การวิเคราะห์ความแปรผันทางมอร์โฟเมตริก และทางพันธุกรรมของผึ้งมิ้มเล็ก *Apis andreniformis* Smith, 1858 ในประเทศไทย

นายอัศเลข รัตนวรรณี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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ANALYSIS OF MORPHOMETRIC AND GENETIC VARIATION OF SMALL DWARF HONEY BEES *Apis andreniformis* Smith, 1858 IN THAILAND

Mr. Atsalek Rattanawannee

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By	Mr. Atsalek Rattanawannee
Field of study	Zoology
Thesis Advisor	Professor Siriwat Wongsiri, Ph.D.
Thesis Co-advisor	Assistant Professor Chanpen Chanchao, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

..... Dean of Faculty of Science

(Professor Piamsak Menasveta, Ph.D.)

THESIS COMMITTEE

..... Chairman

(Assistant Professor Kumthon Thirakhupt, Ph.D.)

..... Thesis Advisor

(Professor Siriwat Wongsiri, Ph.D.)

..... Thesis Co-advisor

(Assistant Professor Chanpen Chanchao, Ph.D.)

..... Member

(Assistant Professor Sureerat Deowanish, D.Agr)

..... Member

(Assistant Professor Jessada Denduangboripant, Ph.D.)

อัศเลข รัตนวรรณี: การวิเคราะห์ความแปรผันทางมอร์โฟเมตริก และทางพันธุกรรมของผึ้งมิ้มเล็ก *Apis andreniformis* Smith, 1858 ในประเทศไทย (ANALYSIS OF MORPHOMETRIC AND GENETIC VARIATION OF SMALL DWARF HONEY BEES *Apis andreniformis* Smith, 1858 IN THAILAND)
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ผึ้งมิ้มเล็ก *Apis andreniformis* จัดเป็นผึ้งพื้นเมืองชนิดหนึ่งของประเทศไทย ซึ่งพบว่ามีการศึกษาทั้งทางด้านมอร์โฟเมตริก และทางพันธุกรรมน้อยมาก ดังนั้นในการศึกษาครั้งนี้ได้ทำการสุ่มเก็บผึ้งมิ้มเล็กจำนวน 30 รังเพื่อใช้ศึกษาความแปรผันทางมอร์โฟเมตริกและเก็บจำนวน 37 รังเพื่อใช้ศึกษาความแปรผันทางพันธุกรรม ในส่วนของความแปรผันทางมอร์โฟเมตริก ทำการวัดและวิเคราะห์ลักษณะทางมอร์โฟเมตริกทั้งหมด 24 ลักษณะในผึ้งงาน จากการใช้ค่าเฉลี่ยของรังในการวิเคราะห์ปัจจัยครั้งที่ที่ 1 พบว่ามี 20 ลักษณะจากทั้งหมด 24 ลักษณะที่ถูกคัดเลือกไว้เป็นปัจจัยใหม่ และเมื่อทำการวิเคราะห์ปัจจัยครั้งที่ 2 สามารถจัดกลุ่มทั้ง 20 ลักษณะที่เลือกมาจากข้างต้นได้เป็น 4 กลุ่มปัจจัยใหม่ จากการนำคะแนนปัจจัยที่ได้มาสร้างกราฟ ผลที่ได้แสดงว่าผึ้งมิ้มเล็กจากประเทศไทย และจากเมืองทีนอม ประเทศมาเลเซียอยู่กลุ่มเดียวกัน นอกจากนี้จากการใช้เดนโดรแกรมที่ได้จากการวิเคราะห์แบบคลัสเตอร์ สามารถจัดกลุ่มผึ้งมิ้มเล็กดังกล่าวนี้เป็น 1 กลุ่มเช่นเดียวกัน แต่ผลจากการวิเคราะห์ความถดถอยเชิงเส้นของค่าปัจจัยใหม่ทั้ง 4 ปัจจัย กับค่าละติจูด และลองติจูด แสดงถึงการเปลี่ยนแปลงของลักษณะลักษณะทางมอร์โฟเมตริกของผึ้งมิ้มเล็กในประเทศไทย กล่าวคือ ขนาดของผึ้งมิ้มเล็กจากภาคใต้ไปยังภาคเหนือจะมีขนาดเพิ่มขึ้น แต่ขนาดของผึ้งมิ้มเล็กจากภาคตะวันตกไปภาคตะวันออกจะมีขนาดเล็กลง

ศึกษาความหลากหลายทางพันธุกรรมโดย 2 วิธี วิธีแรกโดยการดูรูปแบบของชิ้นส่วนของผลิตภัณฑ์จากปฏิกิริยาลูกโซ่โพลิเมอเรสหลังตัดด้วยเอ็นไซม์ตัดจำเพาะ นำผลิตภัณฑ์พีซีอาร์บางส่วนของยีน *cytb* ที่ได้ (520 คู่เบส) ไปตัดด้วยเอ็นไซม์ตัดจำเพาะ *Dra*I และ *Alu*I พบความแปรผันทางพันธุกรรมของกลุ่มตัวอย่างผึ้งมิ้มเล็กจากบริเวณต่างๆ เมื่อทำการตัดด้วย *Alu*I แบ่งผึ้งมิ้มเล็กเป็น 6 แฮปโลไทป์ แต่เมื่อตัดด้วย *Dra*I สามารถแบ่งผึ้งมิ้มเล็กได้เป็น 3 แฮปโลไทป์ วิธีที่ 2 ทำการหาลำดับเบสบางส่วนของยีน *cytb* จากการวิเคราะห์ลำดับเบสที่ได้ พบว่าผึ้งมิ้มเล็กจากบริเวณแผ่นดินใหญ่ของประเทศไทยมีดีเอ็นเอโพลีมอร์ฟิซึมต่ำกว่าตัวอย่างผึ้งจากบริเวณเกาะภูเก็ตและเชียงใหม่ของประเทศไทย สร้างแผนภูมิต้นไม้แสดงความสัมพันธ์ทางวิวัฒนาการโดยใช้โปรแกรมเอ็นเจและยูพีจีเอ็มเอ พบว่าสามารถแบ่งกลุ่มผึ้งมิ้มเล็กในประเทศไทย ออกได้เป็น 2 กลุ่ม คือ กลุ่ม A ซึ่งพบได้ในตัวอย่างผึ้งมิ้มเล็กจากแผ่นดินใหญ่ของประเทศไทย ส่วนกลุ่ม B พบในตัวอย่างผึ้งจากจังหวัดภูเก็ต และจังหวัดเชียงใหม่

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ATSALEK RATTANAWANNEE: ANALYSIS OF MORPHOMETRIC AND GENETIC VARIATION OF SMALL DWARF HONEY BEES *Apis andreniformis* Smith, 1858 IN THAILAND. THESIS ADVISOR: PROF. SIRIWAT WONGSIRI, Ph.D., THESIS CO-ADVISOR: ASST. PROF. CHANPEN CHANCHAO, Ph.D., 117 pp.
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Small dwarf honey bee, *Apis andreniformis,* is one of native Thai honey bees. Less data on morphometric and genetic variation of this species have been reported. In this investigation, thirty colonies of *A. andreniformis* were collected for morphometric analysis and 37 colonies were collected for genetic analysis. For morphometric analysis, 24 characters of worker bees were measured and analyzed. By using colony means for the 1st factor analysis, 20 out of 24 morphometric characters were selected as new variable. For the 2nd analysis, 20 morphometric characters could be grouped into 4 new factors. Due to graph plotting of factor scores, bees from Thailand and from Tenom, Malaysia belong into one group. In addition, a dendrogram generated from cluster analysis supports that bees from Thailand and Tenom, Malaysia are clumped into one group. However, result on linear regression analysis of factor scores against latitude and longitude shows clinal patterns in morphometric characters of *A. andreniformis* in Thailand. The body size of bees from the south to the north increase but decreased in bees from the west to the east.

Genetic variation was determined into 2 means. First, genetic variation was analyzed by using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). After amplification of cytochrome oxidase subunit b (*cytb*), products of 520 bp were restricted by *Dra*I and *Alu*I. Genetic variation was observed. Six haplotypes were found after *Alu*I digestion while 3 haplotypes were found after *Dra*I digestion. Second, PCR products amplified by *cytb* were sequenced. Based on nucleotide analysis, DNA polymorphism among bees from mainland of Thailand is lower than that from Phuket Island and Chiang Mai. Phylogenetic trees were constructed by Neighbor-joining and UPGMA programs. Two different groups of *A. andreniformis* of Thailand are obtained from both trees. Bees in group A are from mainland while bees in group B are from Phuket Island and Chiang Mai.

Department	Biology	Student's signature Atsalek Rattanawannee
Field of study	Zoology	Advisor' signature
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ABBREVIATIONS

A, T, G, C	deoxy nucleotide triphosphate (dNTP) containing Adenine, Thymine, Cytosine, and Guanine, respectively
bp	base pair
°C	degree Celcius
DNA	deoxyribonucleic acid
cytb	Cytochrome oxidase subunit b
EDTA	Ethylene diamine tetra-acetic acid
HCl	hydrochloric acid
kb	kilobase
mg	milligram
min	minute
ml	milliliter
mM	millimolar
mtDNA	mitochondrial DNA
ND4	NADH dehydrogenase subunit 4
ng	nanogram
NJ	Neighbor Joining
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
rpm	revolution per minute
sec	second
TEMED	N, N, N, N'-tetra methyl ethylene diamine
Tris	tris (hydroxyl methyl) aminomethane
UPGMA	Unweighted Pair Group Method using Arithmetic averages
UV	ultraviolet
V	volt
µg	microgram
µl	microlitre
µM	micromolar

CHAPTER I

INTRODUCTION

Honey bees are one of important economic insects because they give us useful products such as honey, wax, royal jelly, pollens, and bee venom. Honey is always used as an additive in many kinds of food and cosmetics. It is widely used in traditional medicine. Furthermore, bees play an important role as pollinators which can help increase economic crop yield. Beekeeping and use of honey bee products have a long history in Thailand (Wongsiri *et al*., 1989, Oldroyd and Wongsiri, 2006.).

Honey bees are eusocial insects. A social structure of colonies is composed of a single queen, several thousands of female workers, and a few hundreds of drones. A queen and female workers are both developed from fertilized eggs (diploid 2n = 32) while drones or males are hemizygotes (haploid individual) developed from unfertilized eggs (Wongsiri *et al*., 1989). A queen is the only fertile female and belongs to a very important caste in a colony. She is a mother of all members of the colony (Wongsiri *et al.*, 1989). A queen can release queen pheromone from mandibular gland. The pheromone is composed of 9-oxodectans-2-enonic acid and 9-hydroxydec-2-enonic acid (Wongsiri *et al*., 1989). They can control social activities and inhibit development of worker's ovaries. Although workers are sterile, they have many obligations in the colony. For example, during early stages, hypopharyngeal glands of nurse bees are fully active to synthesize royal jelly to feed young larvae and a queen. Next stage, they change to produce wax for building a comb and to clean the colony. At final stage, they serve as foragers those search for nectar and pollens back and act as guarders to defend the colony. Drones are fertile males which are emerged only in mating season (Okada, 1985; Wongsiri, 1988).

Nowadays, there are 9 *Apis* species which are recognized (Oldroyd and Wongsiri, 2006). The newly recognized species were classified into 3 groups (O'Toole and Raw, 1991). *A. andreniformis* Smith, 1858 and *A. florea* Fabricius, 1787 belong to the first group. Their nest is single, small, and free open comb. We always find it as a single comb around a single branch of a small tree. *A. dorsata* Fabricius, 1793 and *A. laboriosa* Smith, 1871 belong to the second group. They are the open-nesting and giant bee species. They always build a single comb under a horizontal and strong support such as a branch of a tree, a rock cliff. In addition, *A. mellifera* Linneaus, 1758, *A. cerana* Fabricius, 1798, *A. nigrocincta* Smith, 1861, *A. koschevnikovi* Buttel-Reepen, 1906, and *A. nuluensis* Tingek, Koeniger and Koeniger, 1996 belong to the last group. Their nests are the cavity-nesting type with multiple combs.

In Thailand, there are 5 *Apis* species which are *A. dorsata*, *A. cerana*, *A. florea*, *A. andreniformis*, and *A. mellifera*. First 4 species are native to Thailand but *A. mellifera* is introduced to the country. Only *A. mellifera* and *A. cerana* can be well managed in hives (Wongsiri *et al*., 1990 and 1996).

A. andreniformis, one of 4 native species in Thailand, is wild and smallest. It is widely distributed throughout tropical areas, especially in the southern part of China, India, Burma, Laos, Vietnam, Malaysia, Indonesia, and Philippines (Wongsiri *et al*., 1996 and 2000).

Due to wide geographical distribution, many different methods are used to investigate biological diversity of honey bees. Morphometrical method was first introduced to study honey bee diversity (Ruttner, 1988). Morphometry is the measurement of morphological structures of organisms and is analysed by statistics (Daly, 1985). Later, various molecular biology techniques have been used to study diversity of *Apis* species at DNA level. These techniques are Random Amplified

Polymorphic DNA (RAPD, Hunt and Page, 1995), Restriction Fragment Length Polymorphism (RFLP, Deowanish *et al*., 1996; De La Rua *et al*., 1998 and 2000; Sihanuntavong *et al*., 1999; Kandemir *et al*., 2000; Sittipraneed *et al*., 2001), Microsatellite (Oldroyd *et al*., 1996; Franck *et al*., 1998; Sittipraneed *et al*., 2001; De La Rua *et al*., 2001), and DNA sequencing (Cameron, 1993 ; Crozier and Crozier, 1993 ; De La Rua *et al*., 2000 ; Sittipraneed *et al*., 2001 ; Arias *et al*., 1996, 2005). DNA analysis is a direct approach to determine genetic variation among honeybee population.

Most researches on morphometric and genetic variation of honey bee have been conducted on *A. mellifera* while few data on native honeybee species in Thailand, especially on *A. andreniformis* have been reported. This rare species is one of important insect pollinators to agricultural production and maintenance of natural ecosystem (Deowanish *et al*., 2001). It is necessary to gain more data, especially on species distribution, habitat diversity, and variation among population.

In this study, we aim to determine the morphometric and genetic variation of *A. andreniformis* population in Thailand. Samples were collected from all over the country except the central and the northeastern parts of Thailand. Twenty four morphometric characters were measured. In addition, variation in partial sequence of Cytochrome oxidase subunit b (*cytb*) and NADH dehydrogenase subunit 4 (*ND4*) of mitochondrial DNA were studied by using PCR-RFLP and DNA sequencing analysis. Molecular phylogenetic relationship among *A. andreniformis* population in Thailand was analysed. The obtained result will provide information on basic biology, biodiversity, geographic variation, and genetic relationship among *A. andreniformis* population in Thailand. In addition, it may apply to conservation biology of *A. andreniformis*.

CHAPTER II

LITERATURE REVIEW

2.1 Taxonomy of *Apis andreniformis*

Taxonomy of *A. andreniformis* has been recognized as follows:

Kingdom **Animalia**

Phylum **Arthropoda**

Class **Insecta**

Order **Hymenoptera**

Family **Apidae**

Genus ***Apis***

Species ***Apis andreniformis* Smith, 1858**

2.2 Biology and distribution of *A. andreniformis* Smith, 1858

Small dwarf honeybee, *A. andreniformis,* is a native wild species in Thailand (Figure 1 and 2).

Figure 1. Small dwarf honey bee worker, *A. andreniformis* Smith, 1858 (http://drone.cyberbee.net/gallery/smallbees/andreniformis_onfinger).

Figure 2. Nest of small dwarf honey bee, *A. andreniformis* in Thailand. It shows a single comb hanging on a branch of a small tree.

A. andreniformis described in 1858 by Smith was recognized as the 2nd dwarf honey bee species. Considering specific species characters, workers have black hairs on a hind tibia and dorsolateral surface of a hind basitarsus but workers of *A. florea* have white hairs instead (Rinderer *et al*., 1996). Due to morphology of an endophallus and a tibia of drones, *A. andreniformis* has recently been reconfirmed to be a separated species from its sympatric species, *A. florea* (Wu and Kuang, 1987; Wongsiri *et al*., 1996). In *A. andreniformis* worker, there is black pigment in congruence which makes the bees look the darkest among other bees. Different in color from other parts, a scutellum likely looks yellowish. In contrast, abdominal segments of a queen and a drone are all black (Wongsiri *et a*l., 1996).

More biological data was provided by Rinderer *et al*. (1993). They reported that mating flights of drones from sympathetic *A. andreniformis* and *A. florea* were temporally separated. Furthermore, *A. andreniformis* virgin queen initiated mating flights between 12.33 and 12.50 p.m. but not in *A. florea* virgin queen (Koeniger *et al*., 2000). Considering a nest building, *A. andreniformis* builds a single-comb nest that its structure looks much different from that of *A. florea* as well (Rinderer, 1996).

A. andreniformis is widely distributed in tropical and sub tropical regions of Asia, especially in the southern part of China, India, Burma, Laos, Vietnam, Malaysia, Indonesia, and the Philippines (Figure 3). It is always found at coastal flats and near foothill areas (1-100 m above sea level) to high mountain and forest areas at about 1600 m attitude (Wongsiri *et al*., 1996).

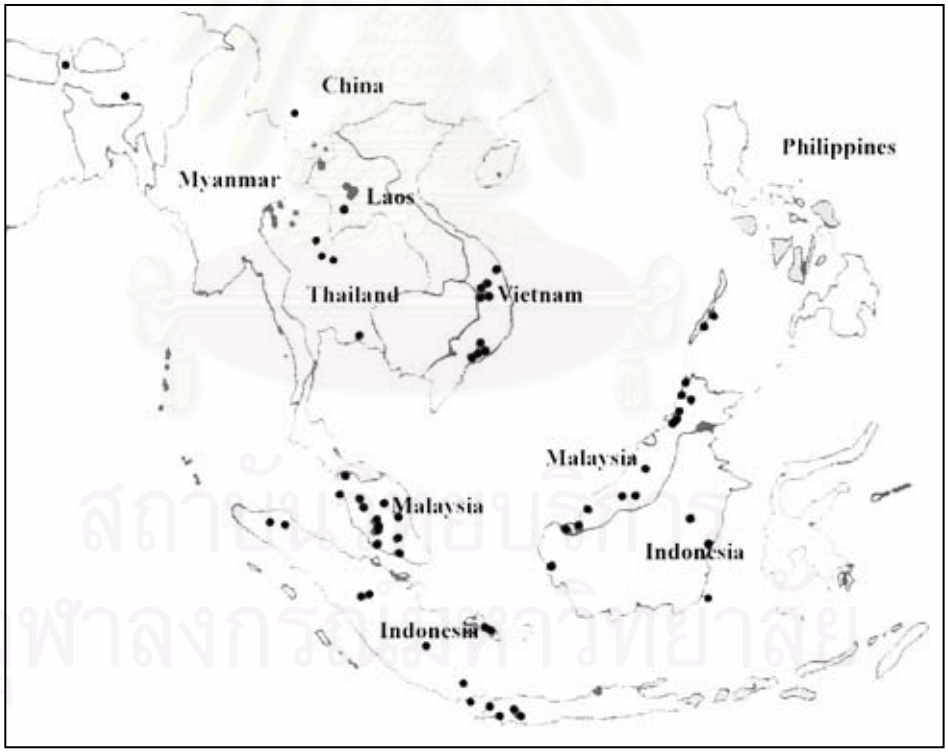

Figure 3. Distribution of *A. andreniformis* in southeast Asia (Wongsiri *et al*., 1996).

2.3 Morphometry of *Apis* spp.

Morphometrical method was first introduced to study diversity and variation of organisms including honey bees and other insects. Morphometry is the measurement of particular structures of organisms and analysed by statistics. In honey bee, the first morphometric study on an adequate scale with honey bees was carried out by Cochor in 1916. This author measured the total length of proboscis of *A. mellifera* among 6 geographic races. It presented that there is a gradual increase in proboscis length of bees collected from north to south plains along a line from the Baltic Sea to Caucasus (Ruttner, 1988). This was the starting point of the first chapter in morphometric research in honey bees.

For morphometric study, 2 below criteria must be considered:

1. Means of colony characters are used as variable parameters in statistical analysis but not characters of individual bees.
2. Numeric data, resulting from exact measurements and analyzed with statistical method, are used for classification (Ruttner, 1988).

Morimoto (1965) reported that there is a significant difference in total length of abdomen between *A. mellifera ligustica* and *A. cerana cerana*. Mattu and Verma (1983) investigated the morphometric variation of *A. cerana indica* in southwest of Himalayan region. They collected bees from various parts of Himalayan and Kashmir, India. A significant difference in a postmontum length, pedicel of antenna length, and total length of antenna among bees from Himachal was reported but the significant difference was found only in postmentum length among bees from Kashmir. In addition, they found that total length of antenna and length of flagellum of bees from Kashmir is larger than of bees from Himachal. Furthermore, Crewe, Hepburn, and Moritz (1994) reported that 10 morphological characters were adequate

to identify and discriminate 2 races of southern African honey bees, *A. mellifera capensis* and *A. mellifera scutellata.* They collected bees from 32 localities which were the subcontinent from the west coast to the east coast and were from Cape town in the south to the north of Johannesburg. Moreover, a comparison of *A. andreniformis* from southeastern Thailand and Palawan, the Philippines and *A. florea* from southeastern Thailand. They found that morphology of *A. andreniformis* is very different from that of sympatric *A. florea*. In addition, there is very few morphological difference of *A. andreniformis* between Thai and the Philippine population as well (Rinderer *et al*., 1996).

Tilde *et al*. (2000) investigated the morphometric diversity of *A. cerana* in the Philippines by using 39 morphometric characters. They collected bees throughout the Philippine archipelago. They reported that bees from Palawan were unequivocally distinct and were separated from the others. Also, bees from the Philippine Islands still showed a high degree of variation. Bees from Luzon were obviously differed from those from Visayas and Mindanao. Moreover, among bees within Luzon, the bees from the highland were obviously differed from those from the lowland. They were considered into separated groups. The diversity of *A. cerana* was supported by Hepburn *et al*. (2001). They collected 3,704 *A. cerana* workers from 279 colonies. They were from 64 localities distributing randomly in southern Himalayan. This area is connected to Pakistan in the west and is connected to Myanmar in the east. Fifty five quantitative morphological characters were used. It revealed that there are 4 major morphoclusters of samples. Among 4 morphoclusters, 2 morphoclusters are further subdivided into 3 biometric subgroups. Morover, they found that bees from the west to the east decrease in size but bees from higher altitude are bigger in size.

In Thailand, Chaiyawong (2001) used 22 morphometric characters to investigate diversity of *A. florea* throughout of Thailand. It shows that they all belong into one group. Until present, analysis of morphometry is still used. Francoy *et al*. (2006) introduced a simple methodology to investigate morphometric diversity of *A. mellifera* (*A. mellifera ligustica, A. mellifera carnica,* and *A. mellifera scutellata*). In each subspecies, 50 workers were sampled. Five identified landmarks on forewing radial cell were taken a photo by digitalized image and were estimated by multivariated analysis. It presents that there are significant differences among these *A. mellifera* subspecies. In addition, it can be concluded that features measured in a single wing cell are sufficient to discriminate these racial honey bee groups.

2.4 Molecular marker for investigating variation in honey bees

DNA is genetic material found in all cells of living organisms and can be recovered. In general, DNA can be classified into 2 categories, chromosomal (nuclear) DNA and extrachromosomal (organelle) DNA. Nuclear DNA is located in nucleus of eukaryotic cell while organelle DNA is located in mitochondria and chloroplast. Alternatively, it is known as mitochondrial DNA (mtDNA) and chloroplast DNA, respectively. Analysis of polymorphism at DNA level is considered to be a direct approach to investigate interspecific and intraspecific genetic variations. Mitochondrial DNA has been widely used in honey bees (Cornuet and Garnery, 1991). Like mtDNA in other organisms, honey bee mtDNA is circular and double stranded. The mtDNA molecules are generally about 16,000 bp. Also, there are 5-10 copies of mtDNA within each cell. The mitochondrial genome is composed of 13 protein coding genes, 2 ribosomal RNAs (rRNAs), 22 transfer RNAs (tRNAs), and non-coding region containing an origin of replication (Figure 4). In addition,

protein coding genes are 3 subunits of cytochrome C oxidase (*COI, COII*, and *COIII*), 7 subunits of NADH dehydrogenase (*ND1-6* and *ND4*L), cytochrome 6, and 2 subunits of ATP synthetase (*ATPase6* and *8*). Unlike nuclear DNA, mtDNA is maternally inherited without recombination (Singh *et al*., 1995). Basically, mutation rate of mtDNA is much more rapid than that of single-copy nuclear genes and it is not sensitive to environmental selection pressure (Franck *et al*., 2000). Hence, that makes mtDNA useful and efficient in studying genetic and phylogeographic variations among bee population (Franck *et al*., 2000; Garnery *et al*., 1993).

At present, various techniques in molecular biology have been used for this purpose such as Restriction Fragment Length Polymorphism (RFLP), DNA sequencing, etc (Hepburn and Radloff, 1998). DNA sequencing is a direct method and is a powerful technique to infer variation in DNA sequence while RFLP is an indirect method to infer DNA variation. RFLP is usually performed in a single gene or other easily isolated piece of DNA such as mtDNA. If there is a sequence difference among 2 or more individuals due to the change of a restricted site of endonuclease (restriction enzyme), different patterns of restriction fragments (DNA polymorphism) will be observed.

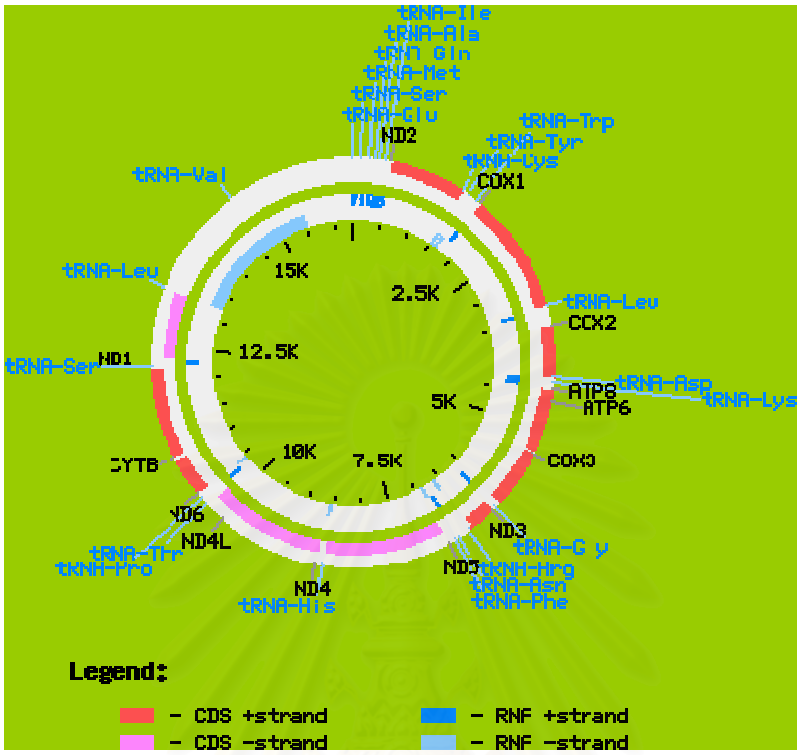

Figure 4. Map of circular mitochondrial genome of *A. mellifera*. It reveals 13 protein coding genes, 2 ribosomal RNA genes (*rRNA*), 22 transfer RNA genes (*tRNA*), and non-coding region (Crozier and Crozier, 1993).

Specific primers will be designed and used in Polymerase Chain Reaction (PCR) in order to amplify a target region. A reaction is composed of DNA template, oligonucleotide primers, deoxynucleotide triphosphates (dNTP), and DNA polymerase (normally, *Taq* DNA polymerase) in suitable buffer. PCR reaction contains 3 important steps: (1) double strand DNA is denatured at high temperature to generate a single stand (2) short oligonucleotide primers bind to a single strand complementary template at lower annealing temperature, and (3) the temperature is raised to synthesize a target sequence by primer extension. During amplification,

these 3 steps will be repeated several times (Hoy, 1994). Moreover, PCR-based techniques such as microsatellites, Random Amplified Polymorphic DNA (RAPD) are widely used to analyse DNA variation. Garnery *et al*. (1991) presented a phylogenetic relationship among *A. florea, A. dorsata, A. cerana,* and *A. mellifera* by using neighbor-joining and parsimony methods. The sequence of 5' end of *COII* was used. The result reveals that *A. cerana* and *A. mellifera* are closely related. In contrast, they are divergent and are separated from *A. florea* and *A. dorsata*. By PCR, Moritz *et al*. (1994) analyzed a variable region between *COI* and *COII* of *A. mellifera* distributed in the southern part of Africa along the 27th latitude. They reported a novel mitotype of *A. mellifera*.

