CHAPTER IV

RESULTS

4.1. Morphological Variation

4.1.1. Morphometric Analysis

All mean morphometric characters were compared among groups using Analysis of Variance (ANOVA). The results showed that fourteen characters were significant different among species and subspecies *i.e.* the shell height (SH), shell width (SW), aperture height (AH), aperture width (AW), body whorl width (BW), spire height (SP), penultimate whorl height (PH), SH/SW, SH/SP, SH/PH, SW/AW, AH/AW, AW/SP, and BW/AW. The mean, standard deviation (SD), and standard error (SE) of all character are given in Appendix I.

Canonical discriminant functions were analysed using fourteen morphological characters, which were selected by one-way ANOVA ($p <$ 0.05). The results showed that *Mekongia* can be divided into five major groups. The first group consisted of *M. sphaericula spiralis*, the second groups consisted of *M. pongensis* and *M. swainsoni kmeriana*, the third group consisted of *M. swainsoni swainsoni*, *M. swainsoni braueri*, *M. lamarcki*, and *M. sphaericula sphaericula*, the forth group consisted of *M. sphaericula* cf. *extensa*, and the last groups consisted of *M. sphaericula extensa*. The group centroids in the area indicated the accuracy of identification (Figure 4-1).
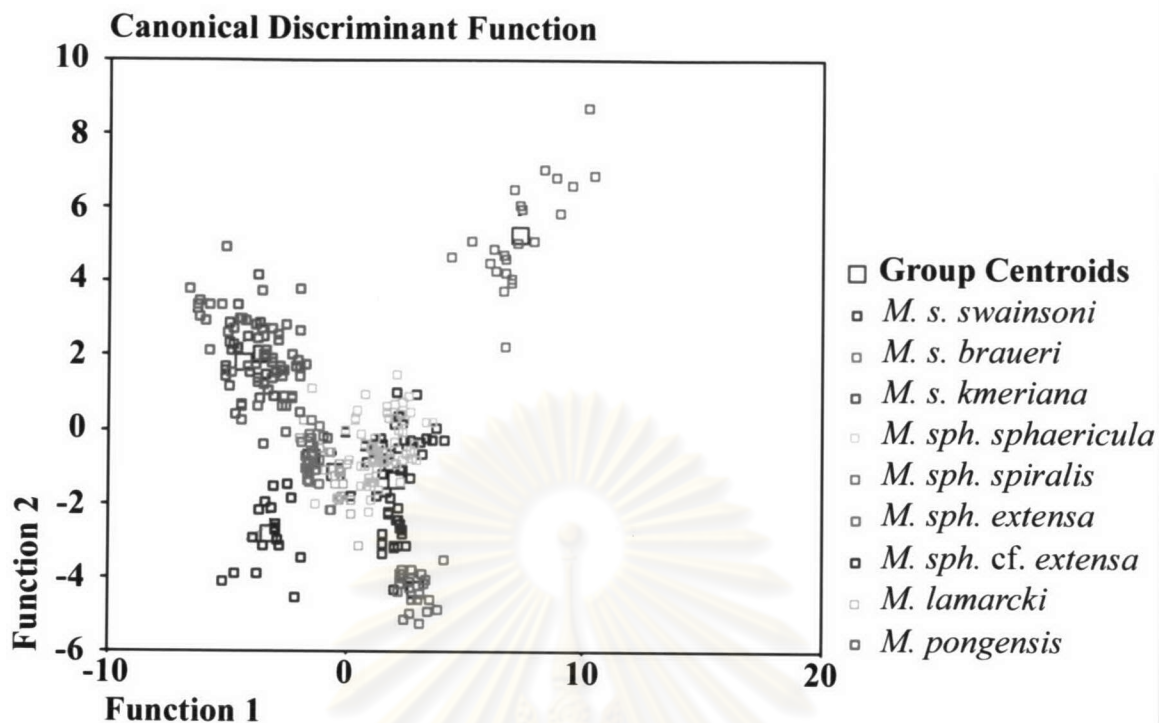
Figure 4-1. Canonical Discriminant Functions, showing the separation of *Mekongia* species by group centroids.

Figure 4-2 and Figure 4-3 revealed canonical discriminant analysis of *M. sphaericula* complex and *M. swainsoni* complex, respectively. From results, Canonical Discriminant Function using fourteen variables were clearly separated the four groups *i.e. M. sphaericula sphaericula* (blue), *M. sphaericula spiralis* (violet), *M. sphaericula extensa* (green), *M. sphaericula* cf. *extensa* (red) (Figure 4-2).

In *M. swainsoni* complex can be clearly divided into three groups with not significant overlap. (Figure 4-3).
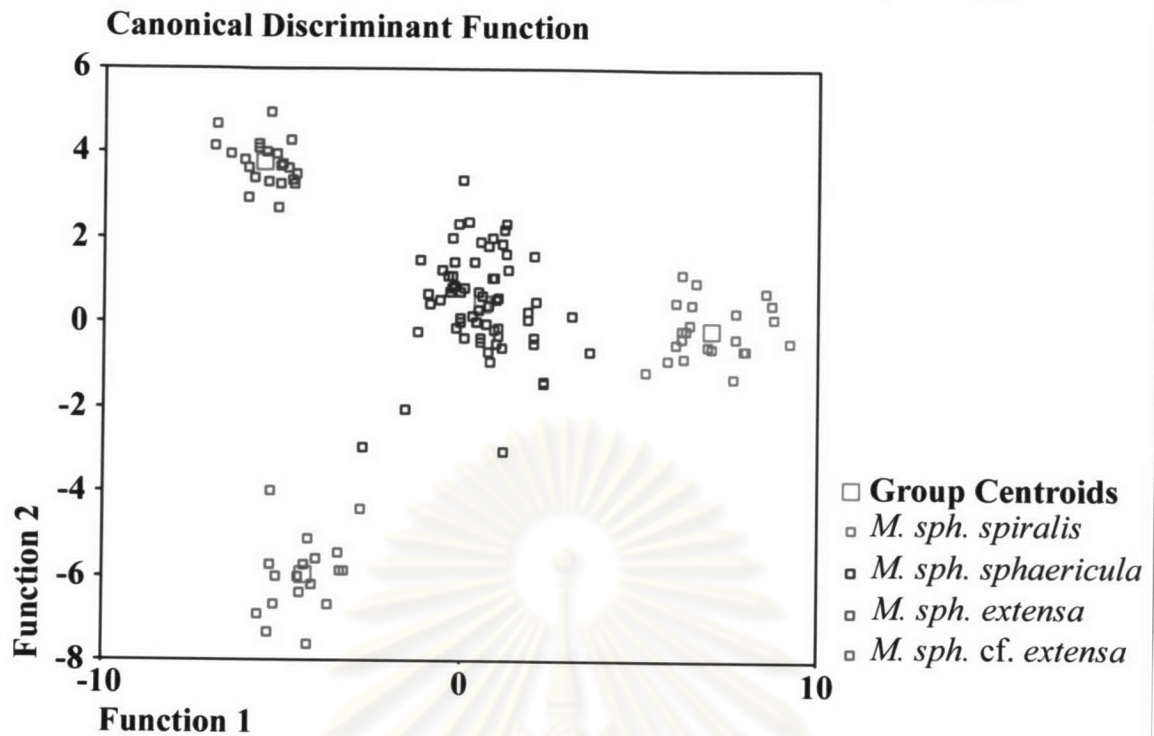
Canonical Discriminant Function

Figure 4-2. Canonical Discriminant Functions, showing the separation of *Mekongia sphaericula* complex by group centroids.

