of water in which they live (Malek and Cheng, 1964).

2.2. Reproductive System Anatomical Study

Mostly, viviparid male can be distinguished by the modified form of the right tentacle that serves as a penis. The testis is a long place along the right edge of the mantle wall and it is separated from the rectum above it by the kidney duct. The vas deferens leaves the testis curves round the mantle wall and enters the posterior end of the ridge, which bears the food groove. Here it enters the prostate gland, which passes forward underneath the food groove to the modified right tentacle (penis) (Brandt, 1974).

The female consists of the enlargement of the pallial oviduct to form a brood-pouch, which usually contains several eggs, embryos and developing juveniles (young snails grow to some 3 to 4 mm and about 3 shell whorls before breaking free from egg capsules and emerging from the brood-pouch). It lies parallel to the rectum at the extreme right edge of the mantle cavity, separated from it by the kidney duct. The albumen gland lies closely pressed to the two limbs of the seminal receptacle. The ovary is very small and hard to find. Generally it is dull orange-brown with a few branches among the tissue of the digestive gland.

The genus *Mekongia* is widely distributed in Thailand. Seven species and seven subspecies were described by Brandt (1974). Their taxonomic positions are shown in Table 1.1, and the distributions are shown in Figure 1-1. Many studies of reproductive organs have been investigated in European viviparid snails and other temperate species but few known about viviparid studies found in Thailand and other tropical regions.

The use of anatomy to classify species and subspecies levels of snails has been studied by many malacologists, for example those belonging in the Bithyniidae (Chung, 1984), the Pilidae (Keawjam, 1986). Vail (1977) reported the reproductive system anatomy in classifying the three European viviparid snails, which was not related to the Asian species.

Berry (1974) studied the reproductive system of West Malaysian snails, suggested that features of anatomy are chiefly important in identification of gastropods, details of the copulatory organs, and details of complex parts of the reproductive system are usually of more urgent concern to the parasitologist. However, previous studies on reproductive anatomy of Asian viviparid snails are not sufficiently details. Thus, the reliability on structural reproductive system characters to be used in taxonomy was left in question because of inadequate information on variation.

2.3. Allozyme Electrophoresis Evidence

Allozyme or protein electrophoresis, the migration of proteins under the influence of an electric field, is among the most cost-effective methods of investigating genetic phenomena at the molecular level (Murphy *et al.*, 1990). Major revolution in understanding micro- and macroevolutionary processes has occurred.

Using enzymatic and non enzymatic proteins, numerous investigations have focused on enzyme efficiency, estimating, and understanding genetic variability in natural populations, gene flow, hybridization, recognition of species boundaries, and phylogenetic relationships, among other problems. Two general forms of protein data can be gathered simultaneously using electrophoretic methods. One is derived from isozymes, which are all

functionally similar forms of enzymes, including all polymers of subunits produced by different gene loci or by different alleles at the same locus (Markert and MoIler, 1959).

The other data set consists of allozymes, a subset of isozymes, which are variants of polypeptides representing different allelic alternatives of the same gene locus. Both forms of data are important in molecular systematics, and both involve proteins that can be separated on the basis of net charge and size. The horizontal starch gel methods are widespread use and efficiency. Ways of avoiding or recovering from common pitfalls are described. The electrophoretic principles and methods described are applicable to all organisms (Murphy *et al.*, 1990).

There are many studies of allozyme differentiation in marine gastropods, but little is known in freshwater gastropod species. Unlike many marine gastropods, none of freshwater gastropod species has a planktonic larval stage; larval development occurs within the eggs and a crawl-away juvenile hatch from egg. Although the eggs of pulmonate snail can be dispersed passively, the eggs of prosobranch snails are either brooded by the female or deposited in large capsules, so dispersal capabilities are even more restricted. The difference inferred level of gene flow between freshwater prosobranch and pulmonate snails may also help explain higher degree of endemism in prosobranchs than in pulmonates (Davis, 1982).

The study of variation, especially geographic variation, has been one of the most important approaches to the study of systematics. Geographic isolates casually have and intermediate status between species and subspecies. When population samples from different portions of the geographic range of a species are compared, smaller or greater differences

are often found. On the basis of some criteria, they would be considered species differentiation but the other basis, they would not. It is usually more convenient for taxonomists or malacologists to attach such doubtful populations as subspecies to the species with which they are most nearly allied (Mayr and Ashlock, 1991).

The technique will be mostly served when the two species are in sympatry. The closely related species in sympatry was usually recognized on morphological criteria alone, or may not. When the populations being studied are allopatric *i.e.* the populations are geographically isolated, their specific status is less practical important than in sympatry condition. The decision, that must be made is weather the populations would or would not be reproductively isolated if they came into sympatry (Baversock and Moritz, 1990).