By determining mtDNA variation, Cornuet and Garnery (1991) categorized *A. melifera* into 3 major lineages: 1) African lineage (lineage A) including *A. mellifera scutellata, A. mellifera capensis, A. mellifera intermissa, A. mellifera adansonii, and A. mellifera monticola*; 2) *mellifera* lineage (lineage M) including *A. mellifera ligustica* and *A. mellifera carnica*; and 3) *caucasic* lineage (lineage C) including *A. mellifera caucasica*. In addition, Deowanish *et al*. (1996) examined mtDNA variation of *A. cerana* from Japan, Korea, Taiwan, Vietnam, Thailand, Nepal, and the Philippines by using RFLP technique. Ten restriction enzymes (*Hae*III, *Hin*fI, *Bcl*I, *Bgl*II, *Eco*RI, *Eco*RV, *Hin*cII, *Hin*dIII, *Nde*I, and *Spe*I) were used. Bees can be classified into 6 groups which are dependent on different localities: 1) Japan; 2) Nepal, Vietnam, and the northern part to the central part of Thailand; 3) Korea-Tsushima; 4) Taiwan; 5) Southern Thailand; and 6) the Philippines.

Instead of using many restriction enzymes, one restriction enzyme is also sufficient to use for a determination. For example, De La Rua, Serano, and Galian (1998) studied *Dra*I restricted patterns of amplified *tRNA*^{leu}-*COII* intergenic regions

in *A. mellifera* from fire Canary Island. They found 5 haplotypes of the African lineage (lineage A) and one of the west European lineage (lineage C). The A14 and A15 haplotypes were firstly described. Furthermore, Sihanuntavong *et al*. (1999) examined genetic variation and population difference of *A. cerana* in Thailand by *Dra*I restriction analysis of amplified *srRNA* and *lrRNA* genes and intergenic *COI-COII* region. They found 12 composite haplotypes. In addition, large genetic differences among *A. cerana* population from the northern part of Thailand and the peninsular Thailand were detected. For another example, Sittipraneed, Sihanuntavong, and Klinbunga (2001) examined genetic difference of *A. cerana* in Thailand by RFLP and DNA sequence analysis of amplified *lrRNA* gene. They found 4 haplotypes of *A. cerana* when considering *Dra*I digested patterns. Haplotype A was found in the northern region, the northeastern region, and the central region whereas haplotyp B was from the peninsular Thailand, Phuket, and Samui Island. Haplotype C was counted as 47.06% of *A. cerana*. They were originated from Samui Island but not from other geographic regions. Haplotype D was also found in the northern part, the northeastern part, and the central part of Thailand but was found in low frequency.

Nanork (2001) determined genetic variation of *A. florea* from various parts of Thailand by PCR-RFLP. There is no variation in a region of *lrRNA* and *cytbI-tRNA*^{ser}. Two different haplotypes were found after *Ase*I digestion of the intergenic *COI-COII* region. However, the different haplotype was detected from only one colony from Prachuab Kiri Khan province.

It has been reported that we can use morphometry together with DNA analysis to support each other in order to determine the variation. For example, Kandemir, Kence, and Kence (2000) used 6 enzyme systems to determine genetic variation and used 10 morphometric characters to determine variation in *A. mellifera* population in

Turkey. The result supports that both morphometric and electrophoretic variation are equally effective in discriminating honey bee population.

CHAPTER III

MATERIALS AND METHODS

2.1 Morphometric analysis

2.1.1 Equipment

- Stereomicroscope (Stemi DV4, Zeiss, Germany)
- Forceps with very fine tips
- Microscope slides (Sail bran, China)
- Incubator BM 400 (Memmert Gamb H, Germany)
- Stirrer/ hotplate, model: PC-320 (Corning, USA)
- Cover glasses (Menzel-glaser, Germany)
- Micrometer
- Brush-pen, No. 0
- Insect pins (the Shiga, Japan)
- Filter paper (4 mm), Whatman (Whatman international Ltd., England)
- 1.5 ml Microcentrifuge tube (Treff lab, Switzerland)
- Dissecting dish

2.1.2 Chemicals

- Gum arabic (Sigma, USA)
- Chloral hydrate (Fluka, Switzerland)
- Glycerine (BDH, England)
- Ethyl acetate (Merck, Germany)
- Ethanol (Merck, Germany)

2.1.3 Collection of bee samples

Apis andreniformis workers were collected from different localities in Thailand and Tenom, Malaysia. Twenty seven colonies were collected from 4 parts of Thailand which were from the northern part (5 colonies), the western part (8 colonies), the eastern part (8 colonies), and the southern part (6 colonies). In addition, 3 colonies were collected from Tenom, Malaysia. Localities and sampling details were shown in figure 16 and appendix II.

At least, 30 worker bees were collected from each colony and immediately anesthesized by ethyl acetate. Then, they would be preserved in 70% (v/v) ethanol.

2.1.4 Dissection

Twenty bees from each colony were dissected. Each of them was put into a dissecting dish containing 70% (v/v) ethanol in order to keep the bee soft and easy to dissect. Dissection was done under a stereo microscope. The used body parts were: antenna, proboscis, forewing, hindwing, hindleg, the 3rd and the 4th tergite (counted from a petiole), and the 3rd, the 4th, and the 6th sternite. These characters are presented in figure 5.

The right forewing and hindwing were pulled by firmly grasping at their attached point. It is important to be aware that wings should not be folded. Also, all required characters must be present.

A whole proboscis consisting of postmentum, mentum, and glossa was pulled by using forceps with very fine tips. Also, an antenna consisting of a scape and a flagellum was used.

In addition, a right hindleg was detached by pulling at a trochanter. After that, a basitarsus would be separated from tibia. The trochanter was also removed from femur which was still attached to tibia.

An abdomen was detached by pulling at a joint between a thorax and an abdomen. The 2nd tergite was removed by inserting a very fine tip of forceps into a hold between the 2nd and the 3rd tergite. The 2nd tergite was then griped and pulled. Later, the 3rd and the 4th tergites were pulled away from the rest and were separated from each other. Muscle and connective tissue attached to the 3rd and the 4th tergites were removed by using a small brush and forceps.

It is difficult to pull sternites because they are easily broken. In order to remove the 3rd sternite, 2 pairs of forceps were used. One pair of forceps was used to pull a petiole from the 3rd sternite while other pair of forceps was used to press the 3rd sternite. The 4th and the 6th sternites were also pulled off by using 2 pairs of forceps. After that, a small brush and forceps were used to make sternites clean.

2.1.5 Making slides of bee body parts

2.1.5.1 Preparing slide

All processes of making slide were done under a stereo microscope. Bee body parts were prepared into 4 sets as below:

Set 1: forewing, hindwing, the 3rd sternite, and the 4th sternite

Set 2: the 3rd tergite, the 4th tergite, and the 6th sternite

Set 3: antenna and proboscis

Set 4: femur, tibia, and basitarsus

Twenty slides and 80 cover glasses were required for 20 workers from one colony.

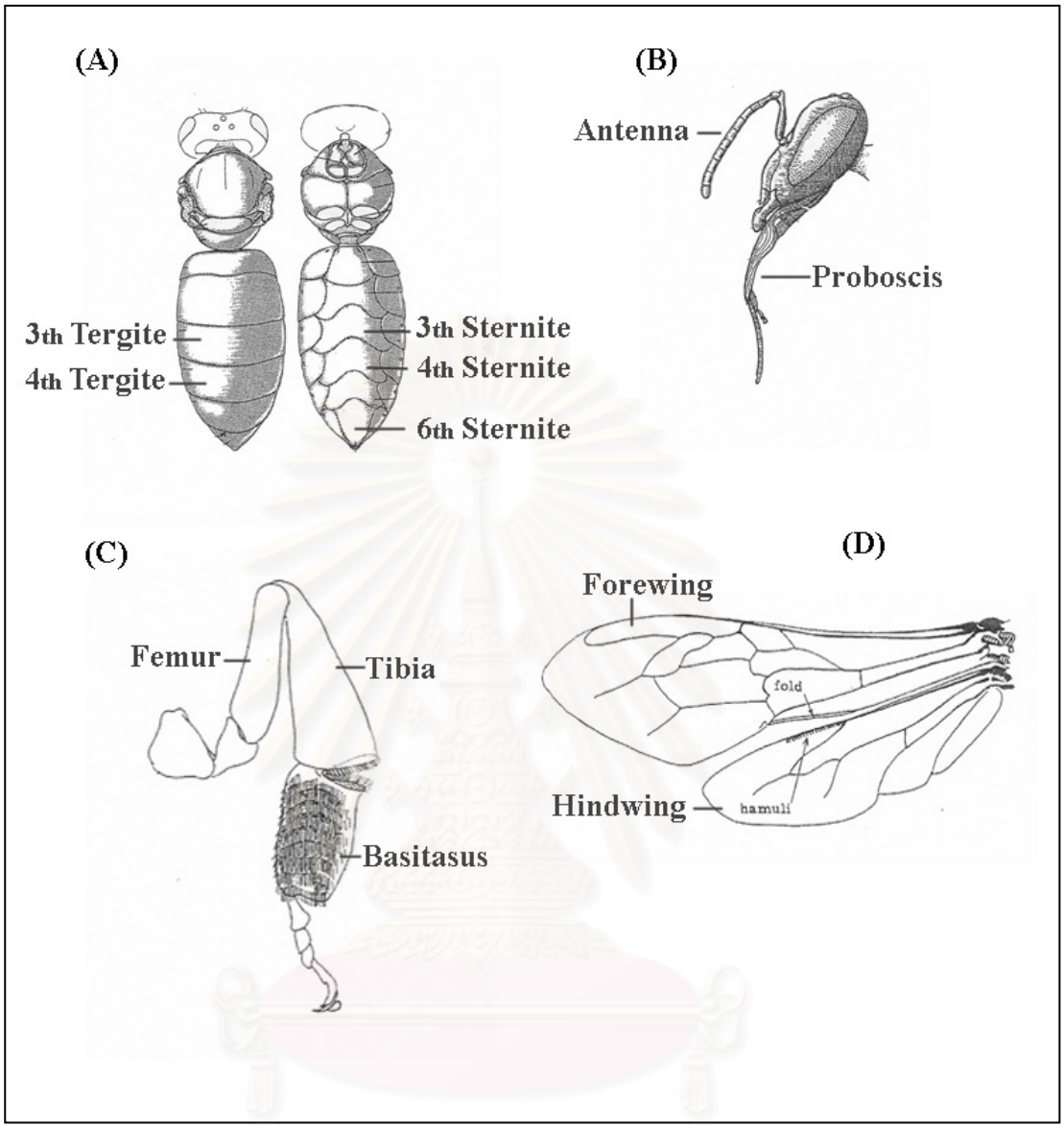

Figure 5. External morphology of honey bee (Dade, 1994):

(A) an abdomen showing the 3rd and the 4th tergites (count from the petiole), together with the 3rd, the 4th, and the 6th sternites (count from the petiole);

(B) a head showing an antenna and a proboscis;

(C) a right hindleg showing femur, tibia, and basitarsus; and

(D) a right forewing and a hindwing.

2.1.5.2 Mounting slides

2.1.5.2.1 Set 1

Hoyer's medium (Krantz, 1978) was dropped on a glass slide. The set 1 bee body parts were placed and set on the above medium drop. After that, a cover glass was placed and sealed on top. Try to avoid air bubbles while sealing.

2.1.5.2.2 Set 2

Set 2 body parts were placed on the Hoyer's medium drop on the same slide as in 2.1.5.2.1. Tergites must be kept unfolded. Then, it was sealed by a cover.

2.1.5.2.3 Set 3

Another drop of Hoyer's medium was put on the same slide as in 2.1.5.2.2. An antenna and a proboscis were placed. The same process of sealing was applied as mentioned before.

2.1.5.2.4 Set 4

The 4th drop of Hoyer's medium was applied on the same slide from 2.1.5.2.4. A femur-tibia and basitarsus were placed on Hoyer drop. The same process of sealing was applied as mentioned before.

In order to point a location precisely, all body parts must be arranged into the same orientation and all required characters must be present. Next, the prepared slide was placed on a hot plate for a few minutes to eliminated air bubbles repeatedly. Finally, a slide was incubated at 50°C for 2 weeks before measurement.

2.1.5.3 Measurement

Bee body parts were photographed by using Digital Photo Marker program. Pictures were saved as JPEG file. Then, 24 characters were measured by using Image-Pro express program. The used characters were:

1. Forewing length (FWL)
2. A line from the outermost end of radial cell to a sharp curve of the inner side of forewing (LFW)
3. Radial cell of fore wing length (RFWL)
4. Hindwing length (HWL)
5. Hindwing width (HWW)
6. The 3rd tergite length (TG3L)
7. The 3rd tergite width (TG3W)
8. The 4th tergite length (TG4L)
9. The 4th tergite width (TG4W)
10. The 3rd sternite width (ST3W)
11. Length of wax mirror on 3rd sternite (ST3WL)
12. Width of wax mirror on 3rd sternite (ST3WW)
13. The 4th sternite width (ST4W)
14. Length of wax mirror on 4th sternite (ST4WL)
15. Width of wax mirror on 4th sternite (ST4WW)
16. The 6th sternite width (ST6W)
17. Length of wax mirror on 6th sternite (ST6WL)
18. Total length of antenna (ANL)
19. Total length of proboscis (PBL)
20. Tibia width (TBW)

21. Tibia length (TBL)

22. Femur length (FML)

23. Basitarsus length (BSTL)

24. Basitarsus width (BSTW)

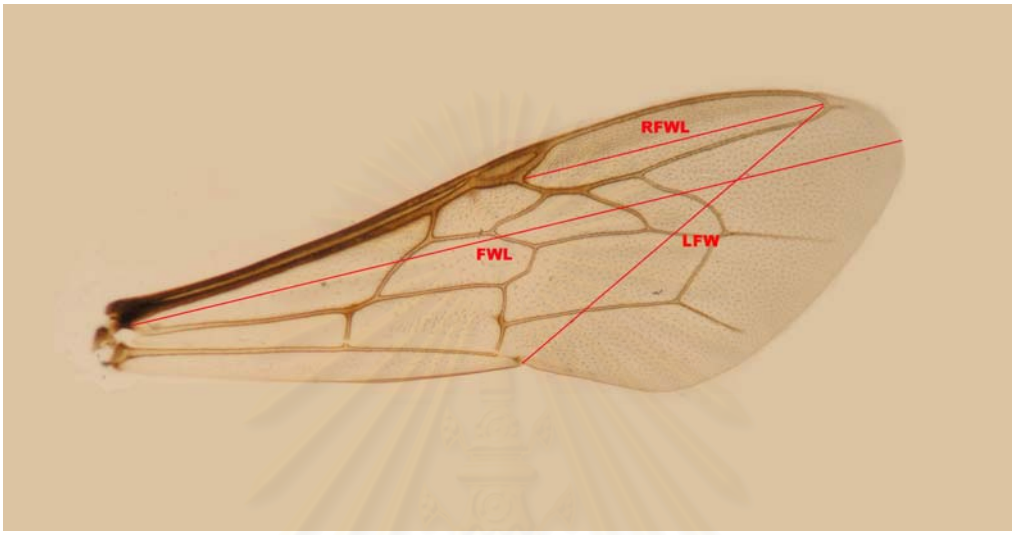

Figure 6. A right forewing of *A. andreniformis* worker. Forewing length (FWL), A line from the outermost end of radial cell to a sharp curve of the inner side of forewing (LFW), and radial cell of forewing length (RFWL) are indicated.

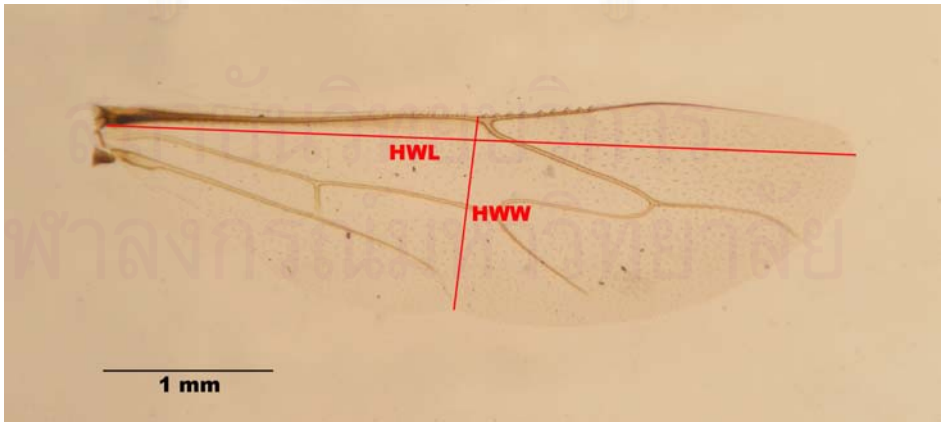

Figure 7. A right hindwing of *A. andreniformis* worker. Hindwing length (HWL) and hindwing width (HWW) are indicated.

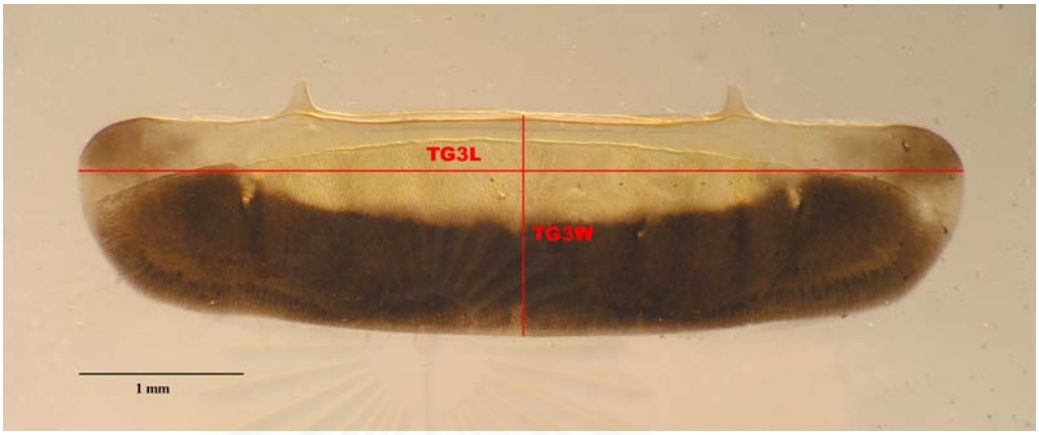

Figure 8. The 3rd tergite of *A. andreniformis* worker**.** The 3rd tergite length (TG3L) and the 3rd tergite width (TG3W) are indicated.

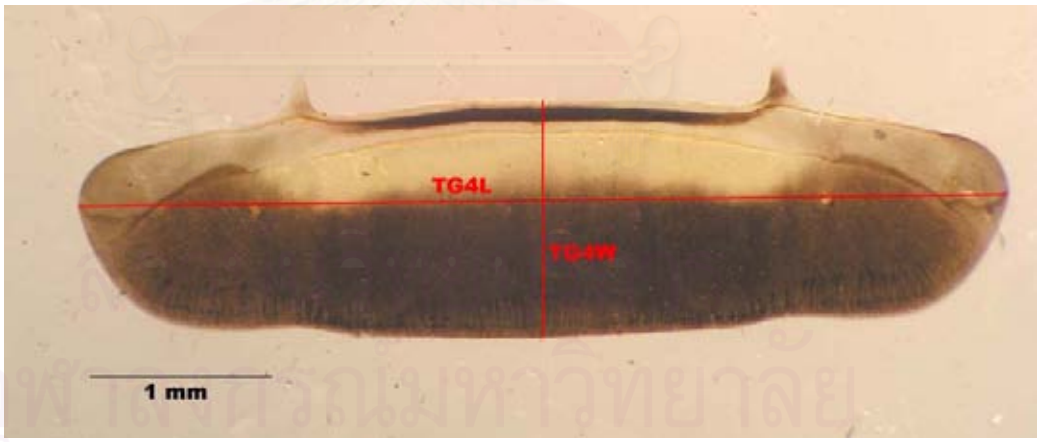

Figure 9. The 4th tergite of *A. andreniformis* worker. The 4th tergite length (TG4L) and the 4th tergite width (TG4W) are indicated.

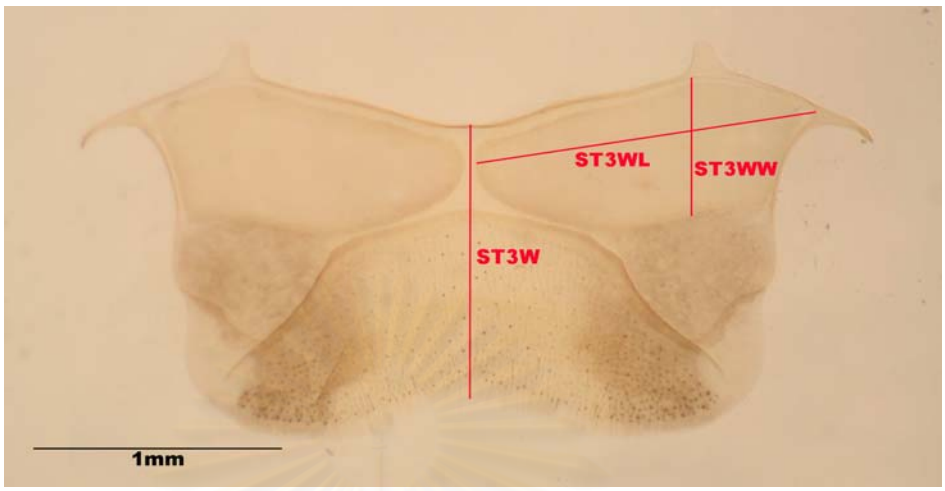

Figure 10. The 3rd sternite of *A. andreniformis* worker. The 3rd sternite width (ST3W), length of wax mirror on the 3rd sternite (ST3WL), and width of wax mirror on the 3rd sternite (ST3WW) are indicated.

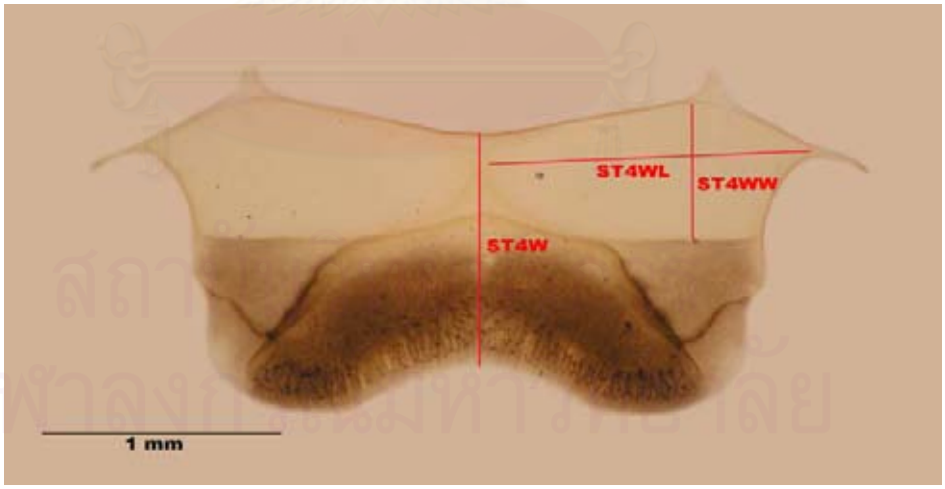

Figure 11. The 4th sternite of *A. andreniformis* worker. The 4th sternite width (ST4W), length of wax mirror on the 4th sternite (ST4WL), and width of wax mirror on the 4th sternite (ST4WW) are indicated.

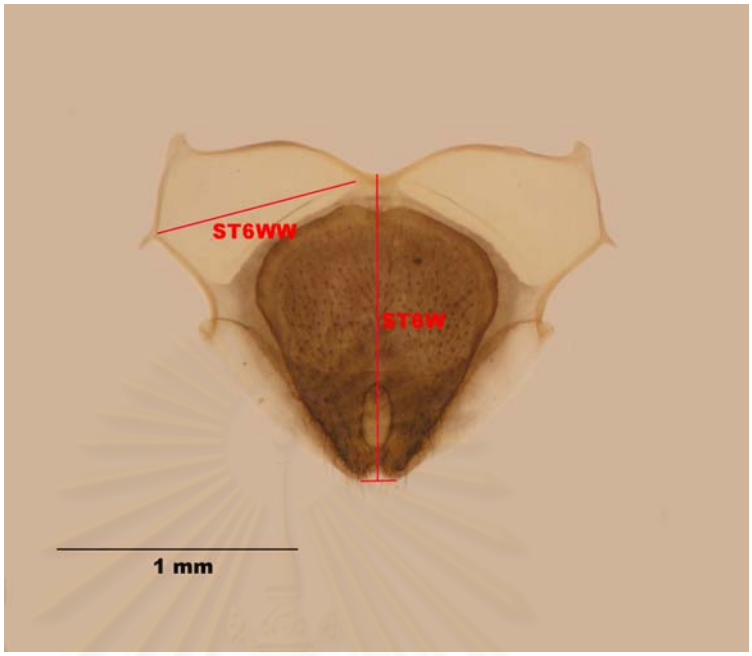

Figure 12. The 6th sternite of *A. andreniformis* worker. The 6th sternite width (ST6W) and width of wax mirror on the 6th sternite (ST6WW) are indicated.

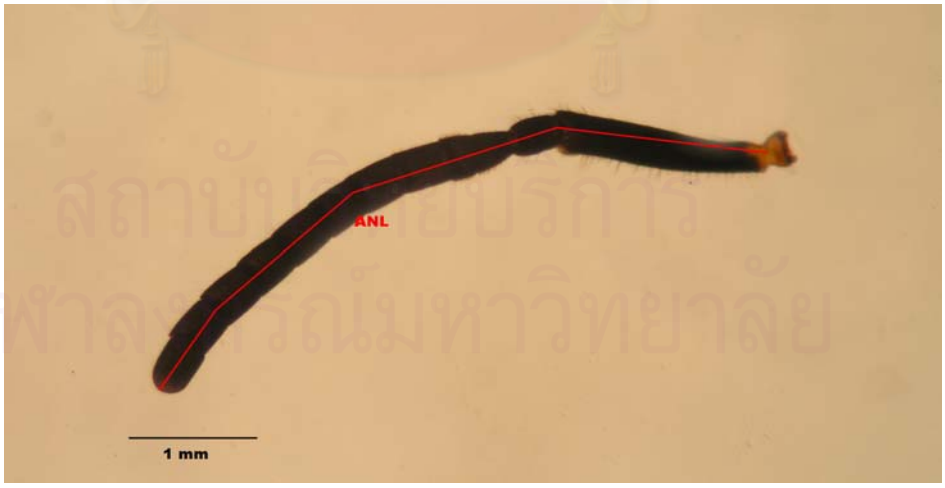

Figure 13. An antenna of *A. andreniformis* worker. Total length of antenna (ANL) is indicated.

Figure 14. A proboscis of *A. andreniformis* worker. Total length of proboscis (PBL) is indicated.

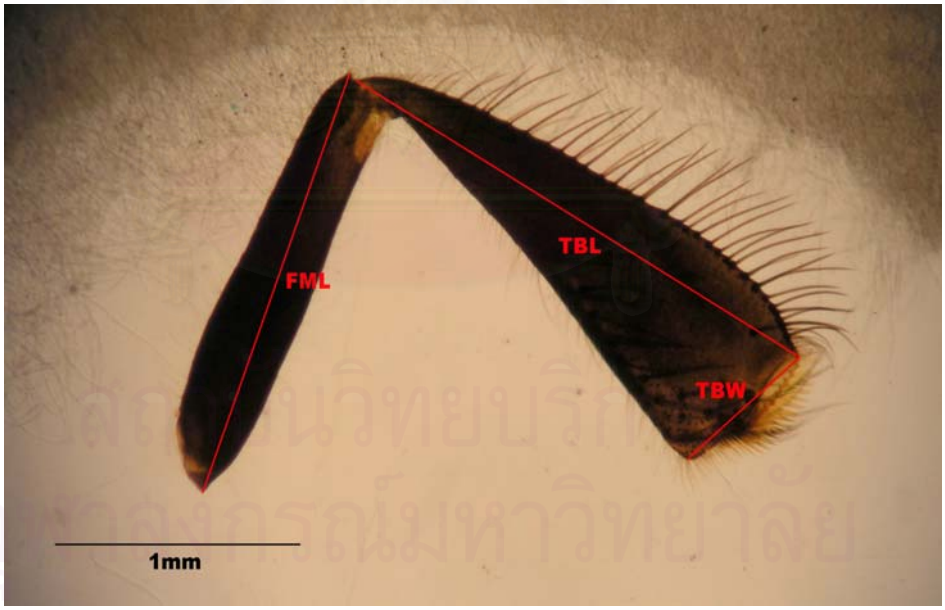

Figure 15. Femur and tibia of right hindleg of *A. andreniformis* worker. Tibia width (TBW), tibia length (TBL), and femur length (FML) are indicated.