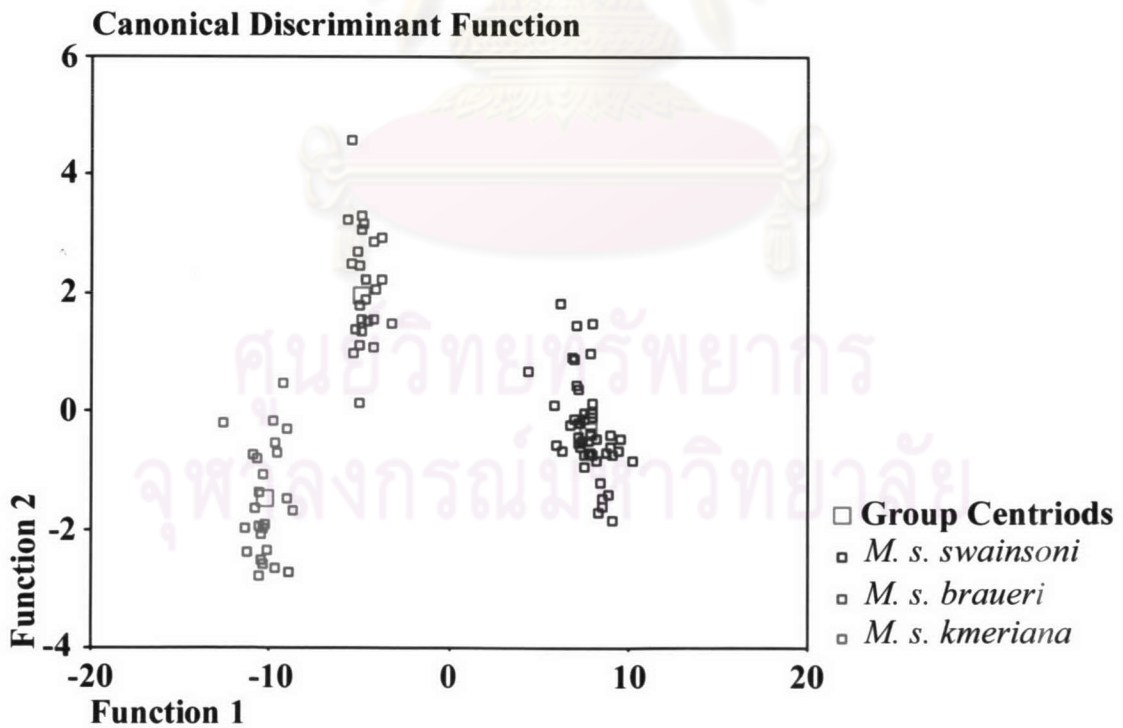**Canonical Discriminant Function**

Figure 4-3. Canonical Discriminant Functions, showing the separation of *Mekongia swainsoni* complex by group centroids.

Table 4-1. Classification results of nine groups. Number 1 to 9 represent *M. pongensis, M. swainsoni kmeriana, M. sphaericula* cf. *extensa, M. sphaericula extensa, M. swainsoni braueri, M. sphaericula sphaericula, M. lamarcki, M. sphaericula spiralis,* and *M. swainsoni swainsoni.*

	Groups	Predicted Group Membership									Total
		1	2	3	4	5	6	7	8	9	
Original Count	1	59	0	0	0	0	0	0	0	0	59
	2	0	25	0	0	0	0	0	0	0	25
	3	0	0	18	0	0	0	0	0	0	18
	4	0	0	0	22	0	0	0	0	0	22
	5	0	0	0	0	25	0	0	0	0	25
	6	0	0	0	0	0	64	0	0	6	64
	7	0	0	0	0	0	0	21	0	0	21
	8	0	0	0	0	0	0	0	23	0	23
	9	0	0	0	0	0	0	0	0	48	48
Percentage	1	100.0	.0	.0	.0	.0	.0	.0	.0	.0	100.0
	2	.0	100.0	.0	.0	.0	.0	.0	.0	.0	100.0
	3	.0	.0	100.0	.0	.0	.0	.0	.0	.0	100.0
	4	.0	.0	.0	100.0	.0	.0	.0	.0	.0	100.0
	5	.0	.0	.0	.0	100.0	.0	.0	.0	.0	100.0
	6	0	.0	.0	.0	.0	100.0	.0	.0	0	100.0
	7	0	.0	.0	.0	.0	.0	100.0	.0	.0	100.0
	8	.0	.0	.0	.0	.0	.0	.0	100.0	.0	100.0
	9	.0	.0	.0	0	.0	.0	.0	.0	100.0	100.0

All nine taxon were finally distinguished by discriminant analysis into nine groups with 100 % correctly classified values (Table 4-1)

In addition, viviparid snails in this genus, *M. swainsoni braueri* from Ayutthaya Province, show sexual size dimorphism (SSD), but their color and microsculpture of shell did not differ. From result of discriminant analysis revealed significantly difference between shell of male and female ($p > 0.05$) (Figure 4-4 and Figure 4-5).
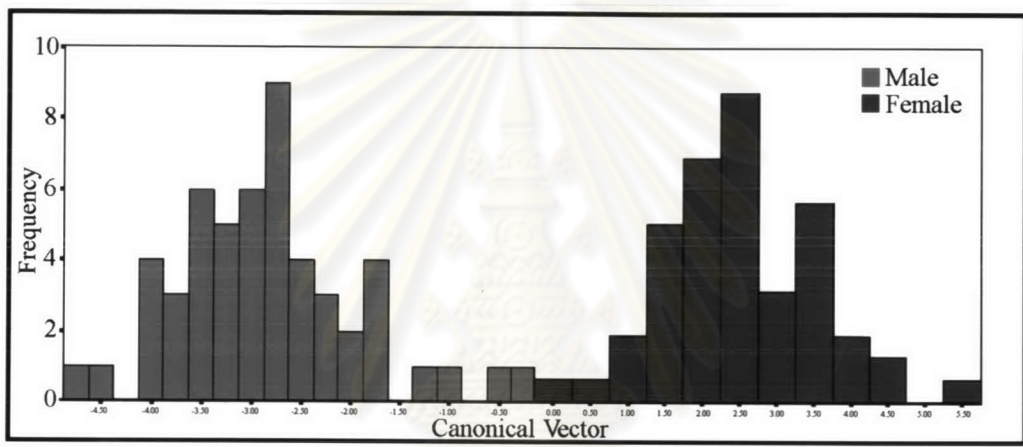

Figure 4-4. Frequency distribution of canonical discriminated scores of male and female, *Mekongia swainsoni braueri*. The mean canonical score are −2.82 for males and 2.53 for females.
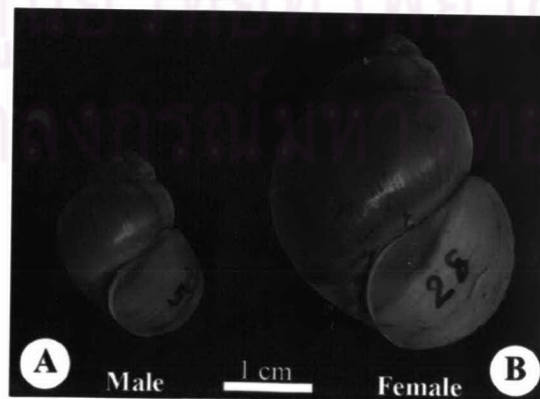

Figure 4-5. Comparisons of adult shells of *Mekongia swainsoni braueri,* male (A) and female (B). Scale bar = 1 cm.

4.1.2. External Shell Morphology and Re-descriptions

Morphology of external shells were observed and identified based on descriptions and key of Brandt (1974). Four species and six subspecies were identified (Figure 4-6), and the character analysis of each species were added up for the following re-description (see below).

Mekongia pongensis Brandt, 1968

Shell is smallest for the genus. Shell shape is subglobose, solid texture. The shell height and width of specimens are 1.7-3.0 and 1.5-2.5 cm, respectively. The periderm is bright yellow colored, malleated surface and dark-violet bordered apical whorl suture and apical whorl. The umbilicus is closed.

Mekongia swainsoni swainsoni (Lea, 1856)

Shell is medium size, subglobose with less elevated spire. The periderm is green to brown. Umbilicus is narrow but opened. The shell height and width of specimens are 2.0-3.1 and 1.6-2.4 cm, respectively. The apical shell is not violet colored.