Allozyme unequivocally distinguished the species and was useful markers in correctly classifying the different species when morphological characters overlapped each other (Mauro *et al.,* 2003).

The studies of viviparid snail genetic variation have been one of the most important approach. The two popular parameters are polymorphism (*P*), the proportion of loci found to be polymorphic in sample, and heterozygosity (*H*), the average frequency of heterozygous loci per individual. On the second level, measurements of electrophoretic similarity can be estimated between populations or species by using techniques such as genetic identity (*I*) or genetic distance (*D*) (Nei, 1972).

An allozyme electrophoresis has been successfully used to detect genetic variations within and among European viviparids at the species level (Katoh and Foltz, 1994; Falniowski *et al.*, 1996; Chiu *et al.*, 2002).

Ayala *et al.* (1974) and Ayala (1974) used the *Drosophila willistoni* species group as a basic model to compare genetic diversity at the stages of taxonomic differentiation. The five increasingly divergent levels of cladogenesis recognized are: 1) between similar geographic populations, no reproductive isolation average I (genetic identity) = 0.970, D (genetic distance) = 0.031; 2) between subspecies average I = 0.795, D = 0.230; 3) between semi-species average I = 0.798, D = 0.226; 4) between sibling species average I = 0.563, D = 0.581; and 5) organisms of the same groups, easily distinguishable by morphological characters average I = 0.325, D = 1.056.

Calow and Calow (1983) reported the distribution viviparid snails, which possess both characters of low mobility as adult and brooding in larval stage and low dispersal ability. The possibility of gene flow is increased because viviparid snails, a common aquaculture species have frequently been transported by humans.

Thorpe (1983) found that conspecific population in several molluscs generally had a genetic identity (I) values greater than 0.9, congeneric species ranged from I = 0.25 to 0.85.

Woodruff *et al.* (1988) studied two subspecies of *Oncomelania hupensis* from China and *O. quadrasi* from the Philippines using allozyme data. Despite their morphological similarity and their lack strong post-mating

reproductive isolation, he suggested that these taxa deserve recognition as full species: *O. hupensis* and *O. quadrasi* ($D = 0.62 \pm 0.20$).

Keawjam (1990) reported the Thai apple genetic distance from allozyme studies in snails, *Pila*. The average genetic distance ranged from 0.921-0.998 and genetic identity ranged from 0.083 to 0.002.

Staub *et al.* (1990) suggested that the highly variation in three races of pomatiopsid gastropod, *Neotricula aperta* from Mekong River and its tributaries, Mun River, in Thailand, should be classified as four new species, with the genetic distance (*D*) ranged from 0.22 to 0.74.

Ponder *et al.* (1996) used allozyme electrophoresis technique to study sibling species of hydrobiid, *Dalhousia globosa* and *D. harrisi*. Two species showed genetic distance ranged from 0.100 to 0.130.

Chiu *et al.* (2000) used allozymes to examine the genetic variation of *Cipangopaludina* spp. (Viviparidae) in Taiwan and detected a very low genetic difference within the species.

2.4. Allozyme Statistical Analysis

In order to quantify the genetic variation of specimens from each locality, the population genetic variability was determined using mean number of alleles per locus, the mean effective number of alleles per locus (Hartle and Clark, 1989), the percentage of polymorphic loci at the 0.95 criterion, and the average observed and expected heterozygosities per locus, according to Nei (1978).

Chi-square goodness of fit tests was computed to determine if there were significant deviations from the Hardy-Weinberg equilibrium between the observed and expected heterozygosities. To determine genetic relationships among populations, Nei's (1978) unbiased genetic distances (D) and genetic similarity (I) were calculated, the fixation index (F_{st}) of Wright (1978) was utilized as a measure to indicate the degree of genetic divergence which found at a locus among local populations. Allelic frequencies were calculated, and a phenogram (dendrogram) was constructed by the UPGMA method using BIOSYS-I computer package (Swofford and Selander, 1989).

Dendrogram is a diagrammatic drawing in the form of branching tree designed to indicate degree of relationships. Two type of dendrogram are phenogram and cladogram. Phenogram is a diagram indicating degrees of overall similarities among populations. Cladogram is a strictly genealogical dendrogram which features the branching points of phyletic lineage.

In this study I have focused on the genetic structure of the Thai genus *Mekongia*. I used allozyme as genetic markers for investigating genetic divergence (genetic variation or genetic diversity) within and between species. Gene frequencies of enzyme loci in populations located in Thailand were estimated. F-statistics is used to estimate the degree of genetic divergence among populations. The genetic differentiation of populations or species was compared using diagnostic loci and Nei's (1978) unbiased genetic distance (D).