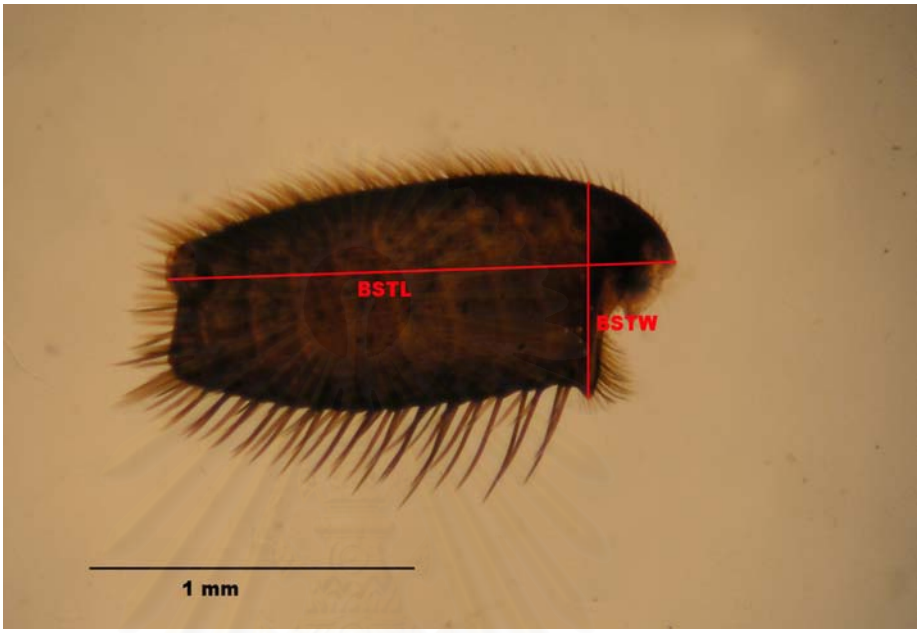

Figure 16. Basitarsus of right hindleg of *A. andreniformis* worker. Basitarsus length (BSTL) and basitarsus width (BSTW) are indicated.

2.1.5.3 Data analysis

A statistic to perform a factor analysis on the colony means using 24 characters for all 600 bees collected from 4 parts of Thailand and Tenom, Malaysia was used. This method provides characters those have larger loadings in various factors and allows the parsimonious reduction in the number of characters needed for further analysis. After that, cluster analysis (SPSS for windows 13.0) was used to investigate the relationship between groups. Finally, linear regression was used to explore clinal patterns in the characteristics of *A. andreniformis* samples in Thailand.

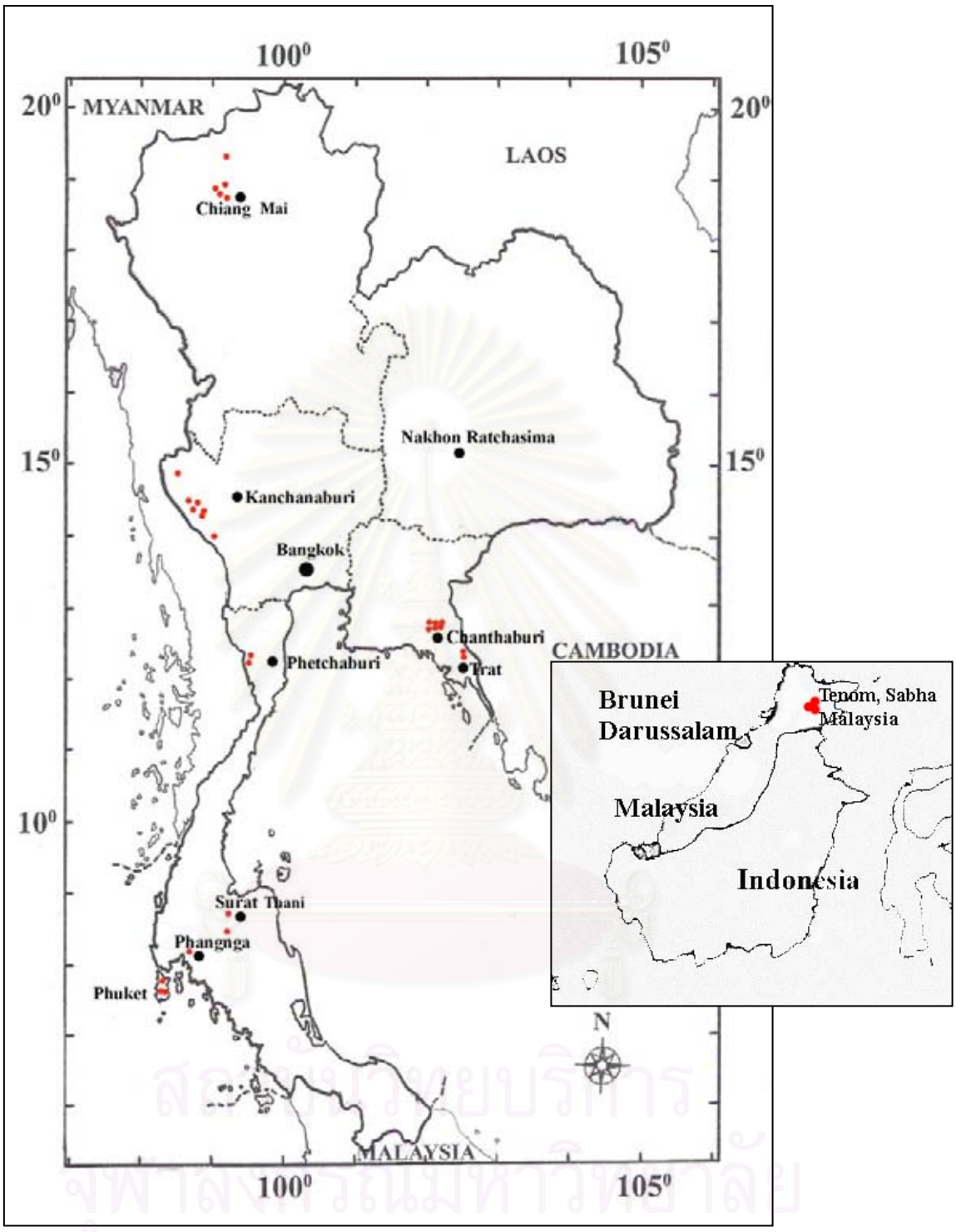

Figure 17. Map of Thailand and Tenom, Malaysia showing sampling sites for *A. andreniformis* for morphometric analysis**.**

2.2 Genetic analysis

2.2.1 Instruments

- Autoclave, model: Conbraco, Conbraco Ind. Inc., USA
- Automatic micropipette P10, P20, P100, P200, and P1000 (Gilson-medical electronics, S.A., France)
- Freezer (-20°C)
- Horizontal gel electrophoresis apparatus, model: Mupid, Advance Co., Ltd., Japan
- High speed microcentrifuge, model: Centrifuge 5410 (Eppendorf, Germany)
- Magnetic stirrer, model: PC-320 (Corning, USA)
- Polaroid camera, model: direct screen instant camera DS 34 H-34 (Peca products, UK)
- Microincubator, model: M-36, Taitec, Japan
- Incubator, model: Memmert, Germany
- Microwave oven, model: Sharp carousel R7456 (Sharp, Thailand)
- PCR machine, model: GeneAmp® PCR system 9700 (Applied Biosystem, Singapore)
- Electronic UV transilluminator (Ultra ium Inc., USA)
- Vortex, model: MS I Minishaker (IKA-works, Inc., USA)

2.2.2 Inventory Supplies

- Black and white pain film
- Filter paper Whatman 3 mm (Whatman international Ltd., England)
- Microcentrifuge tubes (0.5 and 1.5 ml)
- Pipette tips (10, 200, and 1000 μl)

- Thin-wall microcentrifuge tube (0.2 ml)

- Whatman laboratory sealing film (Whatman international Ltd., England)

2.2.3 Chemicals

- Absulute ethanol, CH_3CH_2OH, M. W. = 46.07 (Merck, Germany)

- Acrylamide, M. W. = 71.08 (Promega, USA)

- Agarose (Research organics, USA)

- Boric acid (Research organics, USA)

- Ethidium bromide

- DNA ladder marker 100 bp (catalog # SM0321), Fermentas Life Science

- DNA λ *Hin*dIII marker (catalog # SM0101), Fermentas Life Science

- Ethylene diamine tetra-acetic acid (EDTA), $C_{10}H_{16}N_2O_8$, M. W. = 292.2 (Serve feinbiochemica GmbH & Co., USA)

- 95% Ethyl alcohol, CH_3CH_2OH, M.W. = 46, Thailand

- QIAquick® PCR purification kit (catalog # 28104), Qiagen, Germany

- QIAamp® DNA mini kit (catalog # 51304), Qiagen, Germany

- Sodium chloride, NaCl, M.W. = 58.4, Merck, Germany

- TEMED, Promega, USA

- Tris-(Hydroxymrtyl)-aminomethane, $NH_2C(CH_2OH)_3$, M.W. = 121.14, Pharmacia Biotech, USA

2.2.4 Primers

- All oligonucleotides were synthesized at Bioservice unit of National Science and Technology Development Agency (NSTDA), Bangkok, Thailand.

2.2.5 Enzymes

- Restriction endonucleases

- *Dra*I (catalog# R0129S), Biolabs Inc., New England

- *Alu*I (catalog# R0137S), Biolabs Inc., New England

2.2.6 Sample collection

Adult workers of *A. andreniformis* from 37 colonies were collected from natural colonies throughout 4 parts of Thailand. In each colony, 10-15 bees were sampled. Furthermore, bees while foraging on flowers were sampled from 9 provinces all over Thailand. More *A. andreniformis* from Tenom, Sabah, Malaysia were obtained. Additional details of sample collections are shown in figure 17 and appendix II. Obtained honey bees were preserved in 95% ethanol and were stored at 4°C until DNA extraction.

2.2.7 DNA extraction

Genomic DNA was extracted from an individual thorax of adult worker bees by QIAamp® DNA mini kit (Qiagen). A thorax was cut by a pair of scissors in 180 µl of buffer ATL. Then, the tissue were cut into small pieces and mixed by 20 µl of Proteinase K. It was mixed by vortex and was incubated at 56°C for at least 4 h. After quick spun, the mixture was added by 200 µl of buffer AL, vortexed for 15 sec, and incubated at 70°C for 10 min. After incubation, the mixture was added by absolute ethanol, vortexed for 15 sec, and quick spun. The mixture was transferred to a QIAamp® spin column which was later centrifuged at 8,000 rpm for 1 min. Then, the column was removed to a new clean 2 ml collecting tube while flow through (FT) was discarded. Buffer AW1 of 500 µl was added to the spin column which was later centrifuged at 8,000 rpm for 1 min. The spin column was removed again to a clean 2 ml collecting tube and FT was discarded. Buffer AW2 of 500 µl

was added to the spin column which was later centrifuged at 14,000 rpm for 3 min. After that, the spin column was placed into a 1.5 ml microcentrifuge tube and was added by 50 μl of buffer AE. The spin column was incubated at RT for 2 min and centrifuge at 8,000 rpm for 1 min. The elution containing genomic DNA was saved and stored at -20^{o}C.

2.2.8 Agarose gel electrophoresis

In order to determine the quality of genomic DNA, 0.8% (w/v) agarose gel was prepared. The loading sample was mixed between 5 μl of genomic DNA and 1x loading dye (5x loading dye: 25 mM Tris-HCl at pH 7.0, 0.05% bromophenol blue, 150 mM EDTA, and 25% glycerol). Also, λ *Hin*d III marker (200 ng) was used as a standard marker. Electrophoresis was performed by using 1x TBE buffer (0.05 M Tris-HCl at pH 8.0, 0.05 M Boric acid, and 0.65 M EDTA) as running buffer at 100 V for 50 min. After that, the gel was stained with 10 μg/ml ethidium bromide (EtBr) for 5 min and destained with d-H_2O for 20 min. Genomic DNA was visible under UV light and photographed.

2.2.9 Polymerase Chain Reaction (PCR)

Primers were designed from Cytochrome oxidase subunit b (*cytb*) [NC_001566] and NADH dehydrogenase subunit 4 (*ND4*) [NC_001566] of *A. mellifera* by using Primer 3 program (http://fokker.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Forward primers (*ND4*: 5'- AAAAG CTCAT GTTGA AGCT -3', *cytb*: 5'- TGAAA TTTTG GATCA ATTCT TGG -3') and reverse primers (*ND4*: 5'- TTTTA ACCAC GAAAT TATC -3', *cytb*: 5'- TCCAA GAGGA TTAGA TGATC CAG -3') were synthesized. PCR reaction was carried out in 1x PCR master mix (catalog#

K0171, Fermentas Life Science), 2 μM of each FW and RW primer, and genomic DNA (200 ng). PCR condition by *ND4* amplification was as followed: 94°C for 2 min, 30 sec, followed by 35 cycles of 94°C for 1 min; 58°C for 1 min; and 72°C for 3 min, and a final extension step at 72°C for 10 min. Moreover, PCR condition by *cytb* amplification was submitted to an initial denaturation of 94°C for 2 min 30 sec, followed by 35 cycles of 94°C for 1 min; 50°C for 1 min; and 72°C for 3 min, and a final extension step at 72°C for 10 min. The PCR product was electrophoresed on 1.5% agarose gel at 100 V for 1 h.

2.2.10 Restriction Fragment Length Polymorphism (RFLP)

An amplified product was digested by *Dra*I and *Alu*I restriction endonuclease according to a manufacture's instruction. A reaction was carried out in 20 μl containing 150 ng of PCR products, 1x of recommended buffer, 5 units of restriction enzyme, and d-H_2O. The mixture was incubated at 37°C for at least 1 h. Restriction fragments were separated on 8% acrylamide gel with TBE buffer (89 mM Tris-HCl at pH 8.0, 8.9 mM Boric acid, and 2.5 mM EDTA) at 100 V for about 1.5 h and silver stained.

2.2.11 PCR product purification

Any contaminants in PCR mixture must be removed by purification before sequencing. Purification was performed by using a QIAquick® PCR purification kit. Five times volume of buffer PB were mixed with one volume of PCR product. The mixture was then transferred to a QIAquick® spin column which would be centrifuged at 13,000 rpm for 1 min. Flow through (FT) was discarded. Buffer PE of 750 μl was added to the column which would be centrifuged at 13,000 rpm for 1 min. After that,

FT was discarded again. The column was centrifuged additionally at 13,000 rpm for 1 min. The column was removed to a new 1.5 ml microcentrifuge tube. Buffer EB (30 μl) was added to the center of the column. It was incubated at RT for 2 min and was centrifuged at 8,000 rpm for 1 min.

2.2.12 DNA sequencing and phylogenetic analysis

PCR products amplified by *cytb* were sequenced by Bioservice unit (BSU). Then, partial DNA sequences were aligned initially by using the multiple sequence alignment program CLUSTAL X. The data were saved to NEXUS file formatted for further phylogenetic tree construction. Phylogenetic analyses were performed by using neighbor-joining (NJ) and UPGMA (PAUP*4.0b10) (Swofford, 2000). In order to investigate support for nodes estimated in a parsimony tree, bootstrap analysis with 100 replicates were undertaken by PAUP*4.0b10.

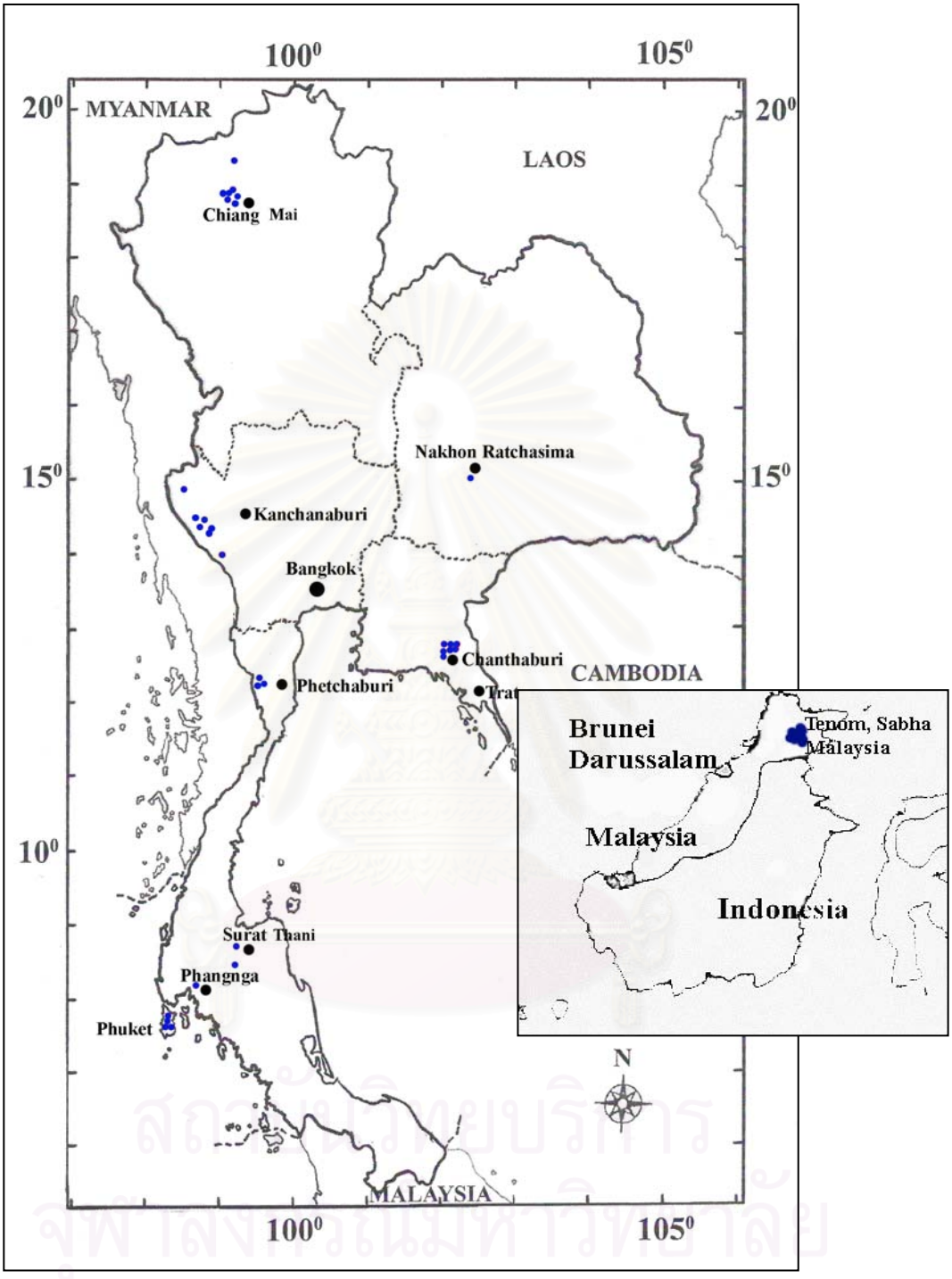

Figure 18. Map of Thailand and Tenom, Malaysia shows sampling sites for *A. andreniformis* for genetic analysis.

CHAPETR IV

RESULTS

4.1 Morphometry

4.1.1 Factor analysis

A. andreniformis workers were collected from 4 parts (north, east, west, and south) of Thailand and Tenom, Malaysia. In each colony, factor analyses were performed by using means of each of 24 morphometric characters. After that, factor loadings would be obtained. Since only factor loading greater than 0.6 would be selected for further analysis, there are only qualified 20 morphometric characters as indicated below:

1. Forewing length (FWL)
2. A line from the outermost end of radial cell to a sharp curve of the inner side of forewing (LFW)
3. Forewing length of radial cell (RFWL)
4. Hindwing length (HWL)
5. Hindwing width (HWW)
6. The 3rd tergite length (TG3L)
7. The 3rd tergite width (TG3W)
8. The 4th tergite length (TG4L)
9. The 4th tergite width (TG4W)
10. The 3rd sternite width (ST3W)
11. Length of wax mirror on the 3rd sternite (ST3WL)
12. The 4th sternite width (ST4W)
13. Width of wax mirror on the 4th sternite (ST4WW)

14. The 6th sternite width (ST6W)
15. Length of wax mirror on the 6th sternite (ST6WW)
16. Total length of antenna (ANL)
17. Tibia width (TBW)
18. Tibia length (TBL)
19. Femur length (FML)
20. Basitarsus length (BSTL)

The 2nd factor analysis using colony means of selected 20 morphometric characters can divide them into 4 groups. A group where variable belong to depends on factors with Eigen values greater than 0.6 and highest among other 3 groups. First factor was accounted for 38.98% of total variation and was mainly associated with body size (TG4L, TG4W, ST3W, ST3WL, ST4W, ST6W, and ST6WW), hindwing size (HWL and HWW), antenna length (ANL), and hindleg size (TBL, FML, and BSTL). The 2nd factor was mainly associated with forewing size (FWL, LFW, and RFWL). This factor was accounted for 11.45% out of total variation. The 3rd factor was mainly associated with the size of the 3th tergite (TG3L and TG3W) and was accounted for 9.45% of total variation. Furthermore, the 4th factor was accounted for 7.58% of total variation and was mainly associated with tibia width (TBW). These 4 factors were accounted for 67.47% of total variation.

Figure 19 to 24 show plots of 4 factor scores generated by principal component analysis (PCA). Bees were coded by 5 major collecting localities which are the northern part, the eastern part, the western part, and the southern part of Thailand and Tenom, Malaysia.

1. Figure 19 presents a plot of factor 1 (x-axis) versus factor 2 (y-axis). Principal components were obtained from colony means of 20

morphometric characters. All characters were measured from each bee. A graph shows one cluster of bees.

2. Figure 20 presents a plot of factor 1 (x-axis) versus factor 3 (y-axis). Principal components were obtained from colony means of 20 morphometric characters. All characters were measured from each bee. A graph shows one cluster of bees.
3. Figure 21 presents a plot of factor 1 (x-axis) versus factor 4 (y-axis). Principal components were obtained from colony means of 20 morphometric characters. All characters were measured from each bee. Due to the graph, 2 clusters of bees can be distinguished. First cluster contains bees from the northern part, the eastern part, and the western part of Thailand. Second cluster contains bees from the southern part of Thailand and Tenom, Malaysia. However, there is some overlap on each axis.
4. Figure 22 presents a plot of factor 2 (x-axis) versus factor 3 (y-axis). Principal components were obtained from colony means of 20 morphometric characters. All characters were measured from each bee. A graph shows one cluster of bees.
5. Figure 23 presents a plot of factor 2 (x-axis) versus factor 4 (y-axis). Principal components were obtained from colony means of 13 morphometric characters. All characters were measured from each bee. A graph shows one cluster of bees.
6. Figure 24 presents a plot of factor 3 (x-axis) versus factor 4 (y-axis). Principal components were obtained from colony means for 13 morphometric characters. All characters were measured from each bee. A graph shows one cluster of bees.

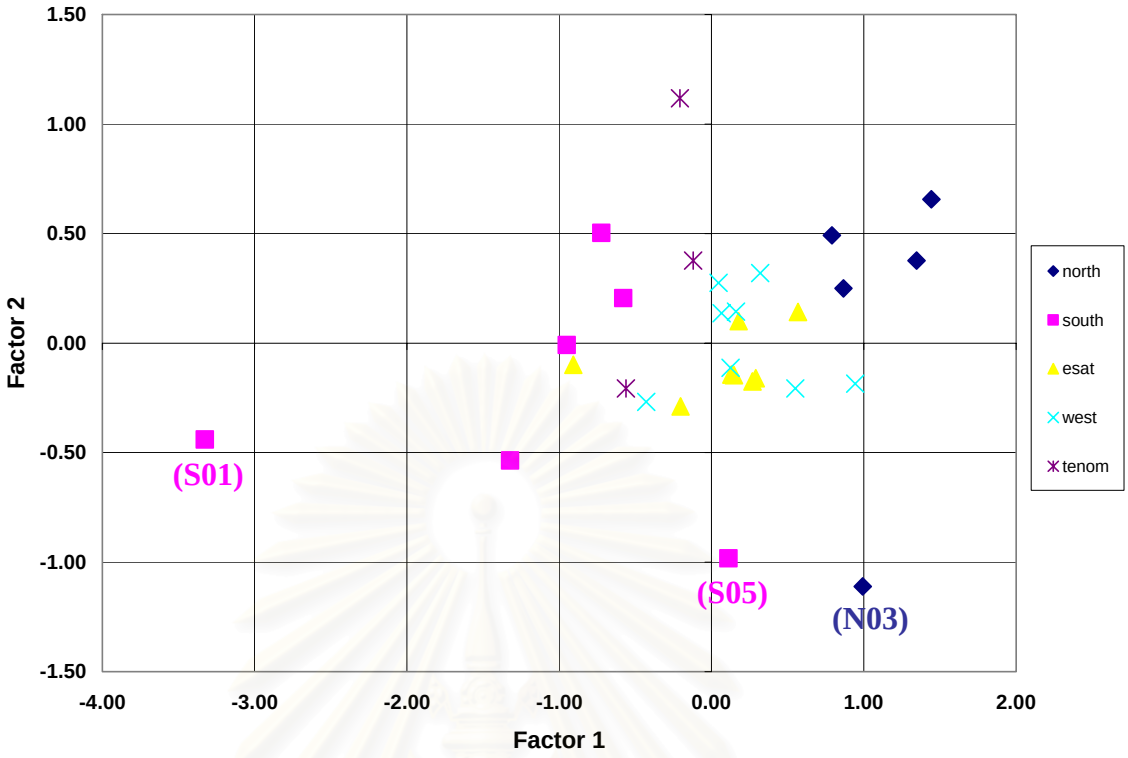

Figure 19. Position of *A. andreniformis* in Thailand and Tenom, Malaysia. Factor axes were derived from factor analysis of morphometric analysis: ordinate; factor 1 and abscissa; factor 2.

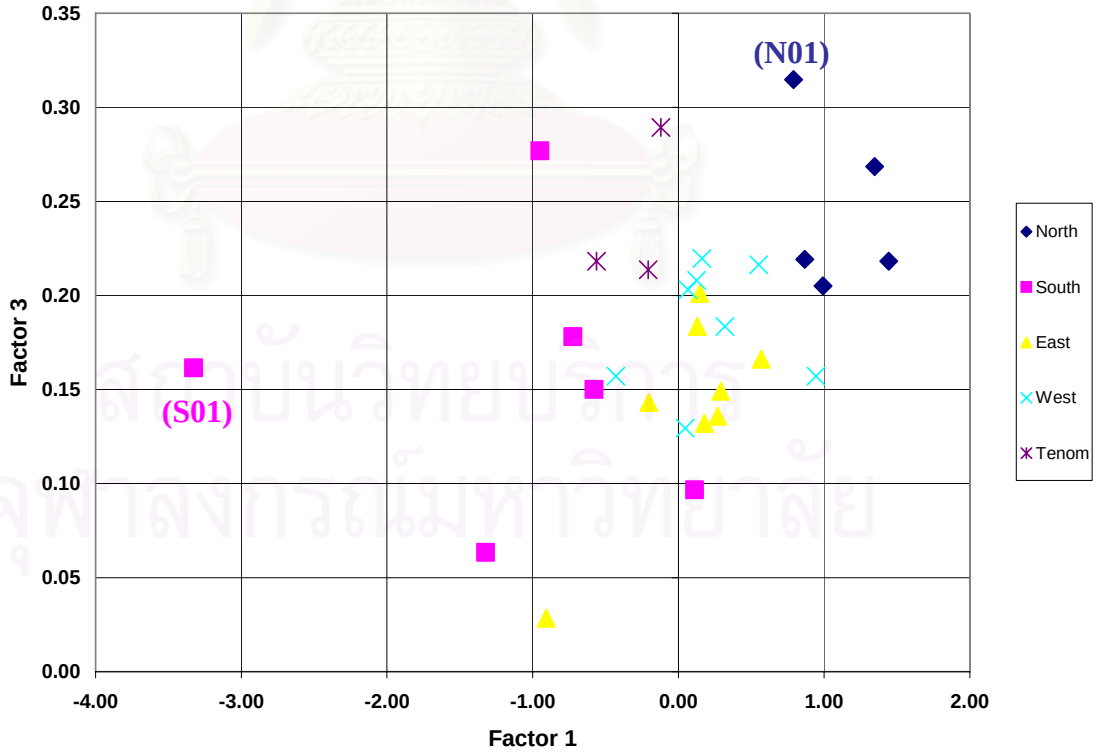

Figure 20. Position of *A. andreniformis* in Thailand and Tenom, Malaysia. Factor axes were derived from factor analysis of morphometric analysis: ordinate; factor 1 and abscissa; factor 3.