Mekongia swainsoni braueri (Kobelt, 1908)

Shell is medium. Shell shape is more ovate-conoidal. It looks very similar to the nominotypical subspecies, but the spire of shell is more elongated with distinct pointed apex. The periderm is bright green to green. The shell height and width of specimens are 2.6-3.4 and 1.9-2.3 cm, respectively.

Mekongia swainsoni kmeriana (Morlet, 1890)

Shell is small. The apical whorl is very low spire, Shell shape is subglobose. The periderm is dark-green to brown colored. The shell height and width of specimen are 2.2-2.6 and 1.8-2.2 cm, respectively.

Mekongia lamarcki (Deshayes, 1876)

Shell is large. Shell is solid and ovoidal. The apical whorl is dark violet. The suture is deep, the first 1 to 3 whorls elevated with distinct pointed apex, and very elevated. The umbilicus is opened. The periderm is bright green to yellow. The shell height and width of specimen are 2.3-3.3 and 1.8-2.4 cm, respectively.

Mekongia sphaericula sphaericula (Deshayes, 1876)

Shell is large. Shell shape is subglobose with short, depressed spire and inflated body whorl. The apical whorl is dark violet. The periderm is dark green to brown. The shell is either smooth or sculptured with irregular spiral lines. The shell height and width of specimen are 2.3-3.8 and 1.9-2.7 cm, respectively.

Mekongia sphaericula spiralis Brandt, 1974

Shell is the largest averaged size for the genus. The shell height and width are 2.5-3.4 and 2.3-3.0 cm, respectively. The shell is very strong solid and with obtuse spiral ridges. The periderm is bright-brown to brown. Apical whorl is not violet colored. In addition, almost adult shells were usually found attached with unknown species of freshwater sponge.

Mekongia sphaericula extensa **Brandt, 1974** (Nong Khai)

Shell is medium. The shell ovate conoidal shape. The shell length and width of specimen are 2.2-2.8 and 1.6-1.9 cm, respectively. The periderm is green to brown. This subspecies differs from other subspecies by its elongate shell. Actually, the apical whorl and second whorl are dark violet.

Mekongia sphaericula **cf.** *extensa* **Brandt, 1974** (Nakhon Phanom)

This subspecies from Nakhon Phanom population looks very similar to *Mekongia sphaericula extensa* from Nong Khai population (see above), but shells are more depress (low spire) and smaller than Nong Khai population. Thus, they were identified into *Mekongia sphaericula* cf. *extensa*. The shell length and width of specimen are 2.1-2.8 and 1.7-2.1 cm, respectively.
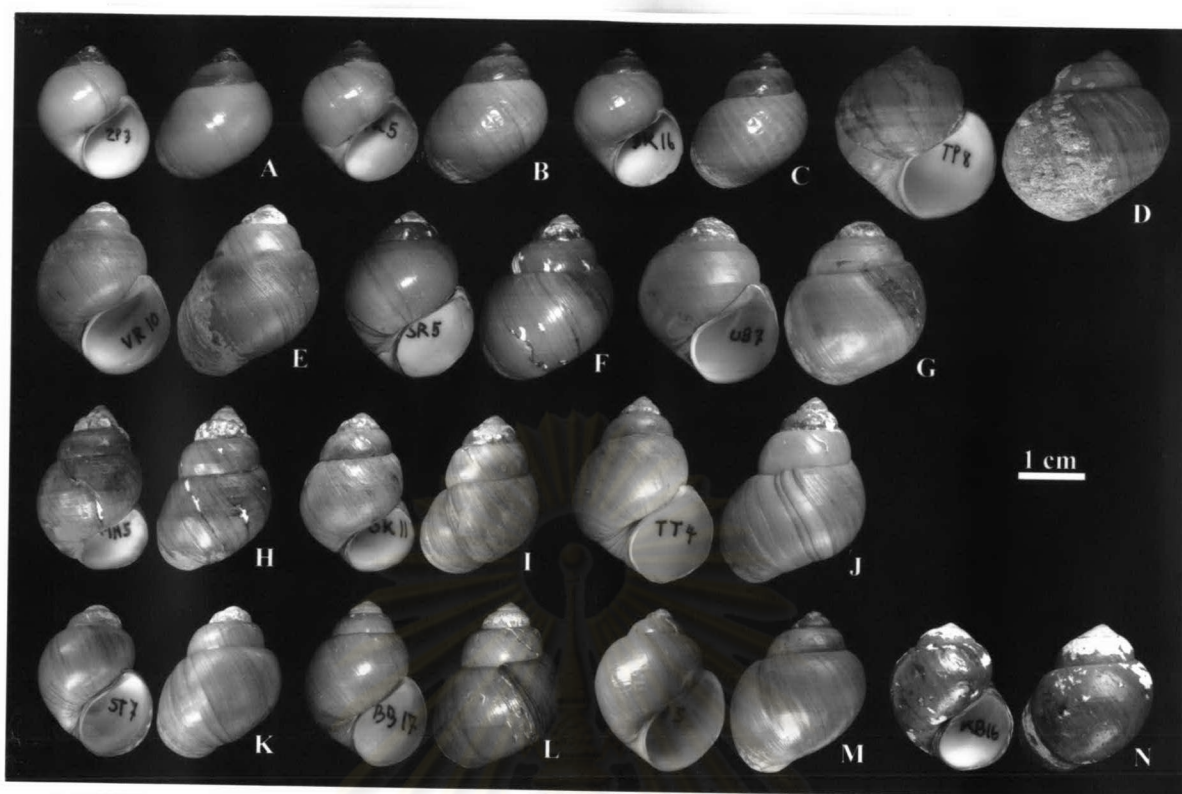
Figure 4-6. Shells of *Mekongia* in Thailand. A = *M. pongensis* from Nakhon Phanom; B = *M. pongensis* from Nong Khai; C = *M. pongensis* from Bueng Kan, Nong Khai; D = *M. sphaericula spiralis* from Nakhon Phanom; E = *M. sphaericula sphaericula* from Varinchamrap, Ubon Ratchathani; F = *M. sphaericula sphaericula* from Surin; G = *M. sphaericula sphaericula* from Ubon Ratchathani; H = *M. sphaericula* cf. *extensa* from Nakhon Phanom; I = *M. sphaericula extensa* from Nong Khai; J = *M. lamarcki* from Nakhon Phanom; K = *M. swainsoni swainsoni* from Ayutthaya; L = *M. swainsoni braueri* from Ayutthaya; M = *M. swainsoni swainsoni* from Phitsanulok; and *M. swainsoni kmeriana* from Phachin Buri. Scale bar = 1 cm.

4.2. Reproductive System Study

Fourteen populations were collected represent four species and seven subspecies, genus *Mekongia* in Thailand. The specimens from all populations were used to study the comparative anatomy of reproductive system. The results revealed no differences in shape and position of the organ among species and subspecies. The descriptions of the reproductive

anatomical features of all species and subspecies are illustrated in Figure 4-8 (male) and Figure 4-9 (female).

4.2.1. Male Reproductive System

Male can be distinguished by the modified form of the right tentacle which serves as a penis (Figure 4-7). The male genital organs of *Mekongia* consist of four parts: the testis (T), the vas deferens (VD), the prostate gland (PG), and the penis (PE), modified right tentacle) are shown in Figure 4-8.

Figure 4-7. The drawing tentacles of *Mekongia swainsoni swaisoni*, male (A) and female (B). Scale bar = 5 mm.

The testis bean-liked shape, distinguished by its yellowish or brownish color. The testis placed along the right edge of the mantle wall. It is separated from the rectum above it by the kidney duct. The gonad is in the visceral spire, close to the digestive gland.

The vas deferens leaves the testis some way behind the middle of its length, curves round the mantle wall, and passes to the posterior floor of the mantle cavity and into the prostate gland. At the anterior end of the vas deferens, a very short, straight duct that is surrounded by muscular tissues. The creamy-orange prostate gland which passes forward underneath the

food-groove towards the right tentacle. It narrows to a terminl vas deferens which passes through the modified right tentacle to open at its tips. The right tentacle is longer and larger than left tentacle. There is no gonopericardial found.