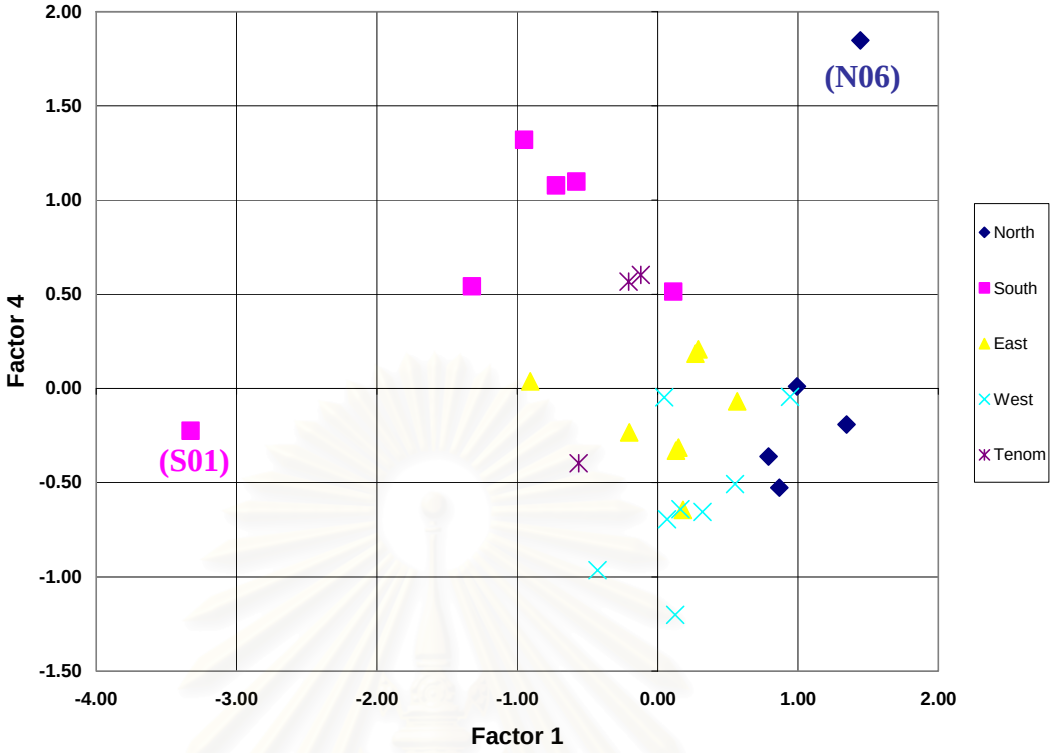

Figure 21. Position of *A. andreniformis* in Thailand and Tenom, Malaysia. Factor axes were derived from factor analysis of morphometric analysis: ordinate; factor 1 and abscissa; factor 4.

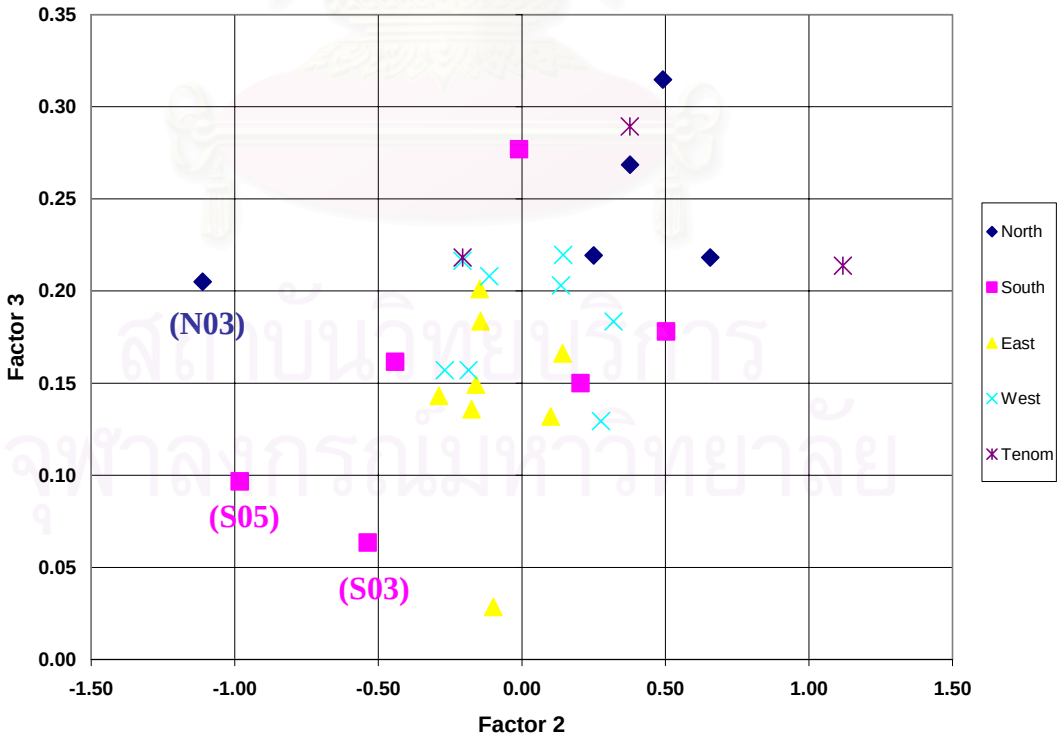

Figure 22. Position of *A. andreniformis* in Thailand and Tenom, Malaysia. Factor axes were derived from factor analysis of morphometric analysis: ordinate; factor 2 and abscissa; factor 3.

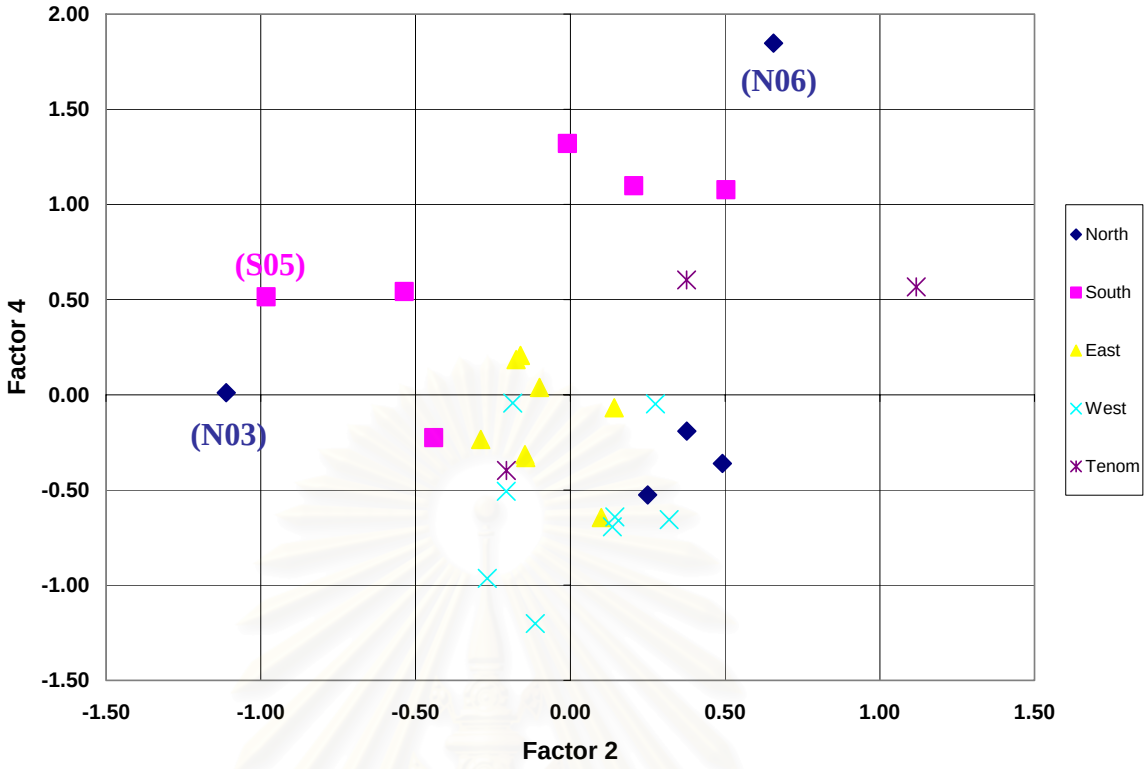

Figure 23. Position of *A. andreniformis* in Thailand and Tenom, Malaysia. Factor axes were derived from factor analysis of morphometric analysis: ordinate; factor 2 and abscissa; factor 4.

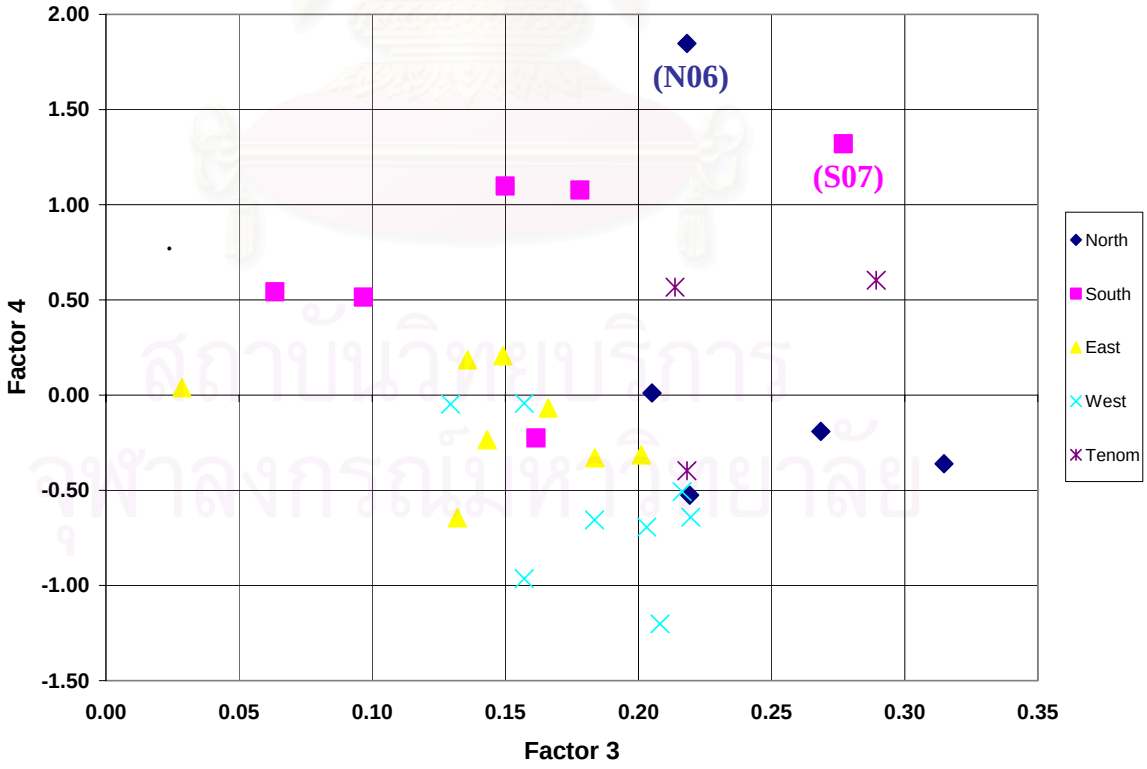

Figure 24. Position of *A. andreniformis* in Thailand and Tenom, Malaysia. Factor axes were derived from factor analysis of morphometric analysis: ordinate; factor 3 and abscissa; factor 4.

4.1.2 Cluster analysis

Figure 25 shows a dendrogram constructed by a cluster analysis of the squeared euclidian distances between means of factor scores. The factor scores were from bees classified by collectable localities. In addition, figure 26 shows a dendrogram of bees grouped by main localities and based on the north and the south 12°N latitude of Thailand, respectively. All 2 dendrograms revealed that these *A. andreniformis* can be clustered into 2 groups. It indicates that 29 colonies were separated into the 1st group while only 1 colony from Kanchanaburi was separated into the 2nd group.

4.1.3 Clinal patterns in the characteristic of *A. andreniformis* in Thailand

To explore clinal patterns in the characteristics of *A. andreniformis*, factor scores were plotted against latitude and longitude. Gradual transitions of characters from the south to the north and the west to the east are indicated in the graph (figure 27-34). Result of linear regression analyses of factor scores against latitude and longitude are summarized in Table 1. A distinct and highly significant slope ($P \leq 0.005$) is observed in latitude for both factor 1 and 4. In addition, the significance ($P \leq 0.025$) is obvious for factor 2. No significance for factor 3 is calculated. A significant slope ($P \leq 0.005$) is observed in longitude for both factor 1 and 4 while there is no significance for factor 2 and 3. According to these results, HWL, HWW, TG4L, TG4W, ST3W, ST3WL, ST4W, ST6W, ST6WW, ANL, TBL, FML, BSTL, FWL, LFW, RFWL, and TBW of *A. andreniformis* increase in size from the south to the north of Thailand. Moreover, HWL, HWW, TG4L, TG4W, ST3W, ST3WL, ST4W, ST6W, ST6WW, ANL, TBL, FML, BSTL, TBW of these bees decrease in size from the west to the east of Thailand.

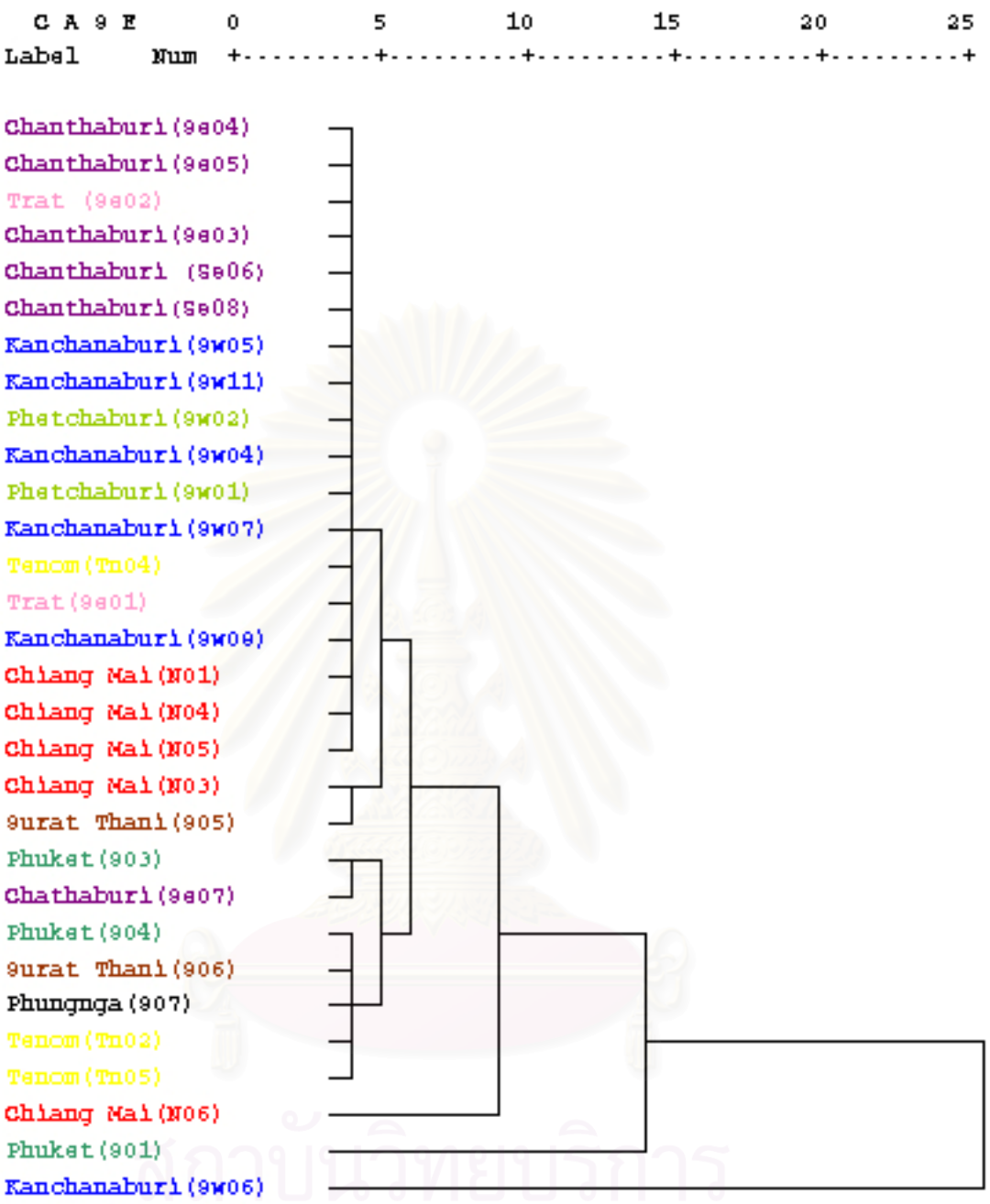

Figure 25. A dendrogram constructed by a cluster analysis. *A. andreniformis* is classified by collection localities.

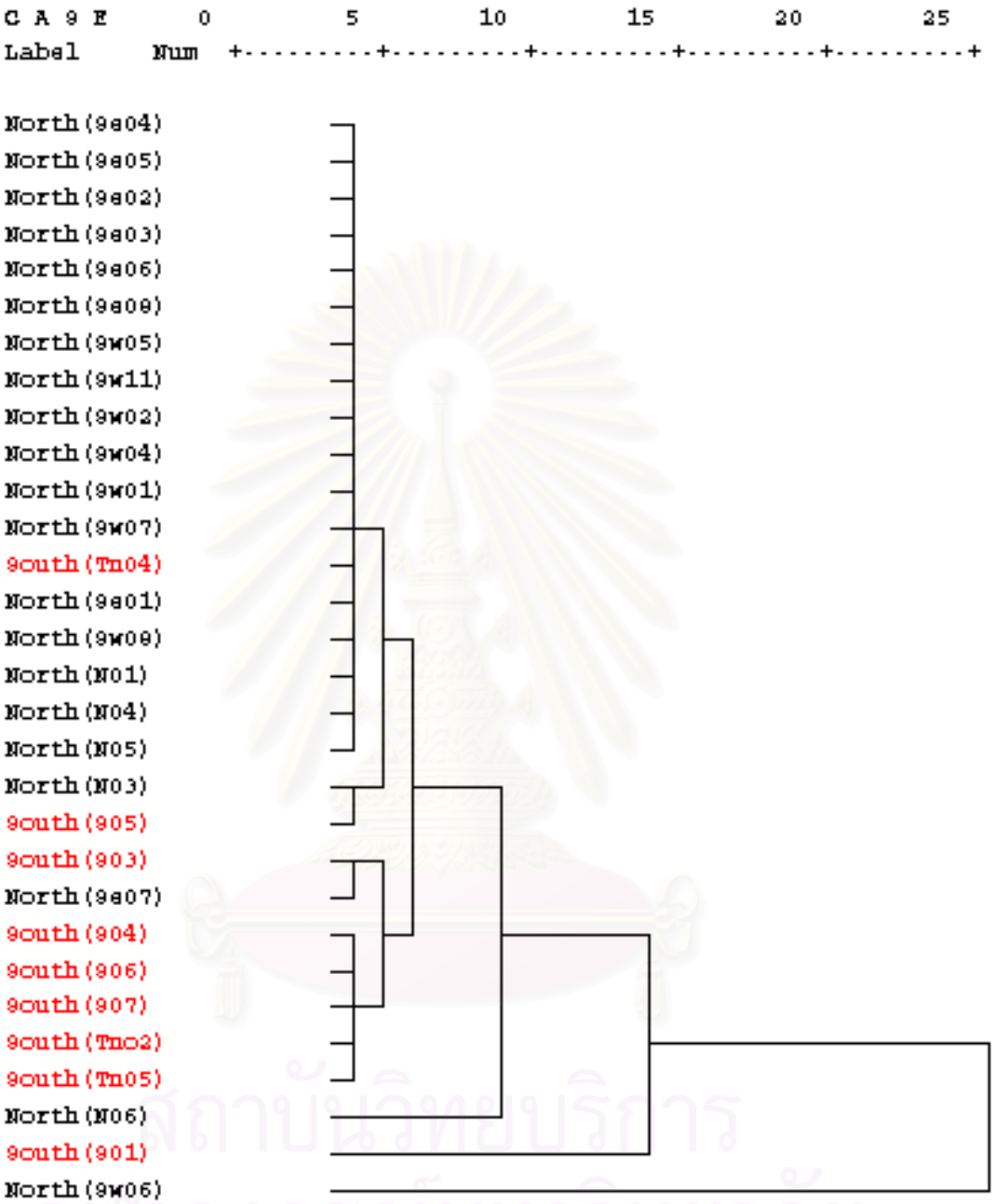

Figure 26. A dendrogram constructed by a cluster analysis. *A. andreniformis* were classified into the north and the south by the north and the south 12°N latitude.

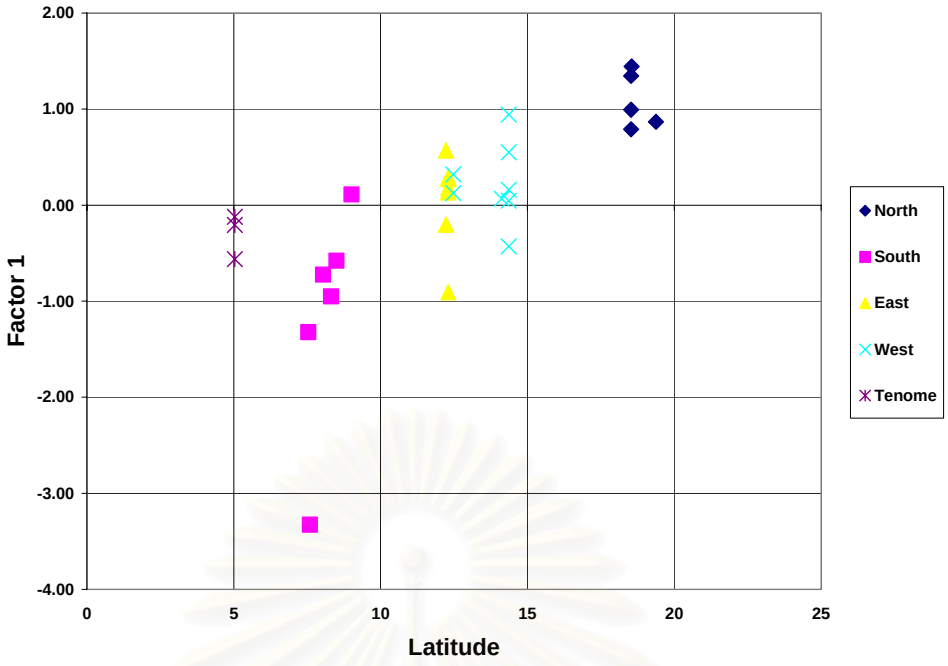

Figure 27. Geographic trends in morphometric characters of *A. andreniformis* in Thailand and Tenom, Malaysia: abscissa; latitude and ordinate; factor score 1 as derived from PCA. Value labels refer to major sampling localities.

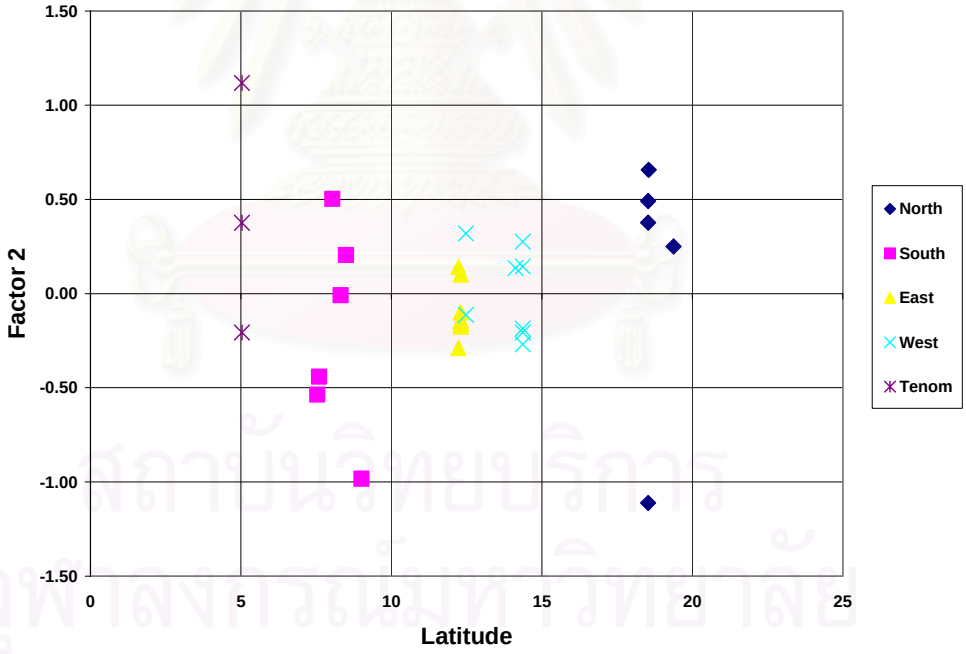

Figure 28. Geographic trends in morphometric characters of *A. andreniformis* in Thailand and Tenom, Malaysia: abscissa; latitude and ordinate; factor score 2 as derived from PCA. Value labels refer to major sampling localities.

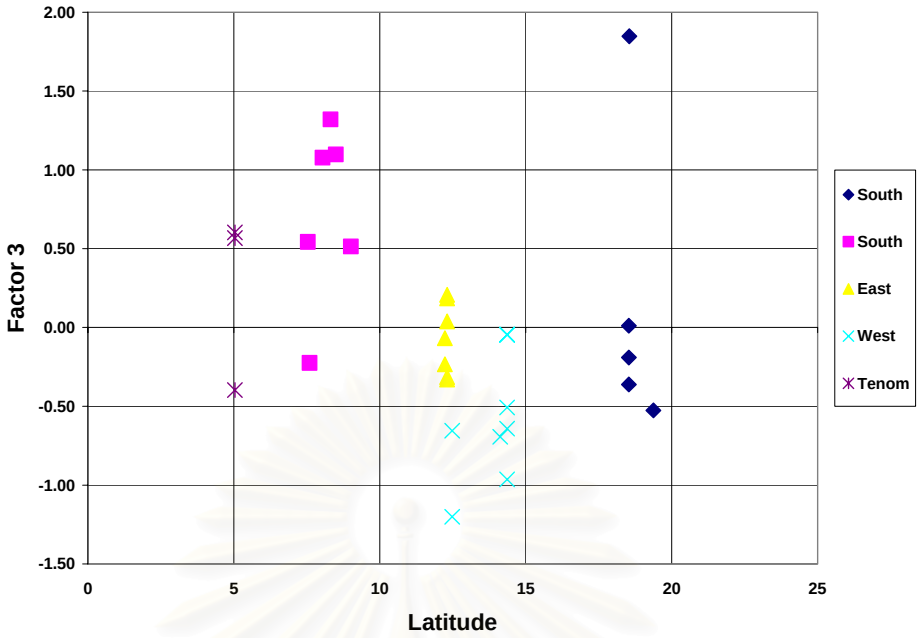

Figure 29. Geographic trends in morphometric characters of *A. andreniformis* in Thailand and Tenom, Malaysia: abscissa; latitude and ordinate; factor score 3 as derived from PCA. Value labels refer to major sampling localities.

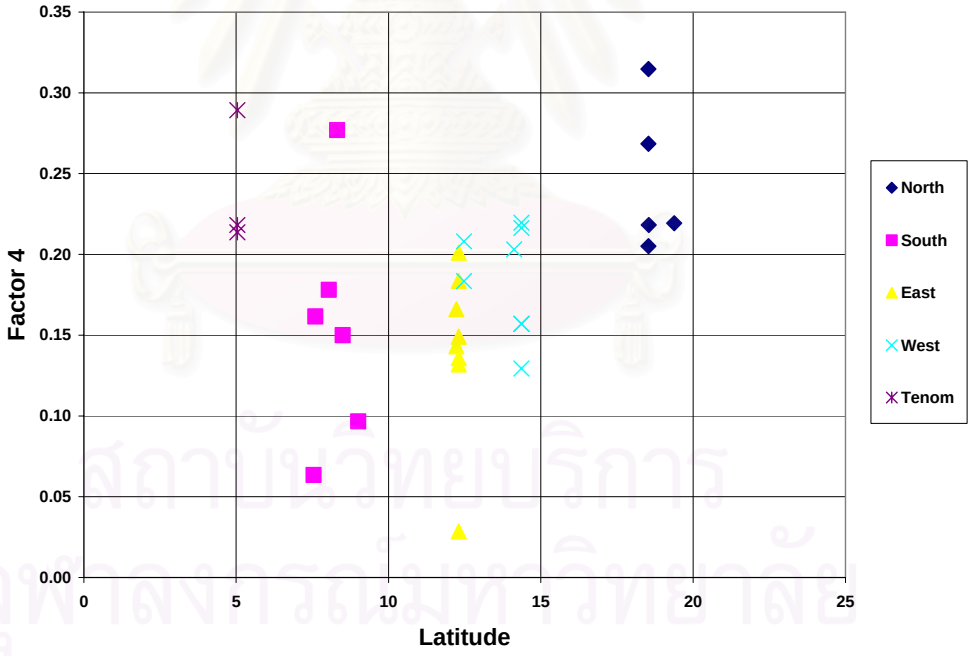

Figure 30. Geographic trends in morphometric characters of *A. andreniformis* in Thailand and Tenom, Malaysia: abscissa; latitude and ordinate; factor score 4 as derived from PCA. Value labels refer to major sampling localities.