Figure 4-8. Male reproductive system. A= *Mekongia lamarcki*, B = *M. pongensis*, C = *M. swainsoni kmeriana*, D = *M. swainsoni swainsoni*, E = *M. swainsoni braueri*, F = *M. sphaericula spiralis*, G = *M. sphaericula sphaericula*, and H = *M. sphaericula extensa*. Abbreviation: PE = penis, PG = prostate gland, T = testis, and VD = vas deferens.

4.2.2. Female Reproductive System

The female genital organs lie in the same location as those of the males. The organs consist of five parts: the ovary (O), the oviduct (OV), the seminal receptacle (SR), the albumen gland (AG) and the pallial oviduct (PO) with the female opening. (Figure 4-9). There is no distinguishable difference among species and subspecies found.

The faintly translucent ovary is vary small, narrow duct and often very hard to fine. It is orange-brown organ with a few branches among the tissue the digestive gland, locating in the apical whorl. The ovarian duct joins the wider duct of the albumen gland, and combined oviduct passed forward close to the kidney and pericardium. The albumen gland lied closely passed to the two limbs of the seminal receptacle.

The pallial oviduct is a long, broad, glandular structure, light yellow color (that part in the mantle roof). It is expanded to form a brood-pouch and usually contains several eggs, embryos and developing young. It lies parallel to the rectum at the right edge of the mantle cavity, separated from it by the kidney duct. The anterior portion of it is modified to form a thin-walled brood-pouch, in which the embryos are developed to young. Along the ventral wall (posterior portion), a sheet-like modified structure occurred as the seminal channel (sperm channel) with two folded walls and leads back to seminal receptacle. The seminal receptacle is located on the albumen gland. It receives the distal end of the oviduct and continuous with the pallial oviduct, from which pallial oviduct is distinguishable by its very narrow diameter.

The large, yellow to gold albumen gland is located in the posterior end of the mantle cavity. It is bordered posterior and dextral portions by the digestive gland, sinistral portion by the intestine, anterior portion by the seminal receptacle, and ventral portion by the posterior edge of the pericardium. The vagina extends from distal end of pallial oviduct. It is detached from the mantle along its entire length, and its extreme distal turns sharply ventral, hangs down into the mantle cavity and terminates at the birth pore.
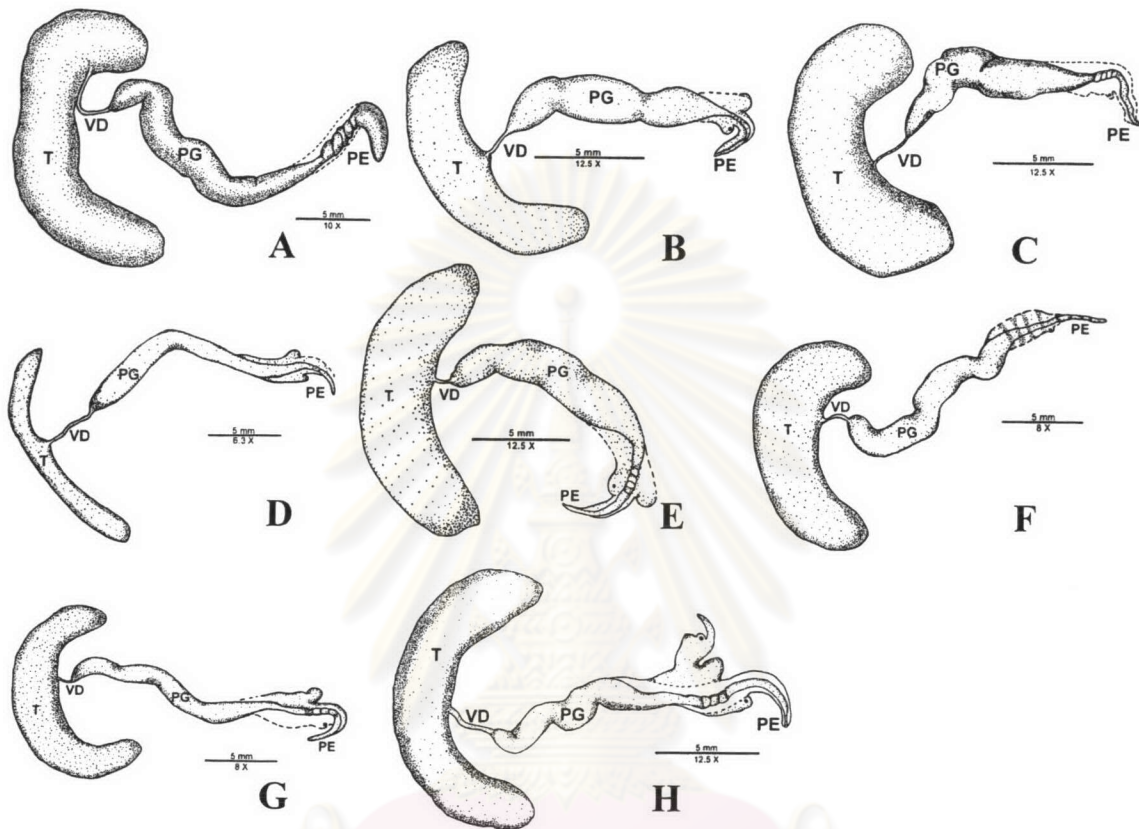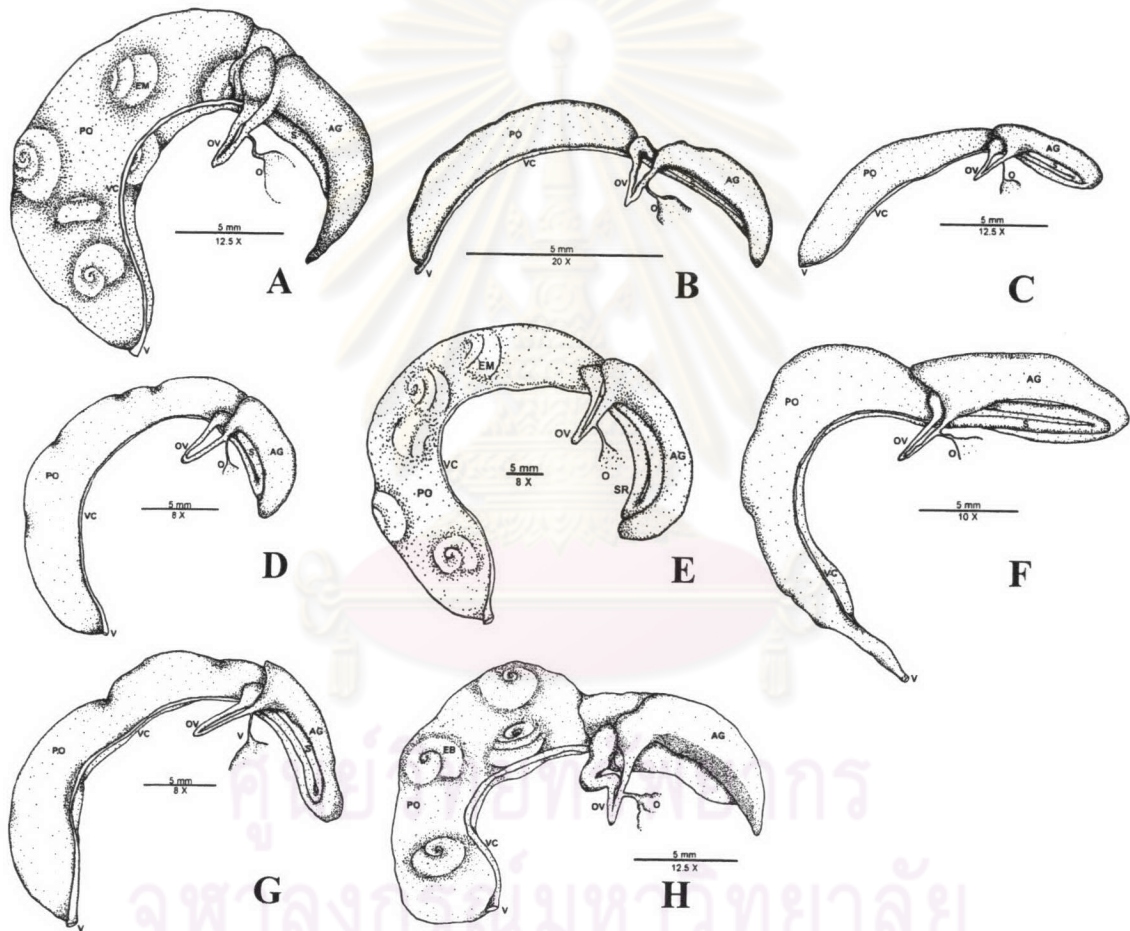