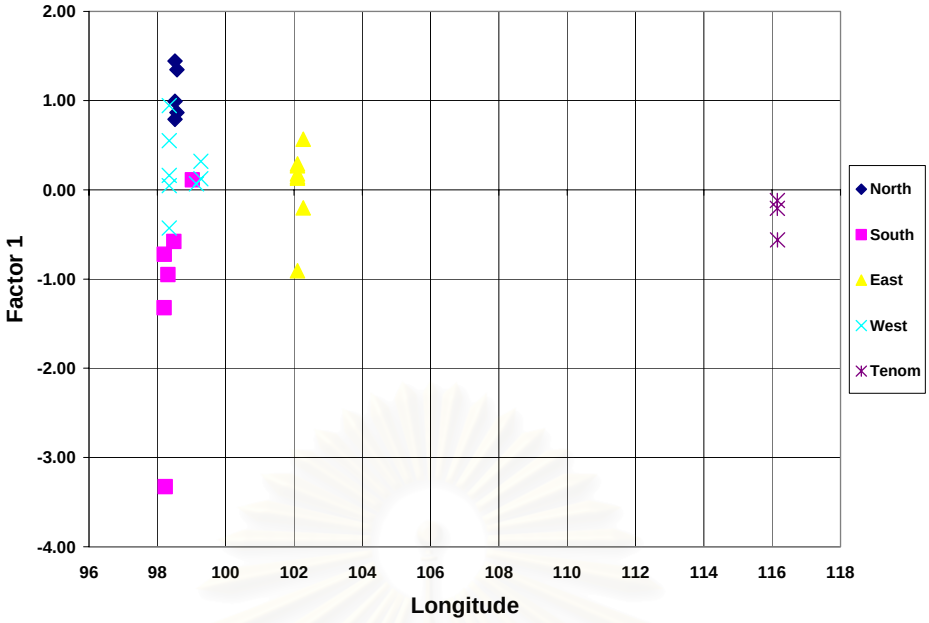

Figure 31. Geographic trends in morphometric characters of *A. andreniformis* in Thailand and Tenom, Malaysia: abscissa; longitude and ordinate; factor score 1 as derived from PCA. Value labels refer to major sampling localities.

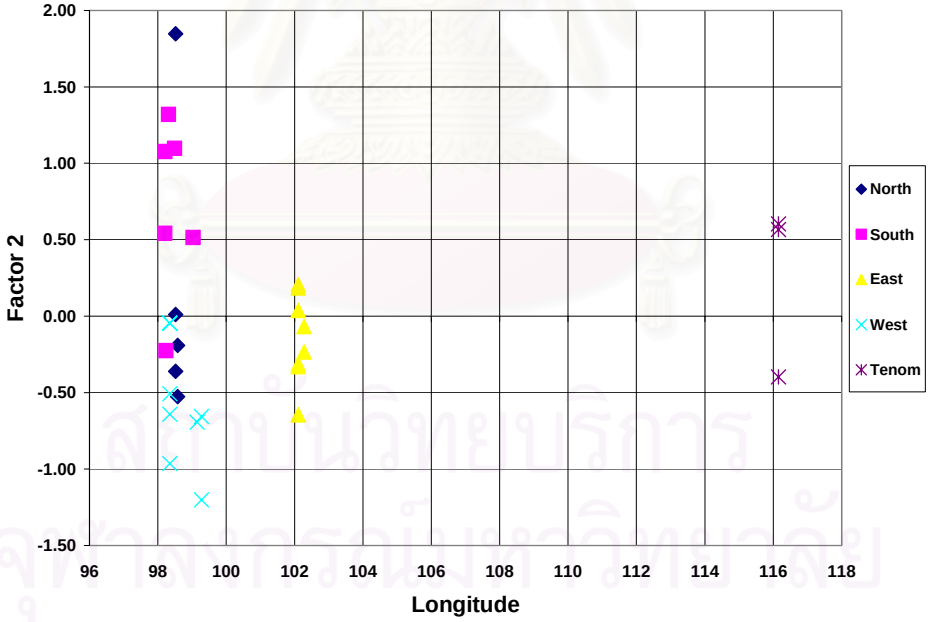

Figure 32. Geographic trends in morphometric characters of *A. andreniformis* in Thailand and Tenom, Malaysia: abscissa; longitude and ordinate; factor score 2 as derived from PCA. Value labels refer to major sampling localities.

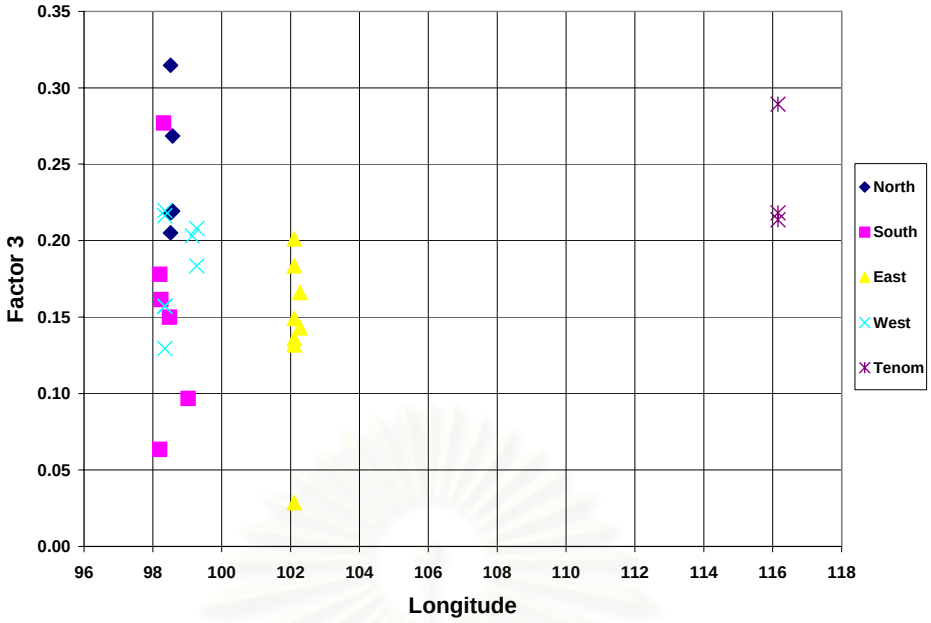

Figure 33. Geographic trends in morphometric characters of *A. andreniformis* in Thailand and Tenom, Malaysia: abscissa; longitude and ordinate; factor score 3 as derived from PCA. Value labels refer to major sampling localities.

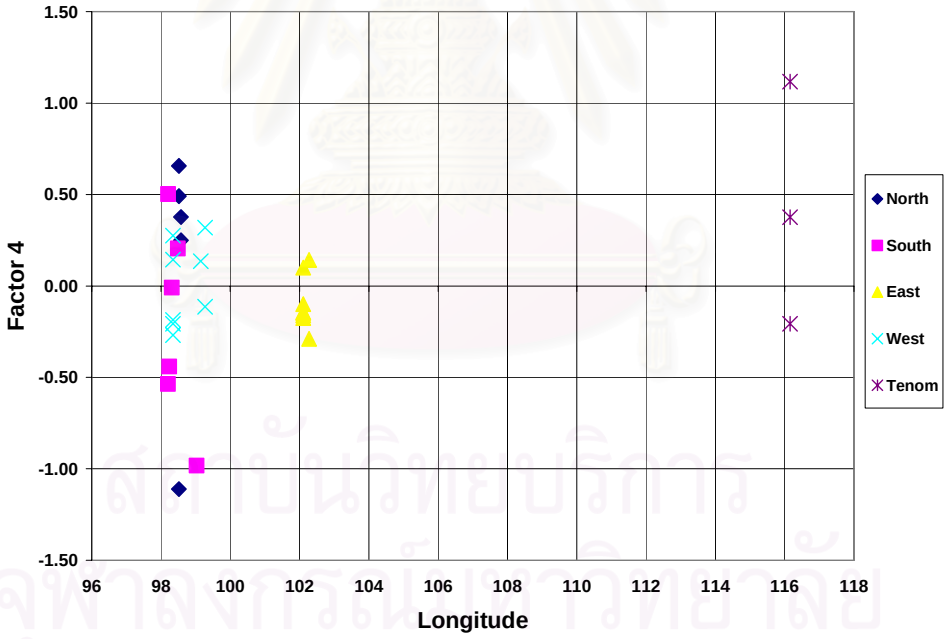

Figure 34. Geographic trends in morphometric characters of *A. andreniformis* in Thailand and Tenom, Malaysia: abscissa; longitude and ordinate; factor score 4 as derived from PCA. Value labels refer to major sampling localities.

Table 1. Linear regression of geographic trends in morphometric characters of *A. andreniformis* from Thailand derived from principal component analysis.

Predictor	Dependent variable	R value	P Significance
Latitude	Factor 1	0.717	0.005
	Factor 2	0.096	0.025
	Factor 3	0.051	0.238
	Factor 4	0.199	0.005
Longitude	Factor 1	0.180	0.005
	Factor 2	0.002	0.972
	Factor 3	0.052	0.232
	Factor 4	0.224	0.005

4.2 Genetic variation analysis

4.2.1 DNA extraction

Genomic DNA of an *A. andreniformis* thorax (30 mg) was extracted by QIAamp® DNA mini kit (Qiagen). Good quality of genomic DNA is determined by sharp and high molecular weight (MW) band on agarose gel. High MW of genomic DNA (about 23 kb in length) is presented (figure 35). Concentration of extracted DNA was estimated by comparing an intensity to bands of λ *Hin*d III DNA as standard marker on agarose gel. Usually, extracted DNA at about 25 ng/μl was obtained per 30 mg tissue.

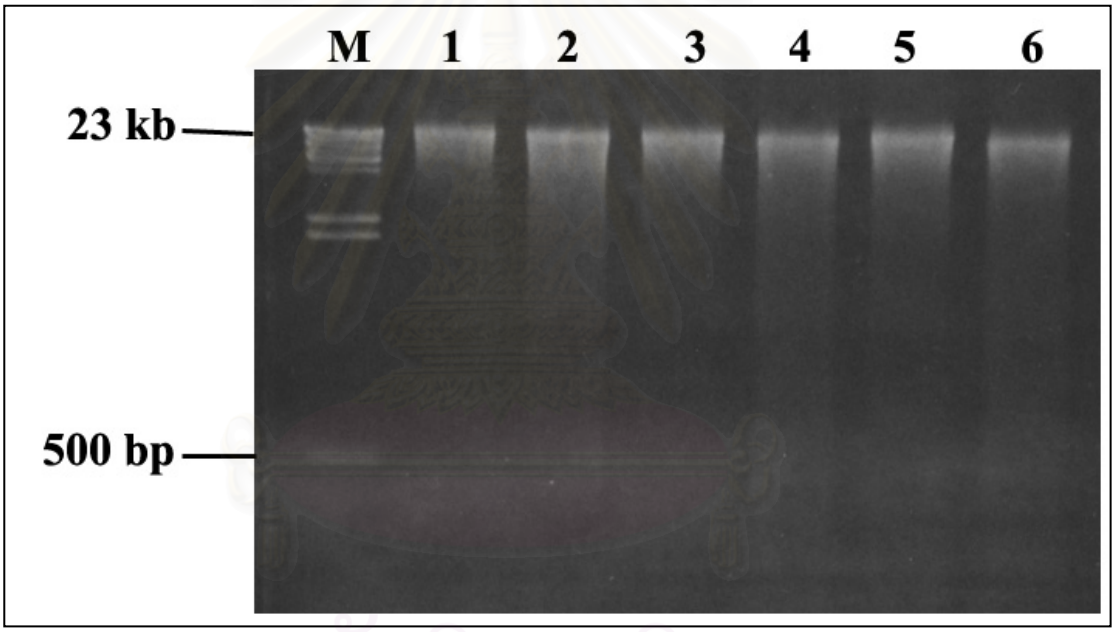

Figure 35. High MW DNA of *A. andreniformis* extracted from thoraxes. On 0.8% agarose gel electrophoresis and EtBr staining, lanes 1-6 indicate individual genomic DNA while lane M represents *λ Hin*d III as standard DNA marker.

4.2.2 PCR amplification

PCR is a technique for *in vitro* DNA amplification of specific sequence by simultaneous primer extension of complementary stand of DNA. After electrophoresis on 1.0% agarose gel and EtBr staining, PCR product was visible under UV light. Size of the product was estimated by comparing to 100 bp DNA ladder. Due to primer design, expected PCR products amplified by *ND4* and *cytb* primers were 540 bp and 520 bp, respectively. Under optimum condition as in Materials and Methods, only single band of 520 bp product was obtained by *cytb* amplification while double bands of PCR products (540 and ~550 bp) were obtained by *ND4* amplification (figures 36 - 37). Thus, PCR products by *cytb* were chosen for restriction and DNA analysis.

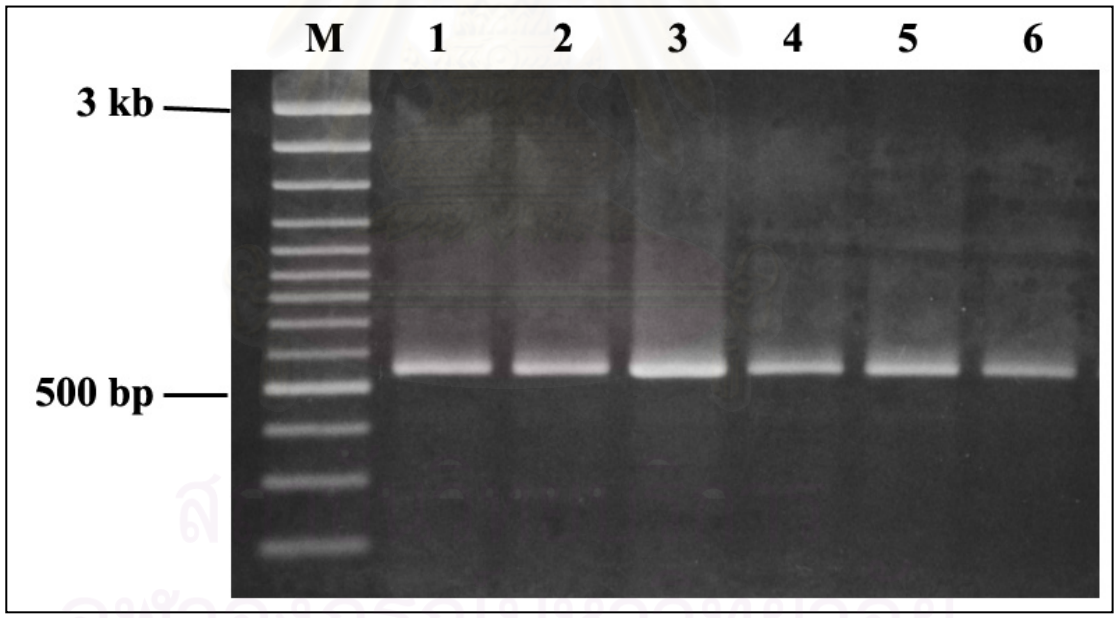

Figure 36. PCR products of *cytb* on 1.5% agarose gel. Lane 1 contains the product of bees from the north. Lanes 2 and 3 contain the products of bees from the east while lanes 4 and 5 contain the products of bees from the west. Furthermore, lane 6 contains the products of bees from Tenom, Malaysia. Lane M represents 100bp ladder as DNA marker.

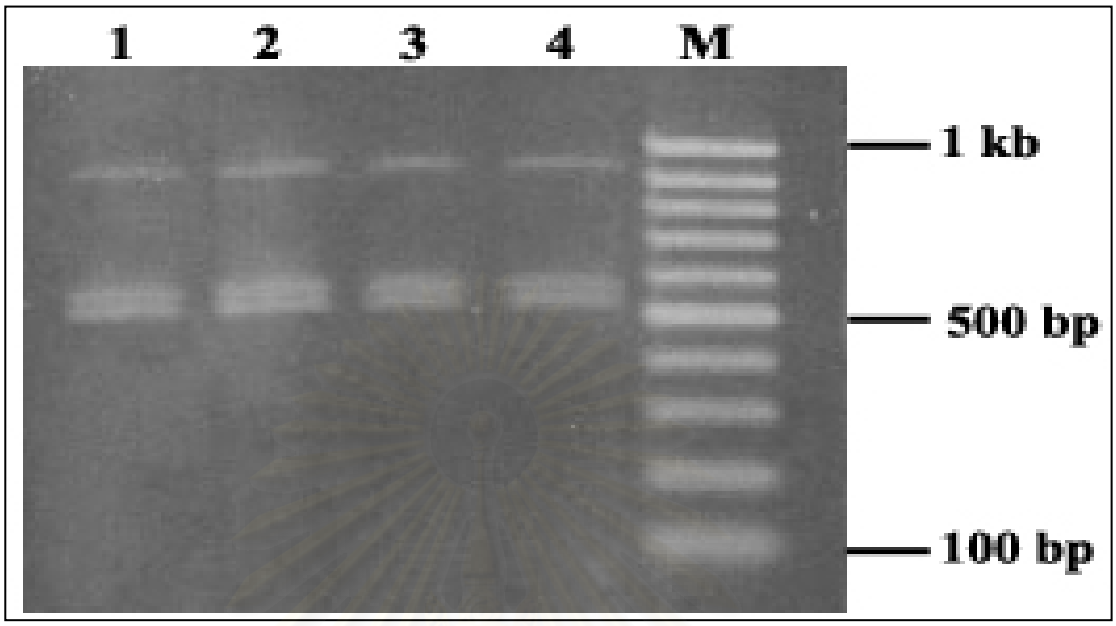

Figure 37. PCR products of *ND4* on 1.5% agarose gel. Lane 1 contains the product of bees from the north. Lanes 2 and 3 contain the products of bees from the east and the west. In addition, lane 4 contains the product of bees from Tenom, Malaysia. Lane M represents 100bp ladder as DNA marker

4.2.3 Restriction analysis

The obtained DNA sequence after *cytb* amplification (at 400 bp) was digested by *Alu*I and *Dra*I restriction endonucleases. Restriction by *Alu*I resulted in 6 different haplotypes (Figure 38). Haplotype 1 (H1) is from bees in Chaing Mai, Chantaburi, Trat, Kanchanaburi, Phetchaburi, Phuket, Pungnga, and Tenom, Malaysia while haplotype 2, 3, and 4 (H2, H3, and H4) is from bees in the southern part of Thailand which are Phuket Island and Surat Thani province, respectively. Moreover, haplotype 5 (H5) is from bee in Chiang Mai (the northern part of Thailand) and in Surat Thani (the southern part of Thailand). At last, haplotype 6 (H6) is found in Chiang Mai province.

```
        10         20         30         40         50         60
ATCTATACAT TATTGTCCTA ATATTGATAT TGCATTTTGA TCAATTACAA ATATTATAAA

        70         80         90        100        110        120
AGATATAAAT TCAGGATGAT TGTTTCGATT AATTCATATA AATGGAGCTT CATTTTATTT

       130        140        150        160        170        180
TTTAATTATA TATATTCATA TTAGACGAAA TATATTTTAT AATTCATTTA AATTAAATAG

       190        200        210        220        230        240
AGTATGAGGA ATTGGAATTT TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA

       250        260        270        280        290        300
TGTTCTTCCA TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC

       310        320        330        340        350        360
AGCTATTCCT TATATTGGAG ATACAGTAGT TCTTTGAATT TGAGGTGGAT TTTCAATTAA

       370        380        390        400
TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
```

Haplotype 1

```
        10          20          30          40          50          60
ATCTCTACAT TATTGTCCTA ATATTGATGT TGCATTTTGA TCAATTGCAA ATATTATAAA

        70          80          90         100         110         120
TGATATTCCT TCTGGATGAT TGTGTCGATT AGTTCCTCCA AATGGAGGTA CATTTTATTT

       130         140         150         160         170         180
TTTAATTATA TATATTGATA CTCCACGAAA TATATTTTAT ACCTCATTTA AATTCAATAG

       190         200         210         220         230         240
CGTATGAGGA ATTGGAATTT TAATTTTATT AATTTCTATG GCAGCTGCAC TTATAGGATA

       250         260         270         280         290         300
TGTTCTTCCT GGAGGACAAA AATCATTTTG AGGAGCAACA GTTATTACAA ATTTATTATC

       310         320         330         340         350         360
AGCTGATCCT CCTTTTGGAG AAACAGAAGC ACTCTGATTT CCAGGAGGAT TTTCTATTAA

       370         380         390         400
TAATGCTGCT TTTGATCGAA TTGTTTCGAC TCATTTTG
```

Haplotype 2

```
        10          20          30          40          50          60
ATCTCTACGT TGTTGTCCTA ATATTGATGT TGCATTTTGA TCAATTGCAA ATATTATAAA

        70          80          90         100         110         120
AGATATAAAT TCAGGATGAT TGTTTCGATC AGTTCCTCCA AATGGAGGTT CATTTTATTT

       130         140         150         160         170         180
TTTAATTGTA TATACTCATA CTCCACGAAA TATATTTTAT ACCTCATTTA AATTAAATAC

       190         200         210         220         230         240
CGTATGAGGA ATTGGAATTT TAATTTTATT AATTTCTATG GCAGCTCCAC TTATAGGATA

       250         260         270         280         290         300
TGTTCTTCCT TGAGGACAAA AATCATTTTG AGGAGCAACA GTTATTACAA ATTTATTATC

       310         320         330         340         350         360
AGCTGTTCCT CCTTTTGGAG AAACAGAAGC ACTCTGATTT CCAGGAGGAT TTTCTATTAA

       370         380         390         400
TAAAGCTGCT TTTGATCGAA TTGTTTCTAC TCATTTTG
```

Haplotype 3

```
        10          20          30          40          50          60
ATCTATACAT TATTGTCCTA ATATTCATAT TGCATTTTGA TCAATTACAA ATATTATAAA

        70          80          90         100         110         120
AGATATTACT TCAGGATGAT TGGTTCGATT AATTCATATA AATGGAGCTT CGTTTTATTT

       130         140         150         160         170         180
TTTAATTATA TATATTGATA TTAGACGAAA TATATTTTAT AATTCATTTA AATTAAATAG

       190         200         210         220         230         240
AGTATGAGGA ATTGGAATTT TAATTTTATT AATTTCTATG GCAGCTGCAC TTATAGGATA

       250         260         270         280         290         300
TGTTCTTCCA TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC

       310         320         330         340         350         360
AGCTAATCCT CATATTGGAG AAACAGTAGT TCCTTGCATT CGAGGTGGAT TTTCAATTAA

       370         380         390         400
TAATGCTACT GTGATTCGAA TTGTTTCTAT TCATTTTG
```

Haplotype 4

```
        10          20          30          40          50          60
ATCTATACAT TATTGTCCTA ATATTCATAT TGCATTTTGA TCAATTACAA ATATTATAAA

        70          80          90         100         110         120
AGATATTCCT TCAGGATGAT TGGTTCGATT AATTCATATA AATGGAGCTT CATTTTATTT

       130         140         150         160         170         180
TTTAATTATA TATATTCATA TTACACGAAA TATATTTTAT AATTCATTTA AATTAAATAG

       190         200         210         220         230         240
AGTATGAGGA ATTGGAATTT TAATTTTATT AATTTCTATG GCAGCTCCAC TTATAGGATA

       250         260         270         280         290         300
TGTTCTTCCT GGAGGACAAA TATCATTTTG AGGAGCAACA GTTATTACAA ATTTATTATC

       310         320         330         340         350         360
AGCTGATCCT CCTTTTGGAG AAACAGAAGC TCCAAGCATT CGAGGTGGAT TTTCTATTAA

       370         380         390         400
TAAAGCTGCT GTGATTCGAA TTGTTTCCAC TCATTTTG
```

Haplotype 5

```
        10         20         30         40         50         60
ATCTATACGT TGTTGTCCTA ATATTGATAT TGCATTTTGA TCAATTGCAA ATATTATAAA

        70         80         90        100        110        120
AGATATAACT TCAGGATGAT TGTTTCGATC AGTTCCTATA AATGGAGCTT CATTTTATTT

       130        140        150        160        170        180
TTTAATTATA TATATTCATA GCTGACGAAA TATATTTTAT ACCTCATTTA AATTCAATAG

       190        200        210        220        230        240
AGTATGAGGA ATTGGAATTT TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA

       250        260        270        280        290        300
TGTTCTTCCA TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC

       310        320        330        340        350        360
AGCTGTTCCT TCTATTGGAG ATACAGAAGT TCTTTGAATT TGAGGTGGAT TTTCAATTAA

       370        380        390        400
TAATGCTGCT TTAGATCGAT TTGTTTCTAT TCATTTTA
```

Haplotype 6

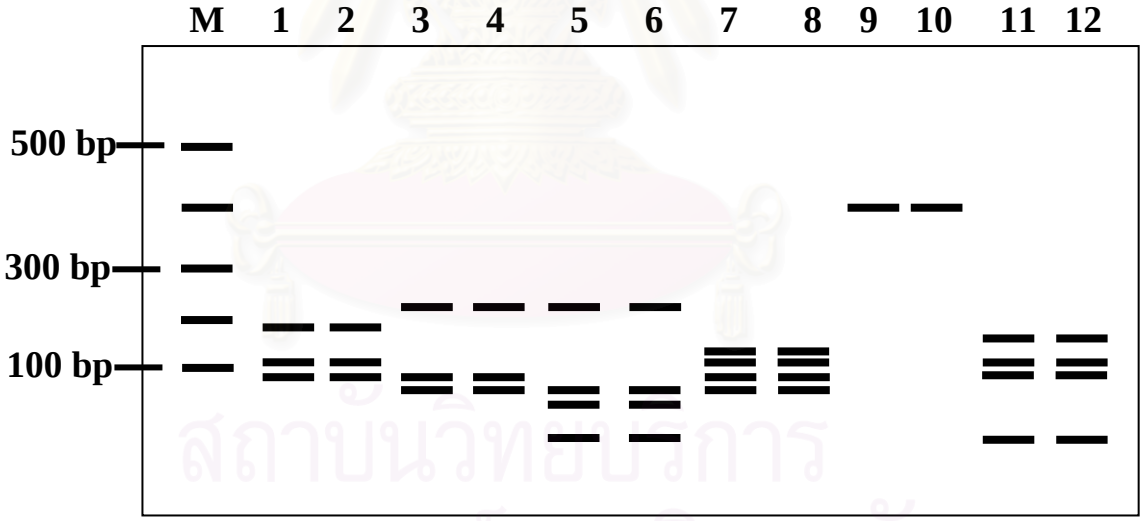

Figure 38. Restriction patterns of the amplified *cytb* gene of *A. andreniformis* digested with *Alu*I. Six mtDNA haplotypes of *A. andreniformis* were observed (H1, lanes 1-2; H2, lanes 3-4; H3, lanes 5-6; H4, lanes 7-8; H5, lanes 9-10; and H6, lanes 11-12). Lane M is 100 bp DNA ladder.

Three restriction patterns of amplified *cytb* of *A. andreniformis* in Thailand and Tenom, Malaysia after *Dra*I digestion were observed (Figure 39). Haplotype 1 (H1) is from bees in Chaing Mai, Chantaburi, Trat, Kanchanaburi, Phetchaburi, Phuket, Pungnga, and Tenom, Malaysia while haplotype 2 (H2) is present in Chaing Mai, Chantaburi, Phetchaburi, Phuket Island, and Surat Thani. Moreover, haplotype 3 (H) is only found in Chiang Mai province (the northern part of Thailand).

Haplotype 1

```
        10          20          30          40          50          60
ATCTCTACAT TATTGTCCTA ATATTGATGT TGCATTTTGA TCAATTGCAA ATATTATAAA

        70          80          90         100         110         120
TGATATTCCT TCTGGATGAT TGTGTCGATT AGTTCCTCCA AATGGAGGTA CATTTTATTT

       130         140         150         160         170         180
TTTAATTATA TATATTGATA CTCCACGAAA TATATTTTAT ACCTCATTTA AATTCAATAG

       190         200         210         220         230         240
CGTATGAGGA ATTGGAATTT TAATTTTATT AATTTCTATG GCAGCTGCAC TTATAGGATA

       250         260         270         280         290         300
TGTTCTTCCT GGAGGACAAA AATCATTTTG AGGAGCAACA GTTATTACAA ATTTATTATC

       310         320         330         340         350         360
AGCTGATCCT CCTTTTGGAG AAACAGAAGC ACTCTGATTT CCAGGAGGAT TTTCTATTAA

       370         380         390         400
TAATGCTGCT TTTGATCGAA TTGTTTCGAC TCATTTTG
```

Haplotype 2

```
        10          20          30          40          50          60
ATCTATACGT GGTTGTCCTA ATTTTGATGT TGCATTTTGA TCAATTGCAA ATATTATAAA

        70          80          90         100         110         120
AGATATAAAT TCAGGATGAC TGTGTCGATC AGTTCCTCCA AATGGAGCTA CATTTGATTT

       130         140         150         160         170         180
TTTAATTGTA TATACTCATA GCTCGCGAAA TATATTTTAG ACCTCATGTA AATTCAATAC

       190         200         210         220         230         240
CGTATGAGGA ATTGGAATTT TAATTTTATT AATTTCTATG GCAGCTCCAC TTATAGGATA

       250         260         270         280         290         300
TGTTCTTCCA GGAGGACAAA TATCATTTTG AGGAGCAACA GTTATTACAA ATTTATTATC

       310         320         330         340         350         360
AGCTGTTCCT CCTTTTGGAG ATACAGAAGT TCTCTGACTT CCAGGAGGAT TTTCAATTAA

       370         380         390         400         410         420
TAATGCTGCT TTAGATCGAT TTGTTTCTAC TCATTTTA
```

Haplotype 3

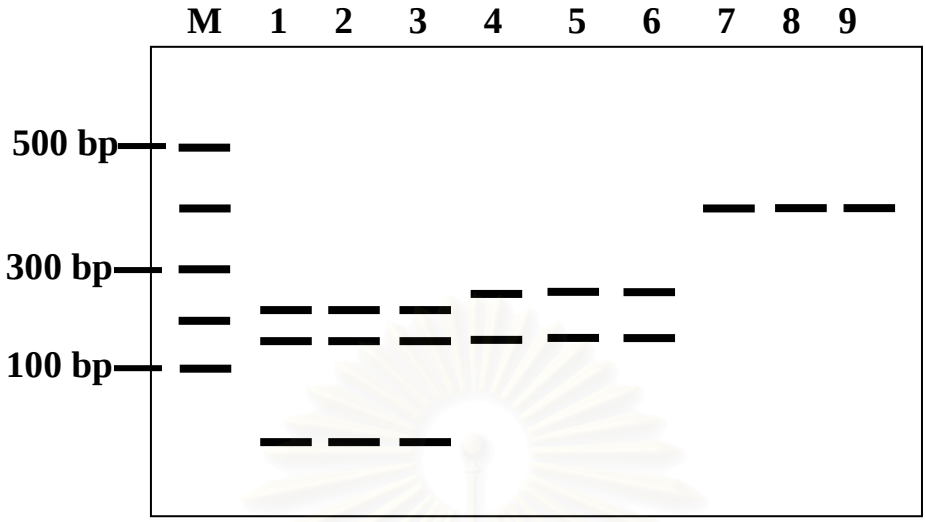

Figure 39. Restriction patterns of the amplified *cytb* gene of *A. andreniformis* digested by *Dra* I. Three mtDNA haplotypes of *A. andreniformis* were observed (H1, lanes 1-3; H2, lanes 4-6; and H3, lanes 7-9). Lane M is 100 bp DNA ladder.