Figure 4-9. Female reproductive system. A= *Mekongia lamarcki*, B = *M. pongensis*, C = *M. swainsoni kmeriana*, D = *M. swainsoni swainsoni*, E = *M. swainsoni braueri*, F = *M. sphaericula spiralis*, G = *M. sphaericula sphaericula*, and H = *M. sphaericula extensa*. Abbreviation: AG = albumen gland, EM = embryo, O = ovary, OV = oviduct, PO = pallial oviduct, SR = seminal receptacle, and VC = seminal channel.

In addition, on dissecting snails for anatomical study, we discovered the water mites, *Unionicola* sp. were found on gill surfaces. This is the first record on the parasitism of *Unionicola* on viviparid snails in Thailand (Srikoom and Panha, 2004). One to two individuals of mite were found on the gills of snails (Figure 4-10).
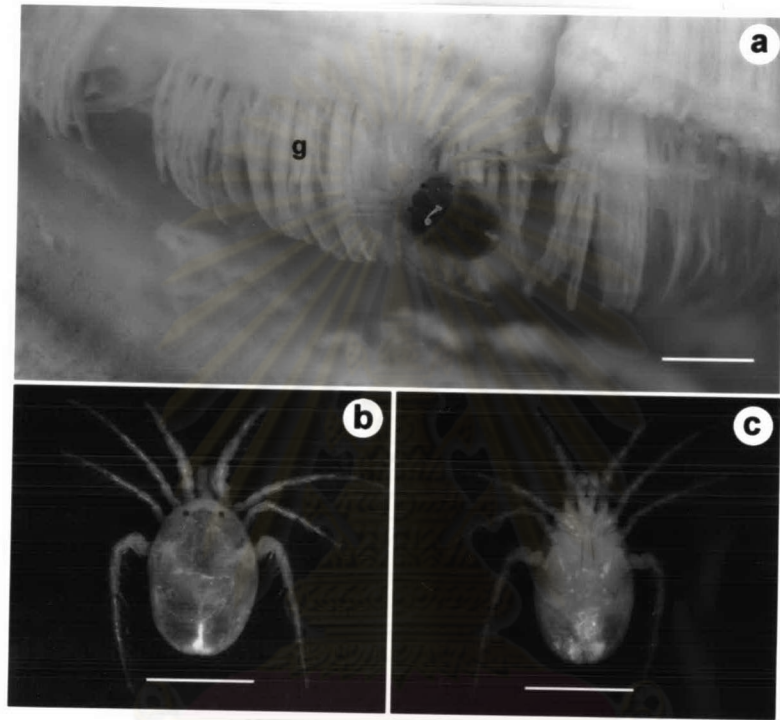
Figure 4-10. *Unionicola* sp. found in *Mekongia sphaericula* (Deshayes, 1876); a, on gill surfaces (g); b, dorsal view; c, ventral view. Scale bars = 1 mm.

4.3. Allozyme Study

Fourteen populations of *Mekongia* snails were collected between March 2004 and July 2004 from five rivers in Thailand (Table 4-2). Each collection was coded by a combination of a capital letter which indicates a river or province names.

4.3.1. Electrophoretic Data

Thirteen enzyme loci were resolved in this study. Multiple loci encoding the same enzyme (isozymes) were designated by consecutive number, with "1" denoting the fastest migrating isozymes in anodal direction. The allozyme abbreviations followed Shaklee *et al.* (1990). Genetically interpretation results on allozyme data, using the technique described by Richardson *et al.* (1986).

Esterase (EST-α) (Figure 4-11)

The results revealed two zone of black band that were clearly and consistently recognizable and all anodal migration. Two characteristics of the esterase zones were encoded as EST-1 and EST-2 (slower migrated). EST-α-1 was found highly variation of electrophoretic bands. Heterozygote genotypes exhibited two bands (monomer) are shown in Figure 4-11. EST-α-2 exhibited the monomorphic phenotype appearing as a single well-defined band occurring among the populations. Electrophoretic variations among species were not found.
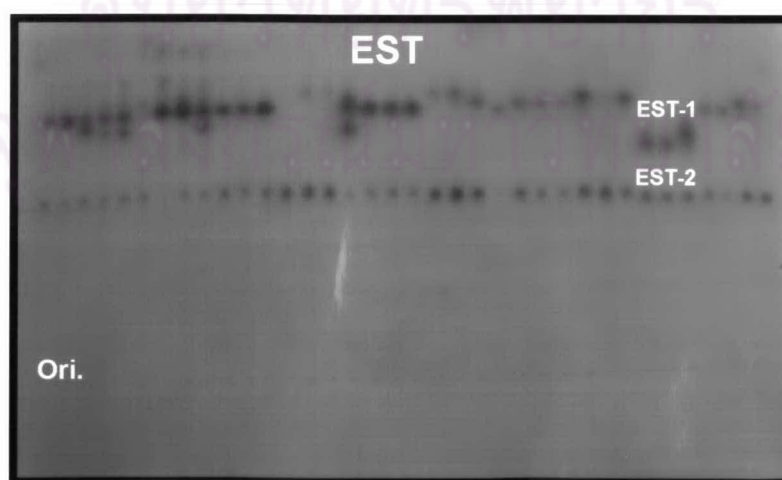
Figure. 4-11. The activity of α-esterase (EST-α). Ori. = Origin.

Glucose-6-phosphate isomerase (GPI) (Figure 4-12)

A form of GPI was detected. Enzyme migrated toward the anode. There are variants in this enzyme. The heterozygous genotype showed three bands pattern, it is dimeric enzyme. On the other hand, homozygous showed only one band.
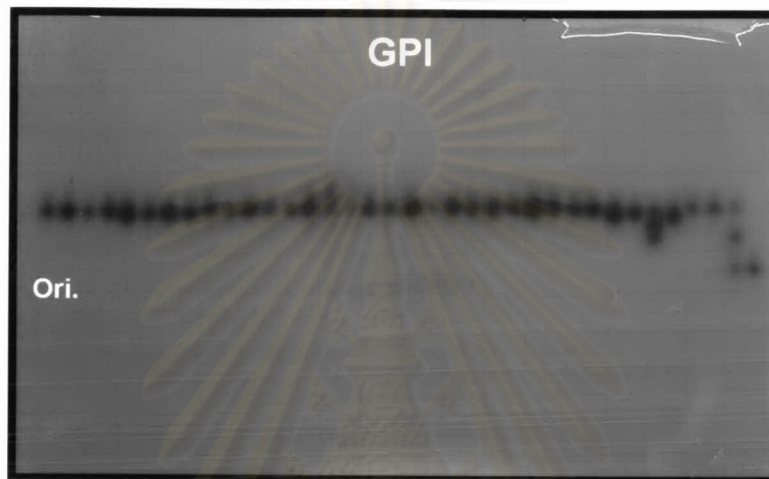
Figure. 4-12. The activity of glucose-6-phosphate isomerase (GPI). Ori. = Origin.