4.2.4 Sequence analysis

PCR products of *cytb* of *A. andreniformis* from all collecting localities in Thailand and Tenom, Sabha, Malaysia were purified and sequenced. The obtained sequence length ranged from 520 to 530 bp. They contain high A+T content with the average of 75.61% (Table 2). The data coincide to a previous report about the whole mtDNA of *A. mellifera* (Crozier and Crozier, 1993). More transitional and transversional events also occur in *A. andreniformis* and other organisms. The similarities in pair of these sequences are 86-100% (Table 3). Pairwise and multi-alignment sequence comparisons revealed nucleotide variation in the form of single base pair substitution. The substitutions can be counted for 73 nucleotide sites (18.25%): 25 sites (34.25%) were transition and 48 sites (65.75%) were transversion (Figure 42). The frequency of A↔G and T↔C transition were 15.07% and 16 44%, respectively. Besides, the frequency of A↔T, A↔C, G↔T, and G↔C transversion were 27.40%, 15.07%, 13.70%, and 9.59%, respectively. The sequence divergence of these sequences is varied from 0-14.32% (Table 4). The mean of sequence divergence among bees from Thailand is 5.70%. The means of sequence divergence within and between groups of bees are shown in Table 5. Considering bees in Thailand, the bees from the west and the east showed lower means of sequence divergence within group, 0.80% and 0.916%, respectively. However, higher mean of sequence divergence within group of bees from the south of Thailand (8.81%) is observed. The mean of sequence divergence between groups of the western and the eastern Thailand is lower (0.96%) as in Table 5. It indicates that bees from both 2 regions are highly related to each other.

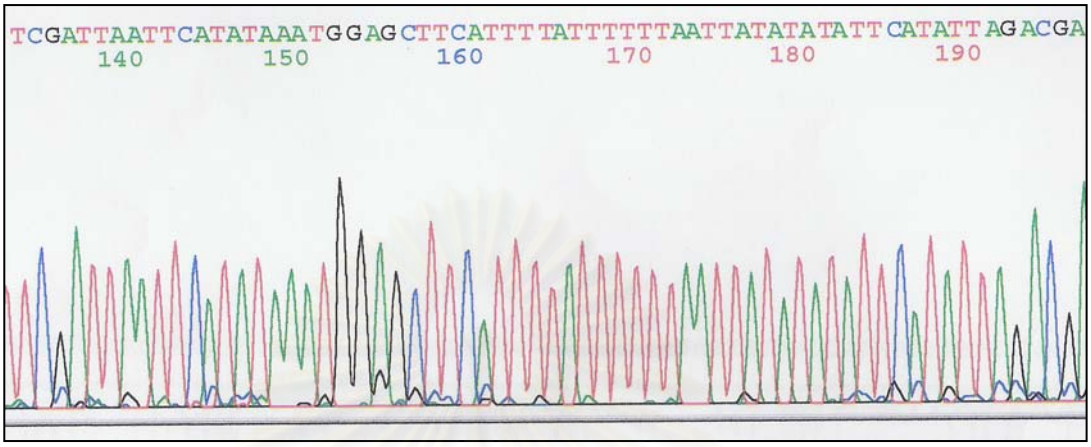

Figure 40. Four colored electropherogram of *cyt*b sequence of *A. andreniformis.* Red peaks indicate Thymine (T). Green peaks show Adenine (A). Blue presents Cytocine (C) and black presents Guanine (G).

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                         ....|....| ....|....| ....|....| ....|....| ....|....|
                                10         20         30         40         50
Chaing Mai 5 (N05)       ATCTATACGT TGTTGTCCTA ATATTGATAT TGCATTTTGA TCAATTGCAA
Chaing Mai 6 (N06)       ATCTATACGT GGTTGTCCTA ATTTTGATGT TGCATTTTGA TCAATTGCAA
Phuket 2 (S02)           ATCTCTACAT TATTGTCCTA ATATTGATGT TGCATTTTGA TCAATTGCAA
Phuket 4 (S04)           ATCTCTACGT TGTTGTCCTA ATATTGATAT TGCATTTTGA TCAATTGCAA
Phuket 1 (S01)           ATCTCTACAT GATTGTCCTA ATATTGATAT TGCATTTTGA TCAATTGCAA
Chaing Mai 4 (N04)       ATCTCTACGT TGTTGTCCTA ATATTGATAT TGCATTTTGA TCAATTGCAA
Kanchanaburi 2 (SW05)    ATCTATACAT TATTGTCCTA ATCTTGATAT TGCATTTTGA TCAATTACAA
Surat Thani 2 (S06)      ATCTATACAT TATTGTCCTA ATATTCATAT TGCATTTTGA TCAATTACAA
Tenom 2 (Tn02)           ATCTATACAT TATTGTCCTA ATATTGATAT TGCATTTTGA TCAATTACAA
Phetchaburi 1 (SW01)     ATCTATACAT TATTGTCCTA ATATTGATAT TGCATTTTGA TCAATTACAA
Tenom 5 (Tn05)           ATCTATACAT TATTGTCCTA ATATTGATAT TGCATTTTGA TCAATTACAA
Tenom 3 (Tn03)           ATCTATACAT TATTGTCCTA ATATTGATAT TGCATTTTGA TCAATTACAA
Tenom 6 (Tn06)           ATCTATACAT TATTGTCCTA ATCTTGATAT TGCATTTTGA TCAATTACAA
Phetchaburi 2 (SW02)     ATCTATACAT TATTGTCCTA ATCTTGATAT TGCATTTTGA TCAATTACAA
Chanthaburi 5 (SE07)     ATCTATACAT TATTGTCCTA ATCTTGATAT TGCATTTTGA TCAATTACAA
Chiang Mai 1 (N01)       ATCTATACAT TATTGTCCTA ATATTGATAT TGCATTTTGA TCAATTACAA
Trat 2 (SE02)            ATCTATACAT TATTGTCCTA ATCTTGATAT TGCATTTTGA TCAATTACAA
Chiang Mai 2 (N02)       ATCTATACAT TATTGTCCTA ATATTGATAT TGCATTTTGA TCAATTACAA
Kanchanaburi 3 (SW06)    ATCTATACAT TATTGTCCTA ATCTTGATAT TGCATTTTGA TCAATTACAA
Kanchanaburi 4 (SW07)    ATCTATACAT TATTGTCCTA ATCTTGATAT TGCATTTTGA TCAATTACAA
Surat Thani 1 (S05)      ATCTATACAT TATTGTCCTA ATATTCATAT TGCATTTTGA TCAATTACAA
Kanchanaburi 1 (SW04)    ATCTATACAT TATTGTCCTA ATCTTGATAT TGCATTTTGA TCAATTACAA
Chanthaburi 6 (SE08)     ATCTATACAT TATTGTCCTA ATCTTGATAT TGCATTTTGA TCAATTACAA
Phetchaburi 3 (SW03)     ATCTATACAT TATTGTCCTA ATATTGATAT TGCATTTTGA TCAATTACAA
Chanthaburi 1 (SE04)     ATCTATACAT TATTGTCCTA ATCTTGATAT TGCATTTTGA TCAATTACAA
Kanchanaburi 5 (SW08)    ATCTATACAT TATTGTCCTA ATATTGATAT TGCATTTTGA TCAATTACAA
Trat 1 (SE01)            ATCTATACAT TATTGTCCTA ATCTTGATAT TGCATTTTGA TCAATTACAA
Chanthaburi 3 (SE05)     ATCTATACAT TATTGTCCTA ATCTTGATAT TGCATTTTGA TCAATTACAA
Chiang Mai 7 (N07)       ATCTATACGT TGTTGTCCTA ATATTGATAT TGCATTTTGA TCAATTGCAA
Pungnga 1 (S07)          ATCTATACAT TATTGTCCTA ATATTGATAT TGCATTTTGA TCAATTACAA
Tenom 4 (Tn04)           ATCTATACAT TATTGTCCTA ATCTTGATAT TGCATTTTGA TCAATTACAA
Kanchanaburi 6 (SW09)    ATCTATACAT TATTGTCCTA ATCTTGATAT TGCATTTTGA TCAATTACAA
Nakhon Ratchasima (E01)ATCTATACAT TATTGTCCTA ATCTTGATAT TGCATTTTGA TCAATTACAA
Chanthaburi 4 (SE06)     ATCTATACAT TATTGTCCTA ATATTGATAT TGCATTTTGA TCAATTACAA
Chanthaburi 1 (SE03)     ATCTATACAT TATTGTCCTA ATCTTGATAT TGCATTTTGA TCAATTACAA
Chanthaburi 7 (SE09)     ATCTATACAT TATTGTCCTA ATCTTGATAT TGCATTTTGA TCAATTACAA
Phuket 3 (S03)           ATCTCTACGT TGTTGTCCTA ATATTGATGT TGCATTTTGA TCAATTGCAA
Clustal Co               **** *** *   ******** ** ** ** * ********** ****** ***
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Figure 41. A 400 bp character matrix of 37 *A. andreniformis* based on partial *cyt*b of mtDNA sequences. Bee code is based on minor collecting localities. Asterisks * indicate that all samples provide nucleotide identity.

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                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                 60         70         80         90        100
Chaing Mai 5 (N05)        ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATT AGTTCATATA
Chaing Mai 6 (N06)        ATATTATAAA AGATATAAAT TCAGGATGAC TGTGTCGATC AGTTCCTCCA
Phuket 2 (S02)            ATATTATAAA TGATATTCCT TCTGGATGAT TGTGTCGATT AGTTCCTCCA
Phuket 4 (S04)            ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATC AGTTCCTCCA
Phuket 1 (S01)            ATATTATAAA AGATATAACT TCTGGATGAT TGTTTCGATC AATTCCTATA
Chaing Mai 4 (N04)        ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATT AGTTCCTCCA
Kanchanaburi 2 (SW05)     ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATT AATTCATATA
Surat Thani 2 (S06)       ATATTATAAA AGATATTACT TCAGGATGAT TGGTTCGATT AATTCATATA
Tenom 2 (Tn02)            ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATT AATTCATATA
Phetchaburi 1 (SW01)      ATATTATAAA AGATATAAAT TCAGGATGAT TGGTTCGATT AATTCATATA
Tenom 5 (Tn05)            ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATT AATTCATATA
Tenom 3 (Tn03)            ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATT AATTCATATA
Tenom 6 (Tn06)            ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATT AATTCATATA
Phetchaburi 2 (SW02)      ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATT AATTCATATA
Chanthaburi 5 (SE07)      ATATTATAAA AGATATAAAT TCAGGATGAT TATTTCGATT AATTCATATA
Chiang Mai 1 (N01)        ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATT AATTCATATA
Trat 2 (SE02)             ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATT AATTCATATA
Chiang Mai 2 (N02)        ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATT AATTCATATA
Kanchanaburi 3 (SW06)     ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATT AATTCATATA
Kanchanaburi 4 (SW07)     ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATT AATTCATATA
Surat Thani 1 (S05)       ATATTATAAA AGATATTCCT TCAGGATGAT TGGTTCGATT AATTCATATA
Kanchanaburi 1 (SW04)     ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATT AATTCATATA
Chanthaburi 6 (SE08)      ATATTATAAA AGATATAAAT TCAGGATGAT TATTTCGATT AATTCATATA
Phetchaburi 3 (SW03)      ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATT AATTCATATA
Chanthaburi 2 (SE04)      ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATT AATTCATATA
Kanchanaburi 5 (SW08)     ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATT AATTCATATA
Trat 1 (SE01)             ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATT AATTCATATA
Chanthaburi 3 (SE05)      ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATT AATTCATATA
Chiang Mai 7 (N07)        ATATTATAAA AGATATAACT TCAGGATGAT TGTTTCGATC AGTTCCTATA
Pungnga 1 (S07)           ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATT AATTCATATA
Tenom 4 (Tn04)            ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATT AATTCATATA
Kanchanaburi 6 (SW09)     ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATT AATTCATATA
Nakhon Ratchasima(E01)ATATTATAAA AGATATAAAT TCAGGATGAT TATTTCGATT AATTCATATA
Chanthaburi 4 (SE06)      ATATTATAAA AGATATAAAT TCAGGATGAT TATTTCGATT AATTCATATA
Chanthaburi 1 (SE03)      ATATTATAAA AGATATAAAT TCAGGATGAT TATTTCGATT AATTCATATA
Chanthaburi 7 (SE09)      ATATTATAAA AGATATAAAT TCAGGATGAT TATTTCGATT AATTCATATA
Phuket 3 (S03)            ATATTATAAA AGATATAAAT TCAGGATGAT TGTTTCGATC AGTTCCTCCA
Clustal Co                ********** *****   * ** ******  *   *****  * *** *  *
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Figure 41. (continued)