Malate dehydrogenase (MDH) (Figure 4-13)

The two activity zones of MDH were observed. They could be assumed to be encoded with two loci, fast and slow zones, as MDH-1 and MDH-2 respectively. MDH-1 migrated to anodal zone. On the other hand, MDH-2 migrated to opposite zone (cathodal zone). Both loci were resolved a single band and no variation in all population, monomorphism. They are monomer.
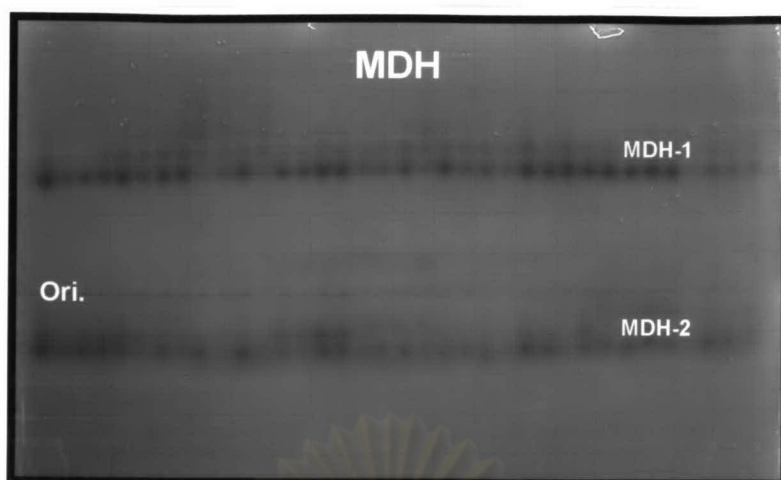
Figure. 4-13. The activity of malate dehydrogenase (MDH). Ori. = Origin.

Mannose-6-phosphate isomerase (MPI) (Figure 4-14)

MPI was encoded by on one locus. A single monomer band migrated to anode direction. Almost of snail populations showed allele B, except the *M. sphaericula spiralis*, they showed allele A.
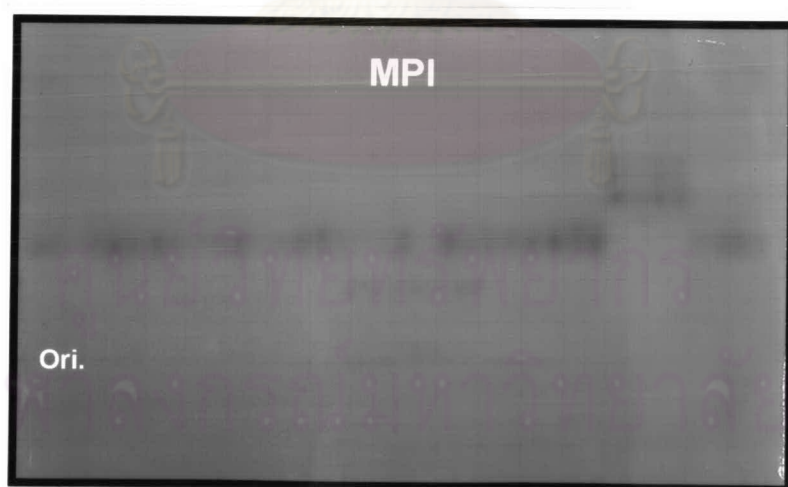
Figure. 4-14. The activity of mannose-6-phosphate isomerase (MPI). Ori. = Origin.

Peptidase-glycyl-L-luecine (PEP-GL) (Figure 4-15)

The activity of PEP-GL was revealed two zones in anode direction. Two loci were coded for PEP-GL-1 and PEP-GL-2. They are monomers.

Variation was found in PEP-GL-2 on *Mekongia sphearicula spiralis*, which was found allele B, but other populations were found allele A only. PEP-GL-1, variations were found in a few populations. PEP-LGG-and PEP-LGG-2 showed two loci represent the slow and fast migrating forms. The PEP-GL-1 loci appeared firstly. Then the PEP-GL-2 loci appeared later.
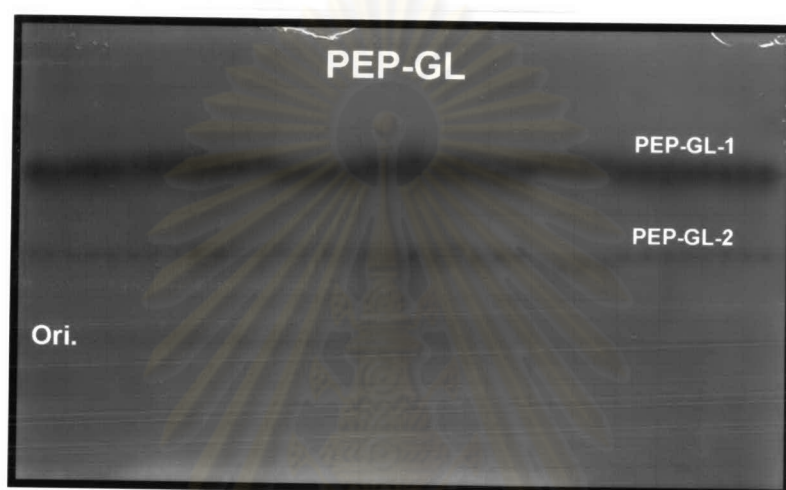
Figure. 4-15. The activity peptidase-glycyl-L-luecine of (PEP-GL). Ori. = Origin.

Peptidase-luecylglycylglycine (PEP-LGG) (Figure 4-16)

The activity of PEP-LGG was revealed two zones in anode direction. Two loci were coded for PEP-LGG-1 and PEP-LGG-2. They are monomers. PEP-LGG-1 PEP-LGG-2 and showed two loci represent the slow and fast migrating forms. The PEP-LGG-1 loci appeared firstly. After that, the PEP-LGG-2 loci appeared.
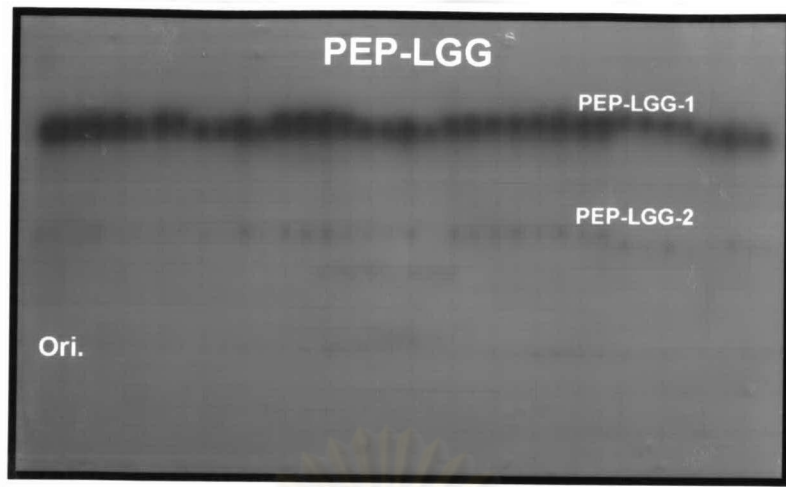
Figure. 4-16. The activity of peptidase-luecylglycylglycine (PEP-LGG). Ori. = Origin.

6-phosphogluconate dehydrogenase (PGD) (Figure 4-17)

PGD was encoded by on only one locus. A single band migrated to anodal zone. They are monomer. The variations were detected in this enzyme.

Figure. 4-17. The activity of 6-phosphogluconate dehydrogenase (PGD). Ori. = Origin.

Phosphoglucomutase (PGM) (Figure 4-18)

PGM was encoded by on locus. A single band migrated to anode zone. The high variations were detected in this enzyme. They are monomer. After

buffer staining, the gel was added MTT by dropped MTT overlay gel, which increased enzyme activity.
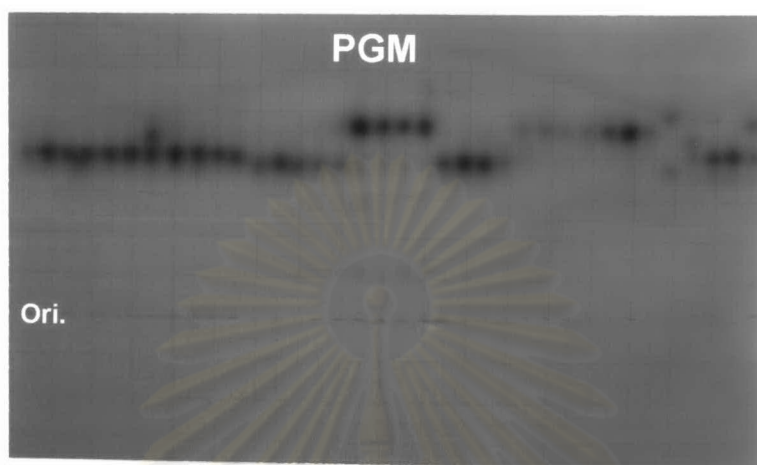
Figure. 4-18. The activity of phosphoglucomutase (PGM). Ori. = Origin.

Sorbitol dehydrogenase (SDH) (Figure 4-19)

SDH was encoded by one locus. A single band migrated to anodal zone. They are monomer. The results were not found heterozygous genotype.

Figure. 4-19. The activity of sorbitol dehydrogenase (SDH). Ori. = Origin.

4.3.2. Data Analysis

Genetic information was obtained from the populations of the mature *Mekongia* snails collecting in the field. The code numbers and abbreviations are shown in Table 4-2.

Thirteen loci were observed from nine enzymes. Allelic frequencies (95% criterion) at 13 polymorphic loci in all samples are presented in Table 4-3. The information on their allelic character state relationship can be explained for the following loci. Between two to six alleles were detected at each locus. Numbers of loci for each enzyme ranged from one to two. Three of the thirteen loci were monomorphic (EST-α-2, MDH-1, and MDH-2). The remaining loci of PGD, EST-α-1, PGM, GPI, MPI, PEP-GL-1, PEP-GL-2, PEP-LGG-1, PEP-LGG-2, and SDH were polymorphic. The samples from TP were distinguished by fixed alleles from other populations by MPI, PEP-GL-1, PEP-GL-2, and SDH. The SR, VR, and UB populations were distinguished by fixed alleles from other samples by PGD and PEP-GL-1.

Table 4-4 shows the 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 ($p = 0.006$), indicated that may be existed the subpopulation within this population. The heterozygous loci were found in the enzyme activities of EST-α, PGM, PGD, GPI, PEP-GL, and PEP-LGG.

Table 4-2. The 14 population of *Mekongia* snails collected from fourteen localities and employed for electrophoretic studies.

Code	Species and/or Subspecies	Locality	Abbreviation
1	*Mekongia swainsoni braueri*	Ayutthaya	BB
2	*M. swainsoni kmeriana*	Prachin Buri	KB
3	*M. swainsoni swainsoni*	Ayutthaya	ST
4	*M. swainsoni swainsoni*	Phitsanulok	VB
5	*M. sphaericula* cf. *extensa*	Nakhon Phanom	MH
6	*M. sphaericula extensa*	Nong Khai	SK
7	*M. pongensis*	Nong Khai	BK
8	*M. pongensis*	Nong Khai	NK
9	*M. pongensis*	Nakhon Phanom	NP
10	*M. sphaericula sphaericula*	Surin	SR
11	*M. sphaericula sphaericula*	Ubon Ratchathani	UB
12	*M. sphaericula sphaericula*	Ubon Ratchathani	VR
13	*M. lamarcki*	Nakhon Phanom	TT
14	*M. sphaericula spiralis*	Nakhon Phanom	TP

Indices of genetic variability are given in Table 4-5. The level of mean observed heterozygosity (H_{obs}) in the population of *M. pongensis* (BK, NK, and NP) ranged from 0.000 to 0.030, *M. swainsoni braueri* (BB) was 0.077, *M. swainsoni swainsoni* (ST and VB) ranged from 0.006 to 0.020, *M. swainsoni kmeriana* (KB) was 0.047, *M. lamarcki* (TT) was 0.015, *M. sphaericula sphaericula* (SR, UB, and VR) ranged from 0.006 to 0.031, *M. sphaericula extensa* ranged from 0.009-0.040, and *M. sphaericula spiralis* was 0.092. Percentage of polymorphic loci ranged from 0% (*M. pongensis* from NK and NP) to 38.5% (*M. sphaericula spiralis* from TP); the mean number of alleles per locus ranged from 1.0 (NK and NP) to 1.5 (TP), indicating a general deficiency of heterozygotes (Table 4-5).

The genetic coefficient indices which consisted of genetic similarity (I) and genetic distance (D) were compiled for pairs of samples (species and subspecies) upon allelic frequencies at both monomorphic and polymorphic loci. Nei's (1978) unbiased coefficient of genetic distance (D_N) and Rogers (1972) coefficient of genetic similarity (I_R) were computed (Table 4-6). The lowest and highest values of D among taxa were 0.010 between the populations of BB and ST and were 1.125 between the population of TP and SR, respectively.

Genetic distances among two populations of *M. swainsoni swainsoni* was 0.000; among three populations of *M. sphaericula sphaericula* ranged from 0.001 between SR and UB, to 0.003 between UB and VR; and among three populations of *M. pongensis* ranged from 0.000 between NK and NP, to 0.008 between BK and NK.

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).

Figure 4-20. Dendrogram, constructed by UPGMA using Nei's unbiased (1978) genetic distance. Two characters in parentheses after the specific names indicate collecting sites as shown in the Table 4-2.

Table 4-3. Allele frequencies at 13 polymorphic loci in fourteen samples of *Mekongia* from Thailand. Population numbers are designated in Table 4-2.

Locus	Population 1	2	3	4	5	6	7	8	9	10	11	12	13	14
PGD														
(N)	25	24	25	25	25	25	25	25	25	25	25	25	25	25
A	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.020	.000	.000	.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.980	1.000	.000	.000
C	.000	.354	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.060
D	1.000	.646	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	.000	.000	1.000	.940
EST-α-1														
(N)	25	25	23	25	25	21	20	25	21	25	21	21	21	25
A	.000	.000	.000	.000	.000	.000	.675	1.000	1.000	.000	.000	.000	1.000	.000
B	.320	.160	.022	.000	1.000	1.000	.325	.000	.000	.020	.167	.000	.000	.000
C	.540	.820	.891	.980	.000	.000	.000	.000	.000	.980	.833	1.000	.000	.000
D	.140	.020	.087	.020	.000	.000	.000	.000	.000	.000	.000	.000	.000	.360
E	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.640
EST-α-2														
(N)	25	25	25	25	25	25	25	25	25	25	25	25	25	25
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGM														
(N)	25	25	24	25	25	25	25	25	25	25	25	25	25	25
A	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.080	.260
B	.000	.000	.000	.000	.140	.900	1.000	1.000	1.000	.000	.000	.000	.900	.000
C	.000	.000	.000	.000	.020	.080	.000	.000	.000	.000	.000	.020	.000	.240
D	.200	.000	.000	.000	.000	.020	.000	.000	.000	.000	.000	.040	.000	.500
E	.800	1.000	.979	1.000	.840	.000	.000	.000	.000	1.000	.960	.820	.020	.000
F	.000	.000	.021	.000	.000	.000	.000	.000	.000	.000	.040	.120	.000	.000
GPI														
(N)	25	25	25	25	25	25	25	25	25	25	25	25	25	25
A	.000	.000	.000	.000	.000	.000	.020	.000	.000	.000	.020	.000	.000	.000
B	1.000	1.000	1.000	1.000	.340	1.000	.980	1.000	1.000	1.000	.980	1.000	1.000	.940
C	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.060
D	.000	.000	.000	.000	.660	.000	.000	.000	.000	.000	.000	.000	.000	.000
MDH-1														
(N)	25	25	25	25	25	25	25	25	25	25	25	25	25	25
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MDH-2														
(N)	25	25	25	25	25	25	25	25	25	25	25	25	25	25
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MPI														
(N)	25	25	25	25	25	25	25	25	25	25	25	25	25	25
A	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000
PEP-GL-1														
(N)	25	25	25	25	25	25	25	25	25	25	25	25	25	25
A	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	.000	.000	1.000	.000
C	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	1.000	1.000	.000	.000
PEP-GL-2														
(N)	25	25	25	25	25	25	25	25	25	25	25	25	25	25
A	.000	.000	.000	.020	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	1.000	1.000	1.000	.980	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000
C	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000
PEP-LGG-1														
(N)	25	25	25	25	25	25	25	25	25	25	25	25	25	25
A	.000	.000	.000	.000	.000	.000	.000	.000	.000	.020	.000	.040	.000	1.000
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.980	1.000	.960	1.000	.000
PEP-LGG-2														
(N)	25	25	25	25	25	25	25	25	25	25	25	25	25	25
A	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	1.000	.980	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.020
C	.000	.020	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.980
SDH														
(N)	25	25	25	25	25	25	25	25	25	25	25	25	25	25
A	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.000
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	.000
C	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000