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                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                 110        120        130        140        150
Chaing Mai 5 (N05)        AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTAGGCGAAA
Chaing Mai 6 (N06)        AATGGAGCTA CATTTGATTT TTTAATTGTA TATACTCATA GCTCGCGAAA
Phuket 2 (S02)            AATGGAGGTA CATTTTATTT TTTAATTATA TATATTGATA CTCCACGAAA
Phuket 4 (S04)            AATGGAGGTT CATTTGATTT TTTAATTGTA TATACTCATA CTCCACGAAA
Phuket 1 (S01)            AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA CTCCACGAAA
Chaing Mai 4 (N04)        AATGGAGCTT CATTTTATTT TTTAATTATA TATACTCATA GCTCACGAAA
Kanchanaburi 2 (SW05)     AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Surat Thani 2 (S06)       AATGGAGCTT CGTTTTATTT TTTAATTATA TATATTGATA TTAGACGAAA
Tenom 2 (Tn02)            AATGGAGCTT CATTCTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Phetchaburi 1 (SW01)      AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTACACGAAA
Tenom 5 (Tn05)            AATGGAGCTT CATTCTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Tenom 3 (Tn03)            AATGGAGCTT CATTCTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Tenom 6 (Tn06)            AATGGAGCTT CATTCTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Phetchaburi 2 (SW02)      AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Chanthaburi 5 (SE07)      AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Chiang Mai 1 (N01)        AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Trat 2 (SE02)             AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Chiang Mai 2 (N02)        AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Kanchanaburi 3 (SW06)     AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Kanchanaburi 4 (SW07)     AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Surat Thani 1 (S05)       AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTACACGAAA
Kanchanaburi 1 (SW04)     AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Chanthaburi 6 (SE08)      AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Phetchaburi 3 (SW03)      AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Chanthaburi 2 (SE04)      AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Kanchanaburi 5 (SW08)     AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Trat 1 (SE01)             AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Chanthaburi 3 (SE05)      AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Chiang Mai 7 (N07)        AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA GCTGACGAAA
Pungnga 1 (S07)           AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Tenom 4 (Tn04)            AATGGAGCTT CATTCTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Kanchanaburi 6 (SW09)     AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Nakhon Ratchasima(E01)AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Chanthaburi 4 (SE06)      AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Chanthaburi 1 (SE03)      AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Chanthaburi 7 (SE09)      AATGGAGCTT CATTTTATTT TTTAATTATA TATATTCATA TTAGACGAAA
Phuket 3 (S03)            AATGGAGGTT CATTTTATTT TTTAATTGTA TATACTCATA CTCCACGAAA
Clustal Co                ******* *  * **  **** ******* ** **** * ***      *****
```

Figure 41. (continued)

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                         ....|....| ....|....| ....|....| ....|....| ....|....|
                                160        170        180        190        200
Chaing Mai 5 (N05)       TATATTTTAT AACTCATGTA AATTCAATAG AGTATGAGGA ATTGGAATTT
Chaing Mai 6 (N06)       TATATTTTAG ACCTCATGTA AATTCAATAC CGTATGAGGA ATTGGAATTT
Phuket 2 (S02)           TATATTTTAT ACCTCATTTA AATTCAATAG CGTATGAGGA ATTGGAATTT
Phuket 4 (S04)           TATATTTTAG ACTTCATTTA AATTAAATAC CGTATGAGGA ATTGGAATTT
Phuket 1 (S01)           TATATTTTAT ACCTCATTTA AATTCAATAG CGTATGAGGA ATTGGAATTT
Chaing Mai 4 (N04)       TATATTTTAG AATTCATGTA AATTCAATAC AGTATGAGGA AGTGGAATTT
Kanchanaburi 2 (SW05)    TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Surat Thani 2 (S06)      TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Tenom 2 (Tn02)           TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Phetchaburi 1 (SW01)     TATATTTTAT AATCCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Tenom 5 (Tn05)           TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Tenom 3 (Tn03)           TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Tenom 6 (Tn06)           TATATTTTAA AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Phetchaburi 2 (SW02)     TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Chanthaburi 5 (SE07)     TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Chiang Mai 1 (N01)       TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Trat 2 (SE02)            TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Chiang Mai 2 (N02)       TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Kanchanaburi 3 (SW06)    TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Kanchanaburi 4 (SW07)    TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Surat Thani 1 (S05)      TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Kanchanaburi 1 (SW04)    TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Chanthaburi 6 (SE08)     TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Phetchaburi 3 (SW03)     TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Chanthaburi 2 (SE04)     TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Kanchanaburi 5 (SW08)    TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Trat 1 (SE01)            TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Chanthaburi 3 (SE05)     TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Chiang Mai 7 (N07)       TATATTTTAT ACCTCATTTA AATTCAATAG AGTATGAGGA ATTGGAATTT
Pungnga 1 (S07)          TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Tenom 4 (Tn04)           TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Kanchanaburi 6 (SW09)    TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Nakhon Ratchasima(E01)TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Chanthaburi 4 (SE06)     TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Chanthaburi 1 (SE03)     TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Chanthaburi 7 (SE09)     TATATTTTAT AATTCATTTA AATTAAATAG AGTATGAGGA ATTGGAATTT
Phuket 3 (S03)           TATATTTTAT ACCTCATTTA AATTAAATAC CGTATGAGGA ATTGGAATTT
Clustal Co               *********  *   *** ** **** ****  ********* * ********
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Figure 41. (continued)

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                         ....|....| ....|....| ....|....| ....|....| ....|....|
                               210        220        230        240        250
Chaing Mai 5 (N05)       TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA TGTTCTTCCA
Chaing Mai 6 (N06)       TAATTTTATT AATTTCTATG GCAGCTCCAC TTATAGGATA TGTTCTTCCA
Phuket 2 (S02)           TAATTTTATT AATTTCTATG GCAGCTGCAC TTATAGGATA TGTTCTTCCT
Phuket 4 (S04)           TAATTTTATT AATTTCTATG GCAGCTCCAC TTATAGGATA TGTTCTTCCT
Phuket 1 (S01)           TAATTTTATT AATTTCTATG GCAGCACCAT TTATAGGATA TGTTCTTCCA
Chaing Mai 4 (N04)       TAATTTTATT AATTTCTATG GCAGCTCCAT TTATAGGATA TGTTCTTCCA
Kanchanaburi 2 (SW05)    TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA TGTTCTTCCA
Surat Thani 2 (S06)      TAATTTTATT AATTTCTATG GCAGCTGCAC TTATAGGATA TGTTCTTCCA
Tenom 2 (Tn02)           TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGTTA TGTTCTTCCA
Phetchaburi 1 (SW01)     TAATTTTATT AATTTCTATG GCAGCACCAT TTATAGGATA TGTTCTTCCA
Tenom 5 (Tn05)           TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGTTA TGTTCTTCCA
Tenom 3 (Tn03)           TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGTTA TGTTCTTCCA
Tenom 6 (Tn06)           TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGTTA TGTTCTTCCA
Phetchaburi 2 (SW02)     TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA TGTTCTTCCA
Chanthaburi 5 (SE07)     TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA TGTTCTTCCA
Chiang Mai 1 (N01)       TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA TGTTCTTCCA
Trat 2 (SE02)            TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA TGTTCTTCCA
Chiang Mai 2 (N02)       TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA TGTTCTTCCA
Kanchanaburi 3 (SW06)    TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA TGTTCTTCCA
Kanchanaburi 4 (SW07)    TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA TGTTCTTCCA
Surat Thani 1 (S05)      TAATTTTATT AATTTCTATG GCAGCTCCAC TTATAGGATA TGTTCTTCCT
Kanchanaburi 1 (SW04)    TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA TGTTCTTCCA
Chanthaburi 6 (SE08)     TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA TGTTCTTCCA
Phetchaburi 3 (SW03)     TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA TGTTCTTCCA
Chanthaburi 2 (SE04)     TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA TGTTCTTCCA
Kanchanaburi 5 (SW08)    TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA TGTTCTTCCA
Trat 1 (SE01)            TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA TGTTCTTCCA
Chanthaburi 3 (SE05)     TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA TGTTCTTCCA
Chiang Mai 7 (N07)       TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA TGTTCTTCCA
Pungnga 1 (S07)          TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA TGTTCTTCCA
Tenom 4 (Tn04)           TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGTTA TGTTCTTCCA
Kanchanaburi 6 (SW09)    TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA TGTTCTTCCA
Nakhon Ratchasima(E01)TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA TGTTCTTCCA
Chanthaburi 4 (SE06)     TAATTTTATT AATTTCTATG GCAGCACCAT TTATAGGATA TGTTCTTCCA
Chanthaburi 1 (SE03)     TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA TGTTCTTCCA
Chanthaburi 7 (SE09)     TAATTTTATT AATTTCTATG GCAGCAGCAT TTATAGGATA TGTTCTTCCA
Phuket 3 (S03)           TAATTTTATT AATTTCTATG GCAGCTCCAC TTATAGGATA TGTTCTTCCT
Clustal Co               ********** ********** *****  **  ******* ** *********
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Figure 41. (continued)

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                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                 260        270        280        290        300
Chaing Mai 5 (N05)        TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Chaing Mai 6 (N06)        GGAGGACAAA TATCATTTTG AGGAGCAACA GTTATTACAA ATTTATTATC
Phuket 2 (S02)            GGAGGACAAA AATCATTTTG AGGAGCAACA GTTATTACAA ATTTATTATC
Phuket 4 (S04)            GGAGGACAAA AATCATTTTG AGGAGCAACA GTTATTACAA ATTTATTATC
Phuket 1 (S01)            TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Chaing Mai 4 (N04)        TGAGGACAAA TATCATTTTG AGGAGCAACA GTTATTACAA ATTTATTATC
Kanchanaburi 2 (SW05)     TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Surat Thani 2 (S06)       TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Tenom 2 (Tn02)            TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Phetchaburi 1 (SW01)      TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Tenom 5 (Tn05)            TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Tenom 3 (Tn03)            TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Tenom 6 (Tn06)            TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Phetchaburi 2 (SW02)      TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Chanthaburi 5 (SE07)      TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Chiang Mai 1 (N01)        TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Trat 2 (SE02)             TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Chiang Mai 2 (N02)        TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Kanchanaburi 3 (SW06)     TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Kanchanaburi 4 (SW07)     TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Surat Thani 1 (S05)       GGAGGACAAA TATCATTTTG AGGAGCAACA GTTATTACAA ATTTATTATC
Kanchanaburi 1 (SW04)     TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Chanthaburi 6 (SE08)      TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Phetchaburi 3 (SW03)      TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Chanthaburi 2 (SE04)      TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Kanchanaburi 5 (SW08)     TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Trat 1 (SE01)             TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Chanthaburi 3 (SE05)      TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Chiang Mai 7 (N07)        TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Pungnga 1 (S07)           TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Tenom 4 (Tn04)            TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Kanchanaburi 6 (SW09)     TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Nakhon Ratchasima(E01)TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Chanthaburi 4 (SE06)      TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Chanthaburi 1 (SE03)      TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Chanthaburi 7 (SE09)      TGAGGACAAA TATCATATTG AGGAGCAACA GTTATTACAA ATTTATTATC
Phuket 3 (S03)            TGAGGACAAA AATCATTTTG AGGAGCAACA GTTATTACAA ATTTATTATC
Clustal Co                 *********  ***** *** ********** ********** **********
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Figure 41. (continued)

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                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                310        320        330        340        350
Chaing Mai 5 (N05)        AGCTATTCCT TATATTGGAG ATACAGTAGT TCTTTGAATT TGAGGTGGAT
Chaing Mai 6 (N06)        AGCTGTTCCT CCTTTTGGAG ATACAGAAGT TCTCTGACTT CCAGGAGGAT
Phuket 2 (S02)            AGCTGATCCT CCTTTTGGAG AAACAGAAGC ACTCTGATTT CCAGGAGGAT
Phuket 4 (S04)            AGCTGTTCCT CCTTTTGGAG AAACAGAAGC ACTCTGATTT CCAGGAGGAT
Phuket 1 (S01)            AGCTATTCCT TCTTTTGGAG ATACAGAAGT TCTTTGACTT TCAGGCGGAT
Chaing Mai 4 (N04)        AGCTGTTCCT CCTTTTGGAG ATACAGAAGT TCTCTGACTT TCAGGTGGAT
Kanchanaburi 2 (SW05)     AGCTATTCCT TATATTGGAG ATACAGTAGT TCTTTGAATT TGAGGTGGAT
Surat Thani 2 (S06)       AGCTAATCCT CATATTGGAG AAACAGTAGT TCCTTGCATT CGAGGTGGAT
Tenom 2 (Tn02)            AGCTATTCCT TATATTGGGG ATACAGTAGT TCTTTGAATT TGAGGTGGAT
Phetchaburi 1 (SW01)      AGCTATTCCT CATATTGGAG ATACAGTAGT TCCTTGCATT CGAGGTGGAT
Tenom 5 (Tn05)            AGCTATTCCT TATATTGGGG ATACAGTAGT TCTTTGAATT TGAGGTGGAT
Tenom 3 (Tn03)            AGCTATTCCT TATATTGGGG ATACAGTAGT TCTTTGAATT TGAGGTGGAT
Tenom 6 (Tn06)            AGCTATTCCT TATATTGGGG ATACAGTAGT TCTTTGAATT TGAGGTGGAT
Phetchaburi 2 (SW02)      AGCTATTCCT TATATTGGAG ATACAGTAGT TCTTTGAATT TGAGGTGGAT
Chanthaburi 5 (SE07)      AGCTATTCCT TATATTGGAG ATACAGTAGT TCTTTGAATT CGAGGGGGAT
Chiang Mai 1 (N01)        AGCTATTCCT TATATTGGAG ATACAGTAGT TCTTTGAATT TGAGGTGGAT
Trat 2 (SE02)             AGCTATTCCT TATATTGGAG ATACAGTAGT TCTTTGAATT TGAGGTGGAT
Chiang Mai 2 (N02)        AGCTATTCCT TATATTGGAG ATACAGTAGT TCTTTGAATT TGAGGTGGAT
Kanchanaburi 3 (SW06)     AGCTATTCCT TATATTGGAG ATACAGTAGT TCTTTGAATT TGAGGTGGAT
Kanchanaburi 4 (SW07)     AGCTATTCCT TATATTGGAG ATACAGTAGT TCTTTGAATT TGAGGTGGAT
Surat Thani 1 (S05)       AGCTGATCCT CCTTTTGGAG AAACAGAAGC TCCAAGCATT CGAGGTGGAT
Kanchanaburi 1 (SW04)     AGCTATTCCT TATATTGGAG ATACAGTAGT TCTTTGAATT TGAGGTGGAT
Chanthaburi 6 (SE08)      AGCTATTCCT TATATTGGAG ATACAGTAGT TCTTTGAATT CGAGGGGGAT
Phetchaburi 3 (SW03)      AGCTATTCCT TATATTGGAG ATACAGTAGT TCTTTGAATT TGAGGTGGAT
Chanthaburi 2 (SE04)      AGCTATTCCT TATATTGGAG ATACAGTAGT TCTTTGAATT TGAGGTGGAT
Kanchanaburi 5 (SW08)     AGCTATTCCT TATATTGGAG ATACAGTAGT TCTTTGAATT TGAGGTGGAT
Trat 1 (SE01)             AGCTATTCCT TATATTGGAG ATACAGTAGT TCTTTGAATT TGAGGTGGAT
Chanthaburi 3 (SE05)      AGCTATTCCT TATATTGGAG ATACAGTAGT TCTTTGAATT TGAGGTGGAT
Chiang Mai 7 (N07)        AGCTGTTCCT TCTATTGGAG ATACAGAAGT TCTTTGAATT TGAGGTGGAT
Pungnga 1 (S07)           AGCTATTCCT TATATTGGAG ATACAGTAGT TCTTTGCATT CGAGGTGGAT
Tenom 4 (Tn04)            AGCTATTCCT TATATTGGGG ATACAGTAGT TCTTTGAATT TGAGGTGGAT
Kanchanaburi 6 (SW09)     AGCTATTCCT TATATTGGAG AAACAGTAGT TCTTTGAATT TGAGGGGGAT
Nakhon Ratchasima(E01)AGCTATTCCT TATATTGGAG ATACAGTAGT TCTTTGAATT CGAGGTGGAT
Chanthaburi 4 (SE06)      AGCTATTCCT CATATTGGAG AAACAGTAGT TCTTTGCATT CGAGGTGGAT
Chanthaburi 1 (SE03)      AGCTATTCCT TATATTGGAG AAACAGTAGT TCTTTGAATT CGAGGTGGAT
Chanthaburi 7 (SE09)      AGCTATTCCT TATATTGGAG ATACAGTAGT TCTTTGAATT CGAGGTGGAT
Phuket 3 (S03)            AGCTGTTCCT CCTTTTGGAG AAACAGAAGC ACTCTGATTT CCAGGAGGAT
Clustal Co                **** **** * **** * * **** **  *  *  **  *** ****
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Figure 41. (continued)

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                          ....|....| ....|....| ....|....| ....|....| ....|...
                                 360        370        380        390
Chaing Mai 5 (N05)        TTTCAATTAA TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
Chaing Mai 6 (N06)        TTTCAATTAA TAATGCTGCT TTAGATCGAT TTGTTTCTAC TCATTTTA
Phuket 2 (S02)            TTTCTATTAA TAATGCTGCT TTTGATCGAA TTGTTTCGAC TCATTTTG
Phuket 4 (S04)            TTTCTATTAA TAAAGCTGCT TTTGATCGAA TTGTTTCCAC TCATTTTG
Phuket 1 (S01)            TTTCTATTAA TAATGCTGCT TTAAATCGAA TTGTTTCGAT TCATTTTA
Chaing Mai 4 (N04)        TTTCTATTAA TAAAGCTGCT TTAGATCGAA TTGTTTCTAT TCATTTTA
Kanchanaburi 2 (SW05)     TTTCAATTAA TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
Surat Thani 2 (S06)       TTTCAATTAA TAATGCTACT GTGATTCGAA TTGTTTCTAT TCATTTTG
Tenom 2 (Tn02)            TTTCAATTAA TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
Phetchaburi 1 (SW01)      TTTCAATTAA TAATGCTACT TTGAATCGAT TTTTTTCTAT TCATTTTG
Tenom 5 (Tn05)            TTTCAATTAA TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
Tenom 3 (Tn03)            TTTCAATTAA TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
Tenom 6 (Tn06)            TTTCAATTAA TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
Phetchaburi 2 (SW02)      TTTCAATTAA TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
Chanthaburi 5 (SE07)      TTTCAATTAA TAATGCTACT GTAAATCGAT TTTTTTCTAT TCATTTTA
Chiang Mai 1 (N01)        TTTCAATTAA TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
Trat 2 (SE02)             TTTCAATTAA TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
Chiang Mai 2 (N02)        TTTCAATTAA TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
Kanchanaburi 3 (SW06)     TTTCAATTAA TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
Kanchanaburi 4 (SW07)     TTTCAATTAA TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
Surat Thani 1 (S05)       TTTCTATTAA TAAAGCTGCT GTGATTCGAA TTGTTTCCAC TCATTTTG
Kanchanaburi 1 (SW04)     TTTCAATTAA TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
Chanthaburi 6 (SE08)      TTTCAATTAA TAATGCTACT GTAAATCGAT TTTTTTCTAT TCATTTTA
Phetchaburi 3 (SW03)      TTTCAATTAA TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
Chanthaburi 2 (SE04)      TTTCAATTAA TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
Kanchanaburi 5 (SW08)     TTTCAATTAA TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
Trat 1 (SE01)             TTTCAATTAA TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
Chanthaburi 3 (SE05)      TTTCAATTAA TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
Chiang Mai 7 (N07)        TTTCAATTAA TAATGCTGCT TTAGATCGAT TTGTTTCTAT TCATTTTA
Pungnga 1 (S07)           TTTCAATTAA TAATGCTGCT TTAAATCGAT TTGTTTCTAT TCATTTTA
Tenom 4 (Tn04)            TTTCAATTAA TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
Kanchanaburi 6 (SW09)     TTTCAATTAA TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
Nakhon Ratchasima (E01)   TTTCAATTAA TAATGCTACT GTAAATCGAT TTTTTTCTAT TCATTTTA
Chanthaburi 4 (SE06)      TTTCAATTAA TAATGCTACT TTGAATCGAA TTTTTTCTAT TCATTTTG
Chanthaburi 1 (SE03)      TTTCAATTAA TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
Chanthaburi 7 (SE09)      TTTCAATTAA TAATGCTACT TTAAATCGAT TTTTTTCTAT TCATTTTA
Phuket 3 (S03)            TTTCTATTAA TAAAGCTGCT TTTGATCGAA TTGTTTCTAC TCATTTTG
Clustal Co                **** ***** *** *** **  *   ****  ** **** *  *******
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Figure 41. (continued)

Table 2. Percentages of base composition of *cytb* sequences of *A. andreniformis* samples.

Samples	A	C	G	T
Chiang Mai 1 (N01)	34.4	9.8	12.8	43
Chiang Mai 2 (N02)	34.4	9.8	12.8	43
Chiang Mai 4 (N04)	30.6	13.6	14.8	41
Chiang Mai 5 (N05)	32.9	10.3	14.3	42.5
Chiang Mai 6 (N06)	29.7	15.3	16.3	38.7
Chiang Mai 7 (N07)	31.4	11.8	15.1	41.7
Trat 1(Se01)	34.2	10	12.8	43
Trat 2 (Se02)	34.2	10	12.8	43
Chanthaburi 1 (Se03)	34.7	10.3	12.6	42.4
Chanthaburi 2 (Se04)	34.2	10	12.8	43
Chanthaburi 3 (Se05)	34.2	10	12.8	43
Chanthaburi 4 (Se06)	34.4	10.8	12.8	42
Chanthaburi 5 (Se07)	34.4	10.3	13.1	42.2
Chanthaburi 6 (Se08)	34.4	10.3	13.1	42.2
Chanthaburi 7 (Se09)	34.4	10.3	12.6	42.7
Phetchaburi 1 (Sw01)	33.7	11.5	13.1	41.7
Phetchaburi 2 (Sw02)	34.2	10	12.8	43
Phetchaburi 3 (Sw03)	34.4	10.3	13.1	42.2
Kanchanaburi 1 (Sw04)	34.2	10	12.8	43
Kanchanaburi 2 (Sw05)	34.2	10	12.8	43
Kanchanaburi 3 (Sw06)	34.2	10	12.8	43
Kanchanaburi 4 (Sw07)	34.2	10	12.8	43
Kanchanaburi 5 (Sw08)	34.4	9.8	12.8	43
Kanchanaburi 6 (Sw09)	34.4	10	13.1	42.5
Phuket 1 (S01)	31.4	13.8	13.3	41.5
Phuket 2 (S02)	29.7	14.6	15.8	39.9
Phuket 3 (S03)	30.1	15.1	15.1	39.7
Phuket 4 (S04)	30.4	15	15.6	39
Surat Thani 1 (S05)	32.4	10	12.8	43
Surat Thani 2 (S06)	33.2	11.3	14.3	41.2
Pungnga 1 (S07)	33.9	10.3	13.3	42.5
Tenom, Malaysia2 (Tn02)	33.9	10	13.1	43
Tenom, Malaysia 3 (Tn03)	33.9	10	13.1	43
Tenom, Malaysia 4 (Tn04)	33.7	10.3	13.1	42.9
Tenom, Malaysia 5 (Tn05)	33.9	10	13.1	43
Tenom, Malaysia 6 (Tn06)	33.9	10.3	13.1	42.7
Means	**33.35**	**10.97**	**13.42**	**42.2**

4.2.5 Phylogenetic analysis

Partial *cytb* sequences of *A. andreniformis* in Thailand (the north, the west, the east, and the south) and in Tenom, Sabha, Malaysia were used for phylogenetic analysis. Phylogenetic trees were constructed by using neighbor-joining (NJ) and unweighted pair-group method using arithmetic averages (UPGMA). Both trees showed the same topology (Figure 42 and 43). Twenty three mitochondrial DNA haplotypes among 37 colonies of *A. andreniformis* were identified. According to the trees, 2 major groups of these bees can be distinguished. The 1st major group (Group A) is composed of bees from all major collecting localities whiles the 2nd major group (Group B) is composed of bees from the north (Chiang Mai 4, 6, and 7) and the south (Phuket) of Thailand (Figure 42 and 43). However, higher variation of sequences is found in the 2nd major group. The 1st major group can be divided into 5 subgroups. The 1st subgroup is mainly composed of bees from the west and the east of Thailand. The 2nd subgroup is composed of bees from the northeast, the east and the west of Thailand. The 3rd subgroup is composed of bees from Tenom, Sabha, Malaysia. The 4th subgroup is composed of bees from the north (Chiang Mai 1 and 2) and the west (Phetchaburi 3 and Kanchanaburi 5) of Thailand. The 5th subgroup is composed of bees from all parts of Thailand and higher variation within this group was observed (Figure 42 and 43). From the above data, it reveals that bees from the west and the east of Thailand and Tenom, Malaysia show low variation within and between groups, especially bees from the west and the east of Thailand.

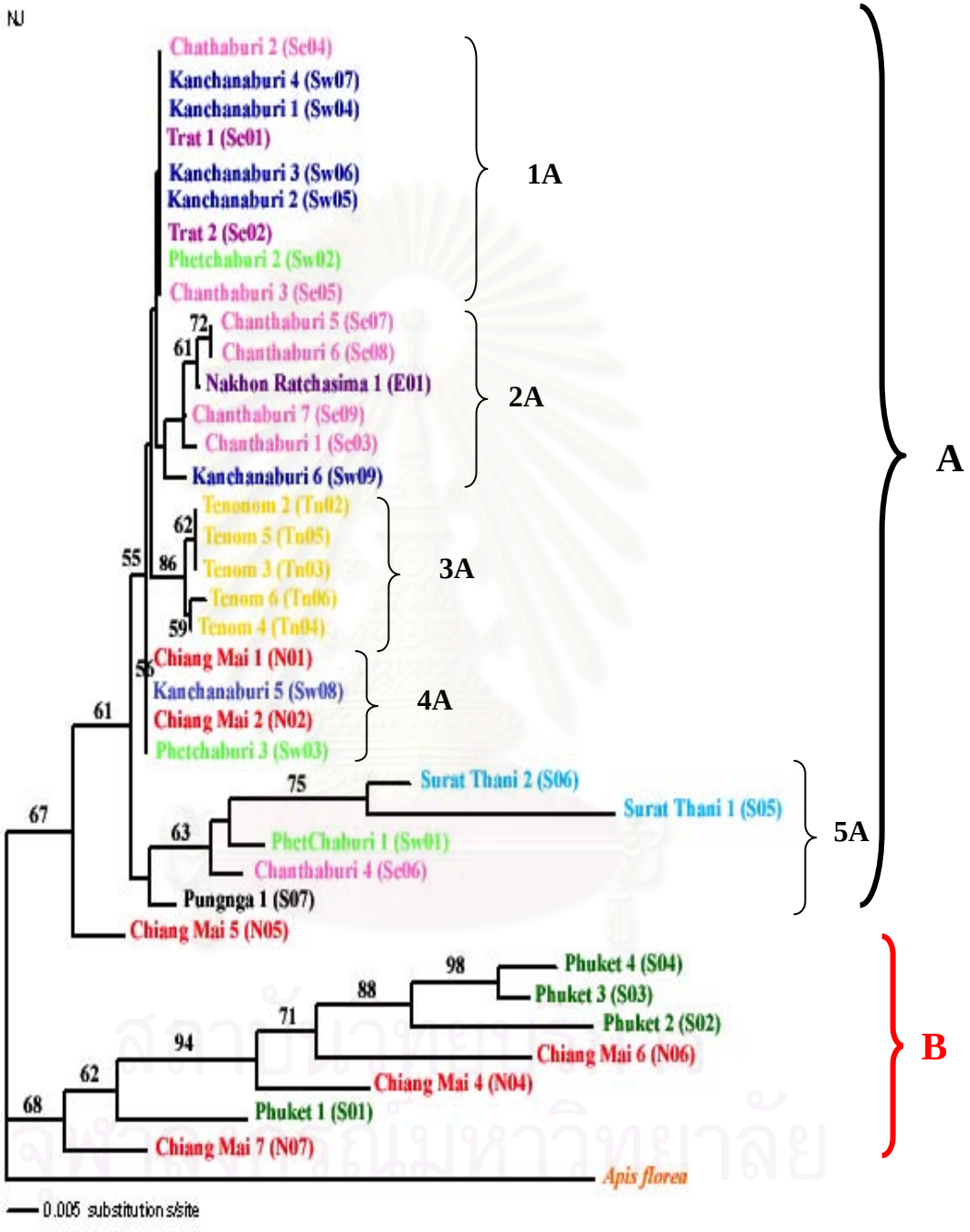

Figure 42. A rooted phylogenetic tree inferred by neighbor-joining method. Confidence probabilities are shown on the branches.

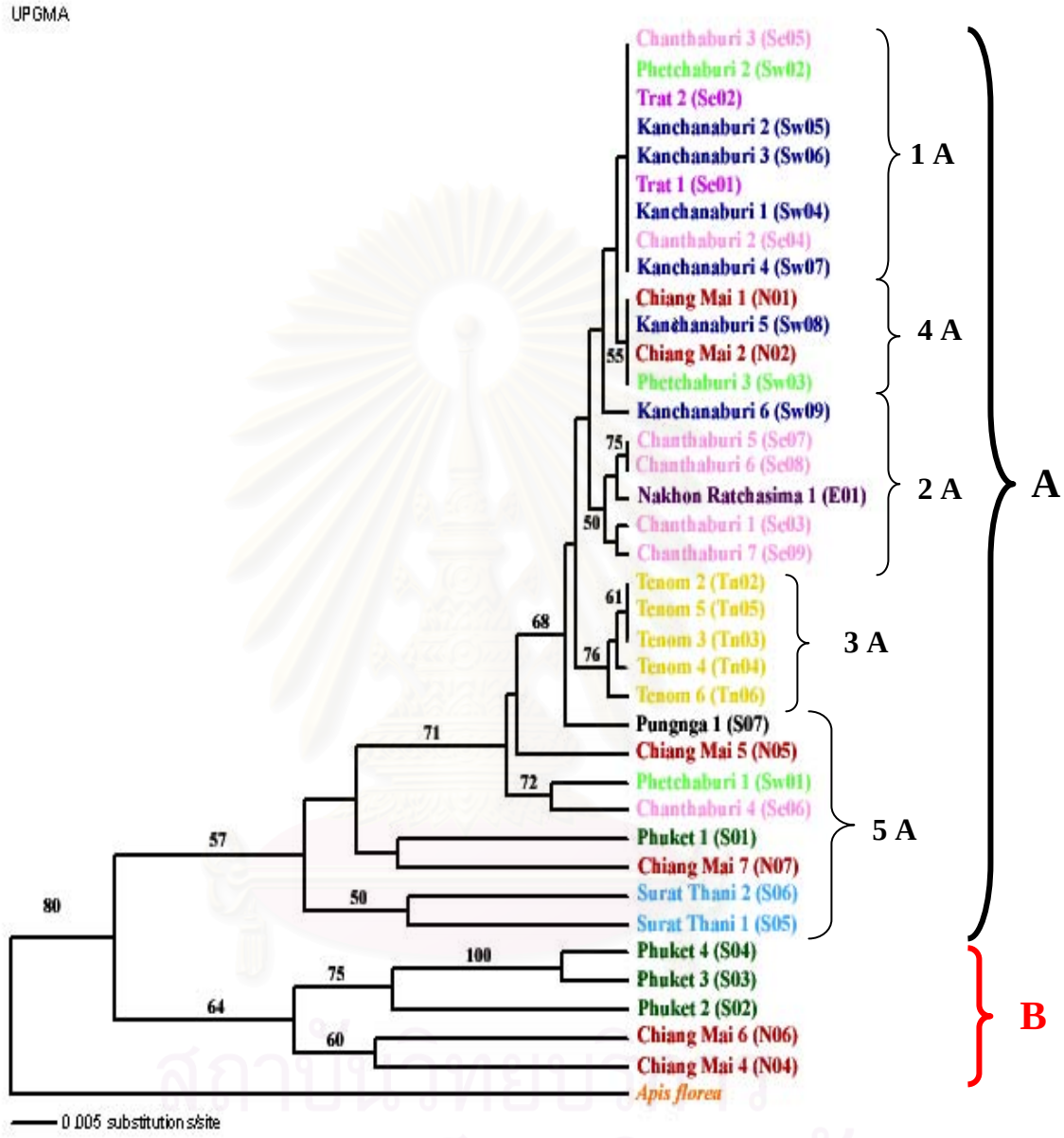

Figure 43. A UPGMA dendrogram. The relationship of *A. andreniformis* population in Thailand and Tenom, Sabha, Malaysia was calculated from genetic distance.

Table 3. The similarity between pair of sequences (%) of *cytb* of *A. andreniformis* samples from Thailand and Tenom, Malaysia (see Table 2 for abbreviated names)

Sample code	N01	N02	N04	N05	N06	N07	Sw01	Sw02	Sw03	Sw04
N01	-	-	-	-	-	-	-	-	-	-
N02	100	-	-	-	-	-	-	-	-	-
N04	91	91	-	-	-	-	-	-	-	-
N05	97	97	92	-	-	-	-	-	-	-
N06	87	87	94	89	-	-	-	-	-	-
N07	95	95	93	96	92	-	-	-	-	-
Sw01	97	97	90	95	87	92	-	-	-	-
Sw02	99	99	90	97	87	94	97	-	-	-
Sw03	100	100	91	97	87	95	97	99	-	-
Sw04	99	99	90	97	87	94	97	100	99	-
Sw05	99	99	90	97	87	94	97	100	99	100
Sw06	99	99	90	97	87	94	97	100	99	100
Sw07	99	99	90	97	87	94	97	100	99	100
Sw08	100	100	91	97	87	95	97	99	100	99
Sw09	99	99	90	97	87	94	96	99	99	99
E01	98	98	90	96	87	94	96	99	98	99
Se01	99	99	90	97	87	94	97	100	99	100
Se02	99	99	90	97	87	94	97	100	99	100
Se03	98	98	90	96	87	94	96	99	98	99
Se04	99	99	90	97	87	94	97	100	99	100
Se05	99	99	90	97	87	94	97	100	99	100
Se06	97	97	90	95	87	92	98	97	97	97
Se07	98	98	89	96	87	93	96	98	98	98
Se08	98	98	89	96	87	93	96	98	98	98
Se09	99	99	90	97	87	94	97	99	99	99
S01	93	93	92	92	90	94	91	93	93	93
S02	86	86	89	86	90	89	86	86	86	86
S03	87	87	93	88	93	90	88	87	87	87
S04	87	87	93	87	93	89	87	87	87	87
S05	90	90	89	88	86	89	92	90	90	90
S06	94	94	88	92	85	91	95	94	94	94
S07	98	98	91	96	88	95	97	98	98	98
Tn02	99	99	90	97	87	94	96	98	99	98
Tn03	99	99	90	97	87	94	96	98	99	98
Tn04	98	98	90	96	87	94	96	99	98	99
Tn05	99	99	90	97	87	94	96	98	99	98
Tn06	98	98	90	96	87	93	96	98	98	98
A. florea	87	87	82	87	81	87	85	87	87	87

Table 3. (continued)

Sample Code	Sw05	Sw06	Sw07	Sw08	Sw09	E01	Se01	Se02	Se03	Se04
N01	-	-	-	-	-	-	-	-	-	-
N02	-	-	-	-	-	-	-	-	-	-
N04	-	-	-	-	-	-	-	-	-	-
N05	-	-	-	-	-	-	-	-	-	-
N06	-	-	-	-	-	-	-	-	-	-
N07	-	-	-	-	-	-	-	-	-	-
Sw01	-	-	-	-	-	-	-	-	-	-
Sw02	-	-	-	-	-	-	-	-	-	-
Sw03	-	-	-	-	-	-	-	-	-	-
Sw04	-	-	-	-	-	-	-	-	-	-
Sw05	-	-	-	-	-	-	-	-	-	-
Sw06	100	-	-	-	-	-	-	-	-	-
Sw07	100	100	-	-	-	-	-	-	-	-
Sw08	99	99	99	-	-	-	-	-	-	-
Sw09	99	99	99	99	-	-	-	-	-	-
E01	99	99	99	98	98	-	-	-	-	-
Se01	100	100	100	99	99	99	-	-	-	-
Se02	100	100	100	99	99	99	100	-	-	-
Se03	99	99	99	99	99	99	99	99	-	-
Se04	100	100	100	99	99	99	100	100	99	-
Se05	100	100	100	97	99	99	100	100	99	100
Se06	97	97	97	98	97	97	97	97	98	97
Se07	98	98	98	98	98	99	98	98	99	98
Se08	98	98	98	99	98	99	98	98	99	98
Se09	99	99	99	93	98	99	99	99	99	99
S01	93	93	93	86	92	92	93	93	92	93
S02	86	86	86	87	86	86	86	86	86	86
S03	87	87	87	87	87	87	87	87	87	87
S04	87	87	87	90	87	86	87	87	87	87
S05	90	90	90	94	90	90	90	90	90	90
S06	94	94	94	98	94	94	94	94	94	94
S07	98	98	98	98	98	98	98	98	98	98
Tn02	98	98	98	99	98	98	98	98	98	98
Tn03	98	98	98	99	98	98	98	98	98	98
Tn04	99	99	99	98	98	98	99	99	98	99