Table 4-4. Chi-square (X^2) values for heterozygous loci in *Mekongia* populations calculated to test the goodness-of-fit from Hardy-Weinberg equilibrium. Populations are designated in Table 4-2. (The significant deviation from Hardy-Weinberg equilibrium, $p < 0.05$, were underlined)

Locus	Population													
	BB	KB	ST	VB	MH	SK	BK	NK	NP	SR	UB	VR	TT	TP
EST-α-1	3.683	4.183	0.342	0.010	-	-	0.818	-	-	0.010	0.429	-	-	0.435
EST-α-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PGD	-	0.780	-	-	-	-	-	-	-	-	0.010	-	-	0.102
PGM	1.563	-	0.011	-	0.907	5.250	-	-	-	-	0.043	4.877	0.309	2.071
GPI	-	-	-	-	<u>7.683</u>	-	0.010	-	-	-	0.010	-	-	0.102
MDH-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MDH-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MPI	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PEP-GL-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PEP-GL-2	-	-	-	0.010	-	-	-	-	-	-	-	-	-	-
PEP-LGG-1	-	-	-	-	-	-	-	-	-	0.010	-	0.043	-	-
PEP-LGG-2	-	0.010	-	-	-	-	-	-	-	-	-	-	-	0.010
SDH	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4-5. Index of genetic variability at 13 loci in all population of *Mekongia*. The population abbreviation are designated in Table 4-2. (standard error in parenthesis)

Population	Mean sample size per Locus	Mean no. of alleles per locus	Percentage of loci polymorphic*	Mean heterozygosity	
				Direct-count	HdyWbg expected**
1. BB	25.0 (.0)	1.2 (.2)	15.4	.077 (.053)	.071 (.051)
2. KB	24.9 (.1)	1.3 (.2)	23.1	.047 (.031)	.063 (.041)
3. ST	24.8 (.2)	1.2 (.2)	15.4	.020 (.017)	.019 (.016)
4. VB	25.0 (.0)	1.2 (.1)	15.4	.006 (.004)	.006 (.004)
5. MH	25.0 (.0)	1.2 (.2)	15.4	.040 (.028)	.057 (.040)
6. SK	24.7 (.3)	1.2 (.2)	7.7	.009 (.009)	.014 (.014)
7. BK	24.6 (.4)	1.2 (.1)	15.4	.030 (.027)	.038 (.034)
8. NK	25.0 (.0)	1.0 (.0)	.0	.000 (.000)	.000 (.000)
9. NP	24.7 (.3)	1.0 (.0)	.0	.000 (.000)	.000 (.000)
10. SR	25.0 (.0)	1.2 (.1)	15.4	.006 (.004)	.006 (.004)
11. UB	24.7 (.3)	1.3 (.1)	30.8	.031 (.019)	.034 (.022)
12. VR	24.7 (.3)	1.3 (.2)	15.4	.025 (.019)	.030 (.025)
13. TT	24.7 (.3)	1.2 (.2)	7.7	.015 (.015)	.014 (.014)
14. TP	25.0 (.0)	1.5 (.2)	38.5	.092 (.047)	.106 (.057)

* A locus is considered polymorphic if more than one allele was detected
** Unbiased estimate (see Nei, 1978)

Table 4-6. Unbiased genetic distance (Nei, 1978) below diagonal and genetic similarity (Rogers, 1972) below diagonal.

Population	BB	KB	ST	VB	MH	SK	BK	NK	TP	SR	UB	VR	TT	TP
1 BB	*****	.937	.960	.953	.888	.886	.880	.865	.865	.799	.812	.801	.793	.430
2 KB	.017	*****	.960	.957	.845	.834	.834	.823	.823	.841	.849	.826	.751	.390
3 ST	.010	.010	*****	.991	.866	.855	.859	.851	.851	.836	.834	.825	.778	.406
4 VB	.014	.011	.000	*****	.860	.849	.853	.845	.845	.842	.829	.828	.773	.401
5 MH	.071	.109	.112	.120	*****	.888	.833	.807	.807	.707	.722	.705	.734	.362
6 SK	.094	.147	.148	.159	.089	*****	.939	.916	.916	.696	.710	.700	.840	.405
7 BK	.103	.147	.140	.148	.137	.037	*****	.973	.973	.699	.713	.703	.889	.410
8 NK	.130	.169	.158	.166	.186	.081	.008	*****	1.000	.692	.699	.695	.916	.400
9 TP	.130	.169	.158	.166	.186	.081	.008	.000	*****	.692	.699	.695	.916	.400
10 SR	.191	.153	.170	.168	.316	.357	.349	.367	.367	*****	.981	.985	.619	.332
11 UB	.183	.152	.171	.171	.288	.327	.330	.353	.353	.001	*****	.972	.626	.339
12 VR	.194	.158	.174	.172	.324	.349	.340	.357	.357	.002	.003	*****	.623	.341
13 TT	.217	.259	.244	.252	.279	.170	.091	.081	.081	.475	.461	.466	*****	.405
14 TP	.853	.947	.904	.915	1.025	.907	.904	.920	.920	1.125	1.120	1.101	.908	*****

Table 4-7. *F*-statistics values for the 9 loci of 14 populations of *Mekongia.*

Locus	F_{IS}	F_{IT}	F_{ST}
PGD	0.122	0.900	0.886
EST-α-1	0.109	0.785	0.759
PGM	0.046	0.767	0.756
GPI	0.375	0.724	0.558
MPI	-	1.000	1.000
PEP-GL-1	-	1.000	1.000
PEP-GL-2	-0.020	0.979	0.979
PEP-LGG-1	-0.034	0.939	0.941
PEP-LGG-2	-0.020	0.957	0.958
SDH	-	1.000	1.00
Mean	0.112	0.877	0.861

Table 4-8. *F*-statistics values for the 2 loci of 3 populations of *M. pongensis.*

Locus	F_{IS}	F_{IT}	F_{ST}
EST-α-1	0.202	0.396	0.243
GPI	-0.020	-0.007	0.013
Mean	0.184	0.370	0.228

Table 4-9. *F*-statistics values for the 5 loci of 3 populations of *M. sphaericula sphaericula*

Locus	F_{IS}	F_{IT}	F_{ST}
PGD	-0.020	-0.007	0.013
EST-α-1	0.123	0.206	0.095
PGM	0.175	0.228	0.064
GPI	-0.020	-0.007	0.013
PEP-LGG-1	-0.034	-.0020	0.014
Mean	0.113	0.170	0.065

Table 4-10. *F*-statistics values for the 2 loci of 2 populations of *M. sphaericula extensa*

Locus	F_{IS}	F_{IT}	F_{ST}
PGM	0.038	0.600	0.584
GPI	0.554	0.774	0.493
Mean	0.294	0.678	0.544

Values of *F* statistics for all *Mekongia* populations were calculated according to Wright (1978) as presented in Table 4-7. The mean $F_{IS} = 0.112$, $F_{IT} = 0.877$, and $F_{ST} = 0.861$. The F_{ST} values of *M. pongensis* (three populations), *M. sphaericula sphaericula* (three populations), and *M. sphaericula extensa* (two populations) are shown in Table 4-8, Table 4-9, and Table 4-10, respectively.