Tn05	98	98	98	99	98	98	98	98	98	98
Tn06	98	98	98	98	98	98	98	98	98	98
A. florea	87	87	87	87	86	87	87	87	87	87

Table 3. (continued)

Sample code	Se05	Se06	Se07	Se08	Se09	S01	S02	S03	S04	S05
N01	-	-	-	-	-	-	-	-	-	-
N02	-	-	-	-	-	-	-	-	-	-
N04	-	-	-	-	-	-	-	-	-	-
N05	-	-	-	-	-	-	-	-	-	-
N06	-	-	-	-	-	-	-	-	-	-
N07	-	-	-	-	-	-	-	-	-	-
Sw01	-	-	-	-	-	-	-	-	-	-
Sw02	-	-	-	-	-	-	-	-	-	-
Sw03	-	-	-	-	-	-	-	-	-	-
Sw04	-	-	-	-	-	-	-	-	-	-
Sw05	-	-	-	-	-	-	-	-	-	-
Sw06	-	-	-	-	-	-	-	-	-	-
Sw07	-	-	-	-	-	-	-	-	-	-
Sw08	-	-	-	-	-	-	-	-	-	-
Sw09	-	-	-	-	-	-	-	-	-	-
E01	-	-	-	-	-	-	-	-	-	-
Se01	-	-	-	-	-	-	-	-	-	-
Se02	-	-	-	-	-	-	-	-	-	-
Se03	-	-	-	-	-	-	-	-	-	-
Se04	-	-	-	-	-	-	-	-	-	-
Se05	-	-	-	-	-	-	-	-	-	-
Se06	97	-	-	-	-	-	-	-	-	-
Se07	98	97	-	-	-	-	-	-	-	-
Se08	98	97	100	-	-	-	-	-	-	-
Se09	99	97	99	99	-	-	-	-	-	-
S01	93	92	92	92	92	-	-	-	-	-
S02	86	87	86	86	86	91	-	-	-	-
S03	87	88	87	87	87	91	94	-	-	-
S04	87	88	86	86	90	90	94	98	-	-
S05	90	92	90	90	94	89	90	89	90	-
S06	94	96	94	94	98	89	88	87	86	94
S07	98	97	98	98	98	93	87	88	87	91
Tn02	98	96	97	97	98	92	86	87	86	90
Tn03	98	96	97	97	98	92	86	87	86	90
Tn04	99	96	98	98	98	92	85	86	86	89
Tn05	98	96	97	97	98	92	86	87	86	90
Tn06	98	96	97	97	98	92	85	86	86	89
A. florea	87	86	86	86	87	84	78	80	79	81

Table 3. (continued)

Sample code	S06	S07	Tn02	Tn03	Tn04	Tn05	Tn06
N01	-	-	-	-	-	-	-
N02	-	-	-	-	-	-	
N04	-	-	-	-	-	-	
N05	-	-	-	-	-	-	-
N06	-	-	-	-	-	-	-
N07	-	-	-	-	-	-	-
Sw01	-	-	-	-	-	-	-
Sw02	-	-	-	-	-	-	-
Sw03	-	-	-	-	-	-	-
Sw04	-	-	-	-	-	-	-
Sw05	-	-	-	-	-	-	-
Sw06	-	-	-	-	-	-	-
Sw07	-	-	-	-	-	-	-
Sw08	-	-	-	-	-	-	-
Sw09	-	-	-	-	-	-	-
E01	-	-	-	-	-	-	-
Se01	-	-	-	-	-	-	-
Se02	-	-	-	-	-	-	-
Se03	-	-	-	-	-	-	-
Se04	-	-	-	-	-	-	-
Se05	-	-	-	-	-	-	-
Se06	-	-	-	-	-	-	-
Se07	-	-	-	-	-	-	-
Se08	-	-	-	-	-	-	-
Se09	-	-	-	-	-	-	-
S01	-	-	-	-	-	-	-
S02	-	-	-	-	-	-	-
S03	-	-	-	-	-	-	-
S04	-	-	-	-	-	-	-
S05	-	-	-	-	-	-	-
S06	-	-	-	-	-	-	-
S07	95	-	-	-	-	-	-
Tn02	94	98	-	-	-	-	-
Tn03	94	98	100	-	-	-	-
Tn04	93	97	99	99	-	-	-
Tn05	94	98	100	100	99	-	-
Tn06	93	97	99	99	99	99	-
A. florea	83	87	87	87	87	87	86

Table 4. The *cytb* sequence divergence (%) based on pairwise comparisons among the *A. andreniformis* samples from Thailand and Tenom, Malaysia (see Table 2 for abbreviated names).

Sample code	N01	N02	N04	N05	N06	N07	Sw01	Sw02	Sw03	Sw04
N01	-	-	-	-	-	-	-	-	-	-
N02	0	-	-	-	-	-	-	-	-	-
N04	8.794	8.794	-	-	-	-	-	-	-	-
N05	2.01	2.01	7.789	-	-	-	-	-	-	-
N06	12.06	12.06	5.779	10.05	-	-	-	-	-	-
N07	4.77	4.774	6.03	3.769	7.789	-	-	-	-	-
Sw01	2.513	2.513	9.779	4.523	12.56	7.286	-	-	-	-
Sw02	0.251	0.251	9.045	2.261	12.06	5.025	2.764	-	-	-
Sw03	0	0	8.794	2.01	12.06	4.774	2.513	0.251	-	-
Sw04	0.251	0.251	9.045	2.264	12.06	5.025	2.764	0	0.251	-
Sw05	0.251	0.251	9.045	2.261	12.06	5.025	2.764	0	0.251	0
Sw06	0.25	0.251	9.045	2.261	12.06	5.025	2.764	0	0.251	0
Sw07	0.251	0.251	9.045	2.261	12.06	5.025	2.764	0	0.251	0
Sw08	0	0	8.794	2.01	12.06	4.774	2.513	0.251	0	0.251
Sw09	0.754	0.754	9.548	2.764	12.31	5.528	3.266	0.53	0.754	0.503
E01	1.005	1.005	9.799	3.015	12.31	5.779	3.015	0.754	1.005	0.754
Se01	0.251	0.251	9.045	2.261	12.06	5.025	2.764	0	0.251	0
Se02	0.251	0.251	9.045	2.261	12.06	5.025	2.764	0	0.251	0
Se03	1.005	1.005	9.799	3.015	12.31	5.779	3.015	0.754	1.005	0.754
Se04	0.251	0.251	9.045	2.261	12.06	5.025	2.764	0	0.251	0
Se05	0.251	0.251	9.045	2.261	12.06	5.025	2.764	0	0.251	0
Se06	2.261	2.261	9.548	4.271	12.81	7.035	1.759	2.513	2.261	2.513
Se07	1.256	1.256	10.05	3.266	12.31	6.03	3.266	1.005	0.251	1.005
Se08	1.256	1.256	10.05	3.266	12.31	6.03	3.266	1.005	1.256	1.005
Se09	0.754	0.754	9.548	2.764	12.06	5.528	2.764	0.503	0.754	0.503
S01	6.533	6.533	7.286	7.035	9.296	5.276	8.04	6.784	6.533	6.784
S02	13.819	13.819	11.56	13.819	10.8	11.558	13.317	14.07	13.819	14.07
S03	12.814	12.814	8.04	12.312	8.291	10.05	11.804	13.065	12.814	13.32
S04	13.317	13.317	8.04	13.317	8.291	11.05	12.315	13.658	13.317	13.57
S05	9.045	9.045	10.55	11.055	13.32	10.804	7.035	9.296	9.045	9.296
S06	5.025	5.025	11.81	2.01	12.06	4.774	2.513	5.276	5.025	5.276
S07	1.005	1.005	8.794	3.015	11.56	4.774	2.513	1.256	1.005	1.256
Tn02	0.754	0.754	9.548	2.764	12.81	5.528	3.266	1.005	0.754	1.005
Tn03	0.754	0.754	9.548	2.764	12.81	5.528	3.266	1.005	0.754	1.005
Tn04	1.005	1.005	9.799	3.015	12.81	5.779	3	0.754	1.005	0.754
Tn05	0.754	0.754	9.548	2.764	12.81	5.528	0.518	1.005	0.754	1.005
Tn06	1.256	1.256	9.799	3.266	12.81	6.03	3.769	1.005	1.256	1.005
A. florea	12.439	12.439	16.9	12.173	18.37	12.634	14.941	12.689	12.439	12.69

Table 4. (continued)

Sample code	Sw05	Sw06	Sw07	Sw08	Sw09	E01	Se01	Se02	Se03
N01	-	-	-	-	-	-	-	-	-
N02	-	-	-	-	-	-	-	-	-
N04	-	-	-	-	-	-	-	-	-
N05	-	-	-	-	-	-	-	-	-
N06	-	-	-	-	-	-	-	-	-
N07	-	-	-	-	-	-	-	-	-
Sw01	-	-	-	-	-	-	-	-	-
Sw02	-	-	-	-	-	-	-	-	-
Sw03	-	-	-	-	-	-	-	-	-
Sw04	-	-	-	-	-	-	-	-	-
Sw05	-	-	-	-	-	-	-	-	-
Sw06	0	-	-	-	-	-	-	-	-
Sw07	0	0	-	-	-	-	-	-	-
Sw08	0.251	0.251	0.251	-	-	-	-	-	-
Sw09	0.503	0.503	0.503	0.754	-	-	-	-	-
E01	0.754	0.754	0.754	1.005	1.256	-	-	-	-
Se01	0	0	0	0.251	0.503	0.754	-	-	-
Se02	0	0	0	0.251	0.503	0.754	0	-	-
Se03	0.754	0.754	0.754	1.005	0.754	0.503	0.754	0.754	-
Se04	0	0	0	0.251	0.503	0.754	0	0	0.754
Se05	0	0	0	0.251	0.53	0.754	0	0	0.754
Se06	2.513	2.513	2.513	2.261	2.513	2.261	2.513	2.513	1.759
Se07	1.005	1.005	1.005	1.256	1.005	0.251	1.005	1.005	0.754
Se08	1.005	1.005	1.005	1.256	1.005	0.251	1.005	1.005	0.754
Se09	0.503	0.503	0.503	0.754	1.005	0.251	0.503	0.503	0.251
S01	6.784	6.784	6.784	6.533	7.035	0.7358	6.784	6.784	7.538
S02	14.07	14.07	14.07	13.819	13.819	14.322	14.07	14.07	13.819
S03	13.07	13.07	13.07	12.814	12.814	13.317	13.065	13.065	12.814
S04	13.57	13.57	13.57	13.317	13.317	13.819	13.568	13.658	13.317
S05	9.296	9.296	9.296	9.045	9.296	9.045	9.296	9.296	9.045
S06	5.276	5.276	5.276	5.025	0.754	1.005	5.276	5.276	1.005
S07	1.256	1.256	1.256	1.005	1.759	1.005	1.256	1.256	1.508
Tn02	1.005	1.005	1.005	0.754	1.508	1.759	1.005	1.005	1.759
Tn03	1.005	1.005	1.005	0.754	1.508	1.759	1.005	1.005	1.759
Tn04	0.754	0.754	0.754	1.005	1.256	1.508	0.754	0.754	1.508
Tn05	1.005	1.005	1.005	0.754	1.508	1.759	1.005	1.005	1.759
Tn06	1.005	1.005	1.005	1.256	1.508	1.759	1.005	1.005	1.759
A. florea	12.69	12.69	12.69	12.439	13.187	12.938	12.689	12.689	12.938

Table 4. (continued)

Sample code	Se04	Se05	Se06	Se07	Se08	Se09	S01	S02	S03
N01	-	-	-	-	-	-	-	-	-
N02	-	-	-	-	-	-	-	-	-
N04	-	-	-	-	-	-	-	-	-
N05	-	-	-	-	-	-	-	-	-
N06	-	-	-	-	-	-	-	-	-
N07	-	-	-	-	-	-	-	-	-
Sw01	-	-	-	-	-	-	-	-	-
Sw02	-	-	-	-	-	-	-	-	-
Sw03	-	-	-	-	-	-	-	-	-
Sw04	-	-	-	-	-	-	-	-	-
Sw05	-	-	-	-	-	-	-	-	-
Sw06	-	-	-	-	-	-	-	-	-
Sw07	-	-	-	-	-	-	-	-	-
Sw08	-	-	-	-	-	-	-	-	-
Sw09	-	-	-	-	-	-	-	-	-
E01	-	-	-	-	-	-	-	-	-
Se01	-	-	-	-	-	-	-	-	-
Se02	-	-	-	-	-	-	-	-	-
Se03	-	-	-	-	-	-	-	-	-
Se04	-	-	-	-	-	-	-	-	-
Se05	0	-	-	-	-	-	-	-	-
Se06	2.513	2.513	-	-	-	-	-	-	-
Se07	1.005	1.005	2.513	-	-	-	-	-	-
Se08	1.005	1.005	2.513	0	-	-	-	-	-
Se09	0.503	0.503	2.01	0.503	0.503	-	-	-	-
S01	6.784	6.784	7.789	7.538	7.538	7.286	-	-	-
S02	14.07	14.07	13.065	14.322	14.322	14.07	9.548	-	-
S03	13.065	13.065	11.558	13.317	13.317	13.065	9.548	5.025	-
S04	13.568	13.568	12.06	13.819	13.819	13.568	10.302	5.779	1.508
S05	9.296	9.296	7.286	9.296	9.296	9.296	10.302	9.296	10.05
S06	0.251	0.251	2.261	5.276	5.276	0.754	6.533	13.189	12.814
S07	1.256	1.256	2.261	1.759	1.759	1.256	6.533	13.065	12.06
Tn02	1.005	1.005	3.015	2.01	2.01	1.508	7.286	14.573	13.568
Tn03	1.005	1.005	3.015	2.01	2.01	1.508	7.286	14.573	13.568
Tn04	0.754	0.754	3.266	1.759	1.759	1.256	7.538	14.824	13.819
Tn05	1.005	1.005	3.015	2.01	2.01	1.508	7.286	14.573	13.568
Tn06	1.005	1.005	3.518	2.01	2.01	1.508	7.789	15.075	14.07
A. florea	12.689	12689	14.189	13.187	13.187	12.689	15.144	21.903	19.891

Table 4. (continued)

Sample code	S04	S05	S06	S07	Tn2	Tn3	Tn4	Tn5	Tn6
N01	-	-	-	-	-	-	-	-	-
N02	-	-	-	-	-	-	-	-	
N04	-	-	-	-	-	-	-	-	
N05	-	-	-	-	-	-	-	-	-
N06	-	-	-	-	-	-	-	-	-
N07	-	-	-	-	-	-	-	-	-
Sw01	-	-	-	-	-	-	-	-	-
Sw02	-	-	-	-	-	-	-	-	-
Sw03	-	-	-	-	-	-	-	-	-
Sw04	-	-	-	-	-	-	-	-	-
Sw05	-	-	-	-	-	-	-	-	-
Sw06	-	-	-	-	-	-	-	-	-
Sw07	-	-	-	-	-	-	-	-	-
Sw08	-	-	-	-	-	-	-	-	-
Sw09	-	-	-	-	-	-	-	-	-
E01	-	-	-	-	-	-	-	-	-
Se01	-	-	-	-	-	-	-	-	-
Se02	-	-	-	-	-	-	-	-	-
Se03	-	-	-	-	-	-	-	-	-
Se04	-	-	-	-	-	-	-	-	-
Se05	-	-	-	-	-	-	-	-	-
Se06	-	-	-	-	-	-	-	-	-
Se07	-	-	-	-	-	-	-	-	-
Se08	-	-	-	-	-	-	-	-	-
Se09	-	-	-	-	-	-	-	-	-
S01	-	-	-	-	-	-	-	-	-
S02	-	-	-	-	-	-	-	-	-
S03	-	-	-	-	-	-	-	-	-
S04	-	-	-	-	-	-	-	-	-
S05	9.548	-	-	-	-	-	-	-	-
S06	13.317	5.025	-	-	-	-	-	-	-
S07	12.563	8.04	1.005	-	-	-	-	-	-
Tn02	14.07	9.799	0.754	1.759	-	-	-	-	-
Tn03	14.07	9.799	0.754	1.759	0	-	-	-	-
Tn04	14.322	10.05	1.005	2.01	0.251	0.251	-	-	-
Tn05	14.07	9.799	5.779	1.759	0	0	0.251	-	-
Tn06	14.322	10.302	1.256	2.261	0.503	0.503	0.251	0.503	-
A. florea	20.39	19.16	12.439	12.934	12.689	12.689	12.93	12.689	13.179

Table 5. Means and standard deviation of sequence divergence (%) between pair of major localities of *andreniformis* samples from Thailand and Tenom, Sabha, Malaysia.

Lacalities	North	West	East	South	Tenom, Malaysia	*A. florea*
North	5.63±3.62					14.16±2.27
West	5.01±3.42	0.8±0.13				12.94±0.89
East	5.32±3.50	0.96±0.13	0.92±0.20			12.81±0.55
South	5.48±4.09	8.83±4.53	8.6±4.62	8.81±3.35		17.41±1.54
Tenom, Malaysia	5.48±4.27	1.2±0.70	1.58±0.43	9.12±3.34	0.25±0.05	12.83±0.10

CHAPTER V

DISCUSSION

Considering sampling collections, *Apis andreniformis* from the east and the west of Thailand are higher abundant than those from the north and the south of Thailand (Figure 17). It might be that first 2 parts of Thailand have abundant food sources and suitable habitats for this honeybee species. *A. andreniformis* was not found in the central and the northeastern parts of Thailand. It may be that these regions have lower abundant forest area. However, more localities such as Nam Nao National Park, Petchaboon, Sakaerat Environmental Research Station, Nakhon Ratchasima, etc should be surveyed. In addition, absence of *A. andreniformis* from the central and the northeastern parts of Thailand might be affected by a migratory season of this honey bee species. Field trip should be performed more often. It should be better if a survey can be performed in all seasons. This result is as same as the result of Wongsiri *et al*. in 1996 (Figure 3). They reported that *A. andreniformis* can be found in at least 7 provinces in Thailand, especially in Chanthaburi province (the eastern part of Thailand).

In this study, selectable morphometric characters (Figure 6-16) were according to Ruttner (1988), Tilde *et al.* (2000), Hepburn *et al*. (2001), and Chaiyavong (2001).

The result of linear regression analysis of factor scores against latitude shows clinal patterns in the characters of *A. andreniformis* in Thailand (Figure 27-34). Bees increase in size from the south to the north of Thailand. In addition, *A. andreniformis* in Thailand decrease in size from the west to the east. A physical factor affects this morphology may be related to altitude of the area more than the east-west direction of the country. Considering geography of Thailand, altitude of the west is higher than

that of the east of Thailand. This result coincides to Bergman's rule that geographic races of one species are larger in the north or higher altitude area than those in the south or lower altitude area (Ruttner, 1988). This rule operates that larger animals have a lower surface area to volume ratio than smaller animals. Thus, they radiate less body heat and stay warmer in cold climates. On the other hand, warmer climates impose the opposite problem. Body heat generated by metabolism needs to be dissipated quickly rather than stored within. Thus, the higher surface area-to-weight ratio in hot and dry climates facilitates heat loss through the skin and helps cooling of the body. Verma (1995) also reported that bees became progressively smaller from the west to the east.

Moreover, the above result is similar to Hepburn *et al.* (2001). They reported that *A. cerana* from the southern Himalayan region decrease in size from the west to the east but increase in size with increasing altitude.

Not only we determine a variation by morphometric analysis, but we also detect genetic variation. First of all, we had to extract mitochondrial DNA (mtDNA) from bees. A thorax had been used in order to avoid pigment contamination (from compound eyes) and plant DNA contamination (from an abdomen). Since mtDNA is very small, we had to assay the quality of genomic DNA instead. High MW and sharp band of genomic DNA should be observed in order to indicate a good quality (Figure 35). It is under an assumption that if genomic DNA is in good condition, so does mtDNA. After that, a part of *ND4* region (with the expected size of 540 bp) on mtDNA was amplified. Although we had tried many PCR conditions, double bands of PCR products were always obtained (Figure 37). We had attempted to obtain 2 bands separatedly. For example, we used higher percent of agarose (1.5% in stead of 0.8%) for electrophoresis. Unfortunatedly, we could not separate 2 bands out of each

other. It might be possible that we should have tried much higher percent of agarose such as 2% or tried to perform electrophoresis under the lower Voltage. It shoud be good if we could reveal that 2 appeared bands came from the same gene or not. Anyway, it might be possible that 2 bands are from heteroplamy due to different copy sizes of mtDNA within a cell. Alternatively, it may be that the specificity of designed primers is not good enough. The primers can amplify more than one subunit of NADH dehydrogenase genes of mtDNA (*ND1-6* and *ND4L*) because the sequence of these subunits shares a lot in common. On the other hand, if we consider the sequence of *ND4* itself, there are nucleotide repeats within the sequence. Thus, the primers might be able to anneal more than one position within the *ND4* sequence. After many attempts, we failed to obtain a single band for *ND4* amplification. Then, we decided to amplify a part of *cytb* in stead. As expected, a product of 520 bp was obtained (Figure 36).

For further experiments, we digested PCR products of *cytb* by 2 restriction endonucleases (*Alu*I and *Dra*I). By *Alu*I, 6 haplotypes of bees could be classified while 3 haplotypes could be classified by *Dra*I (Figure 38-39). The result indicates that polymorphism could be determined among bees, both within Thailand and between Thailand and Tenom, Malaysia. This result supports that RFLP is efficient enough to investigate genetic variation in honey bees. For example, Sihanuntavong *et al*. (1999) found 12 composite haplotypes of *A. cerana* in Thailand by *Dra*I restriction analysis of amplified mitochondrial *srRNA* and *lrRNA* genes and intergenic *COI-COII* region. Sittipraneed *et al*. (2001) also reported 4 haplotypes of *A. cerana* in Thailand after digested PCR product of *lrRNA* by *Dra*I.

For our research, variation could be detected by PCR-RFLP analysis in *cytb* gene among *A. andreniformis* from various parts of Thailand. In contrast, there are

some reports that PCR-RFLP could not be used. In 2001, Nanork found no variation among sympatric species, *A. florea,* in Thailand by PCR-RFLP analysis in *CytbI-tRNA*^{ser} coding gene of mtDNA.

Considering sequences of amplified *cytb,* it indicates low levels of genetic diversity. Its mean of sequence divergence of *A. andreniformis* in Thailand is only 5.07% while mean of sequence divergence between *A. andreniformis* and sympatric species *A. florea* is 14.04% (Table 5). In addition, low polymorphism is observed in *cytb* sequences (73 point mutations from 400 nucleotides in length). Sittipraneed *et al*. (2001) also reported lower level of polymorphism of *lrRNA* coding sequences of *A. cerana* population in Thailand (57 point mutations from 653-654 nucleotides). In contrast, Smith and Hagen (1996) sequenced the non-coding intergenic region of *COI-COII* (68-73 nucleotides) of 110 *A. cerana* individuals. They found 35 point mutations (47.94%-51.47%). It implies that although *cytb* presents low polymorphism, it can be still used for genetic diversity. However, in the future, partial sequence of non-coding regions which can show high polymorphism should be used to determine intraspecific variation.

According to phylogenetic analysis by NJ and UPGMA (Figure 42 and 43), 2 main groups of *A. andreniformis* can be distinguished.

Group A (bees from mainland of Thailand and all bees from Tenom) shows low molecular differentiation between bees from main land of Thailand and Tenom, Malaysia. It is probably that *A. andreniformis* from both 2 regions were colonized by the same ancestor. Alternatively, bees from both areas can fly to both areas so gene flow can still occur in both regions. The obtained result coincides to Oldroyd and Wongsiri (2006). However, both NJ and UPGMA trees reveal that bees of Tenom,

Malaysia have minor separation from bees of main land of Thailand by 86% of boostrap probability.

In addition, Group A shows low genetic variation within *A. andreniformis* from main land of Thailand, especially between bees from the western and the eastern parts of Thailand. The explanation for the low molecular differentiation among these bees of Thailand is probably a result of their migratory behavior (absconding and swarming) throughout the regions. It indicates that bees were not isolated by distance or geographic border. The data coincide to bees in Group B, from Chiang Mai (northern) and Phuket (southern) of Thailand. Although geography of Chiang Mai (native in conserved area and forest) and Phuket (invaded by new building and tourism) are different, genetic diversity of bees are undetectable. There are some reports on migratory behavior in *Apis* spp. Colonies of dwarf honey bees (*A. florea*) are undergoing migration at least one time per year (Wongsiri *et al*., 1996; Oldroyd and Wongsiri, 2006). *A. andreniformis* are prone to abscond after an attack by enemies such as bee mites, ants, nest disturbance, loss of shade (Oldroyd and Wongsiri, 2006). The most dangerous predator of bees is human as bee hunters (Crane, 1993; Wongsiri *et al*., 1996). The maximum distances an *Apis* swarms and absconds are unclear. Due to theoretical calculation, fully laden honeybees which their honey stomach is full of food can fly to the fares distances of about 100 km (Oldroyd, and Wongsiri, 2006).

Moreover, the evolution rate of *cytb* gene which is full of coding regions is slower than non-coding regions (Cornuet and Garney, 1991; Hepburn *et al*., 2001). This may involve the result of low variation among *A. andreniformis* from main land of Thailand and Tenom, Malaysia.

Both NJ and UPGMA trees revealed that genetic variation within group of Group B is higher than the variation in Group A. Remarkably, the sequence divergence between *A. florea* and *A. andreniformis* of Group B were higher than that between *A. florea* and *A. andreniformis* from main land of Thailand (Group A). It implies that bees from Group B have greater mutation accumulation than Group A. This result suggests that *A. andreniformis* from Group B (some colonies from Chiang Mai and all from Phuket Island) are derived from Group A.

Based on morphometric analysis, *A. andreniformis* from Thailand are clumped into one group. It may be possible that colony number is low (30 colonies). Thus, more colonies may be required. In addition, sampling areas should be wider. Alternatively, other regions such as intergenic region, intron of nuclear genes, and other mitochondrial genes should be tried.

In this research, PCR-RFLP and direct sequencing are able to reveal genetic diversity. Nucleotide judgement depends on an obvious peak of electropherogram. Lower peaks of noise on an electropherogram were appeared so the obtained result should be reliable. For future experiments, sequences obtained from cloning should be performed since this technique is very reliable. Nevertheless, patterns of distribution and biological diversity of *A. andreniformis* should be further studied in order that we can conserve them in our ecosystem.

CHAPTER VI

CONCLUSIONS

1. Due to factor analysis, 2 clusters of bees can be distinguished. First cluster contains bees from the north, the east, and the west of Thailand. Second cluster contains bees from the south of Thailand and Tenom, Malaysia. However, there are some overlapping colonies between clusters.

2. Considering to cluster analysis, it demonstrates that *A. andreniformis* from Thailand and Tenom, Malaysia are clumped into one group. Thus, this analysis shows no discernible population structure of bees.

4. By linear regression analysis, clinal patterns in the characters of *A. andreniformis* in Thailand were determined. *A. andreniformis* increase in size from the south to the north of Thailand. In addition, bees from the west to the east of Thailand decrease in size.

5. PCR products of *cytb* of mtDNA were digested by *Alu*I and *Dra*I restriction endonucleases. Six patterns of *Alu*I restricted fragments was observed whereas 3 different patterns of *Dra*I restricted fragments were visible between bees from Thailand and Tenom, Malaysia. Thus, polymorphism can be detected among *A. andreniformis*. Also, higher polymorphism is found in bees in Thailand.

6. Sequences of amplified *cytb* coding gene of *A. andreniformis* indicate low level of genetic diversity among bees originating from different geographic localities in

Thailand and Tenom, Malaysia. The mean of sequence divergence of *cytb* among bees in Thailand is 5.07% whereas that between *A. andreniformis* and sympatric species, *A. florea,* was 14.04%. In addition, a low level of polymorphism is observed in *cytb* sequences (73 point mutations from 400 nucleotides).

7. According to NJ and UPGMA trees, 2 main groups of *A. andreniformis* from Thailand and Tenom, Malaysia can be distinguished. The 1st main group (Group A) is composed of bees from mainland (the north, the west, the east, and the south) of Thailand and all bees from Tenom, Malaysia. The 2nd main group (Group B) is composed of bees from Chiang Mai (the north) and all bees from Phuket (the south) of Thailand.

8. Due to our data, morphometry cannot determine variation of *A. andreniformis* collected in Thailand. In contrast, PCR-RFLP is effective enough in analyzing the difference of bees in Thailand and Tenom, Malaysia. The best analysis for this study is direct sequencing.

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APPENDICES

APPENDIX I

Collection of *Apis andreniformis* from Thailand and Tenom, Sabha, Malaysia

No	Sampling area	code	Coordinate	
			Latitude	Longitude
1	Chiang Mai 1	N01	18’ 53.215 N	98’ 51.677 E
2	Chiang Mai 2	N02	18’ 53.215 N	98’ 51.677 E
3	Chiang Mai 3	N03	18’ 53.215 N	98’ 51.677 E
4	Chiang Mai 4	N04	19’ 37.656 N	98’ 57.591 E
5	Chiang Mai 5	N05	18’ 53.215 N	98’ 51.677 E
6	Chiang Mai 6	N06	18’ 54.731 N	98’ 47.135 E
7	Chiang Mai 7	N07	18’ 52.362 N	98’ 47.637 E
8	Nakhon Ratchasima 1	E01	14’ 48.495 N	101’ 54.631 E
9	Trat 1	Se01	12’ 22. 839 N	102’ 27.426 E
10	Trat 2	Se02	12’ 22. 839 N	102’ 27.426 E
11	Chanthaburi 1	Se03	12’ 30.738 N	102’ 10.583 E
12	Chanthaburi 2	Se04	12’ 30.738 N	102’ 10.583 E
13	Chanthaburi 3	Se05	12’ 30.738 N	102’ 10.583 E
14	Chanthaburi 4	Se06	12’ 30.738 N	102’ 10.583 E
15	Chanthaburi 5	Se07	12’ 30.738 N	102’ 10.583 E
15	Chanthaburi 6	Se08	12’ 30.738 N	102’ 10.583 E
17	Chanthaburi 7	Se09	12’ 30.738 N	102’ 10.583 E
18	Phetchaburi 1	Sw01	12’ 47.830 N	99’ 27. 463 E
19	Phetchaburi 2	Sw02	12’ 47.830 N	99’ 27. 463 E
20	Phetchaburi 3	Sw03	12’ 47.830 N	99’ 27. 463 E
21	Kanchanaburi 1	Sw04	14’ 36.361 N	98’ 34.854 E
22	Kanchanaburi 2	Sw05	14’ 36.361 N	98’ 34.854 E
23	Kanchanaburi 3	Sw06	14’ 36.361 N	98’ 34.854 E
24	Kanchanaburi 4	Sw07	14’ 36.361 N	98’ 34.854 E
25	Kanchanaburi 5	Swo8	14’ 36.361 N	98’ 34.854 E
26	Kanchanaburi 6	Sw09	14’ 36.361 N	98’ 34.854 E
27	Kanchanaburi 7	Sw11	14’ 12.573 N	99’ 14.481 E
28	Phuket 1	S01	07’ 59.853 N	98’ 23.658 E
29	Phuket 2	S02	07’ 59.853 N	98’ 23.658 E
30	Phuket 3	S03	07’ 53.880 N	98’ 19.987 E
31	Phuket 4	S04	08’ 04.111 N	98’ 20.739 E
32	Surat Thani 1	S05	09’ 00.787 N	99’ 02.812 E
33	Surat Thani 2	S06	08’49.377 N	98’ 48.769 E
34	Pung-nga 1	S07	08’ 31.550 N	98’ 31.263 E
36	Tenom 2, Malaysia	Tn02	5' 03.586 N	116' 15.2491E
37	Tenom 3, Malaysia	Tn03	5' 03.573 N	116' 15.55 E
38	Tenom 4, Malaysia	Tn04	5' 03.586 N	116' 15.25 E
39	Tenom 5, Malaysia	Tn05	5' 03.586 N	116' 15.25 E
40	Tenom 6, Malaysia	Tn06	5' 03.586 N	116' 15.25 E

APPENDIX II

Means and Standard Deviation of morphometric characters of *Apis andreniformis* in Thailand and Tenom, Sabha, Malaysia

colony no.		FWL	FWW	RFWL	HWL	HWW	TG3L	TG3W	TG4L
Chiang Mai 1 (n01)	Mean	6.331529	3.164127	2.518400	4.504128	1.241418	5.380964	1.308577	5.122754
	Std. Deviation	0.0869421	0.0498813	0.0402081	0.0926366	0.0241937	0.0971518	0.0252362	0.1035096
Chiang Mai 3 (n03)	Mean	6.021163	3.011490	2.383544	4.532998	1.241854	5.400643	1.320053	5.138175
	Std. Deviation	0.7663295	0.3828385	0.2969553	0.0716998	0.0269171	0.1056293	0.0319790	0.0929655
Chiang Mai 4 (n04)	Mean	6.310125	3.156480	2.492246	4.472927	1.233642	5.374822	1.314464	5.126037
	Std. Deviation	0.0659323	0.0382955	0.0423784	0.0666824	0.0234591	0.0698101	0.0282277	0.0677403
Chiang Mai 5 (n05)	Mean	6.353975	3.193576	2.518085	4.591327	1.219955	5.507033	1.352935	5.236489
	Std. Deviation	0.1173291	0.0571597	0.0436407	0.0868962	0.0188210	0.0959945	0.0198107	0.0898411
Chiang Mai 6 (n06)	Mean	6.394037	3.228499	2.568688	4.639033	1.216924	5.466505	1.335315	5.205319
	Std. Deviation	0.0595436	0.0316842	0.0332045	0.0601391	0.0234223	0.0962846	0.0326803	0.0811487
Phuket 1 (s01)	Mean	5.770400	2.891135	2.332683	4.052752	1.076990	4.840725	1.208715	4.573118
	Std. Deviation	0.0820789	0.0489248	0.0183044	0.0377199	0.0358678	0.0399682	0.0098859	0.0648952
Phuket 3 (s03)	Mean	5.996524	2.972019	2.366762	4.262369	1.113476	5.151868	1.267261	4.932855
	Std. Deviation	0.0728535	0.0347604	0.0478601	0.0427741	0.0132801	0.1047732	0.0218859	0.1037780
Phuket 4 (s04)	Mean	6.231305	3.093857	2.479638	4.394454	1.159265	5.275058	1.289715	4.998551
	Std. Deviation	0.1177995	0.0505068	0.0434330	0.1190755	0.0303962	0.0916081	0.0240013	0.0981397
Surat Thani 1 (s05)	Mean	6.023032	2.981601	2.383695	4.396989	1.157358	5.268669	1.315770	5.003220
	Std. Deviation	0.4521997	0.2317244	0.1878073	0.0803637	0.0244163	0.0894513	0.0422834	0.0849926
Surat Thani 2 (s06)	Mean	6.174393	3.080921	2.453225	4.387846	1.158801	5.258533	1.301882	5.013112
	Std. Deviation	0.1043961	0.0515763	0.0380891	0.0870303	0.0337555	0.0693558	0.0318518	0.0838441
Punganga 1 (s07)	Mean	6.082818	3.050892	2.414464	4.386937	1.154652	5.183924	1.295229	4.890169
	Std. Deviation	0.0541697	0.0357624	0.0448860	0.0751585	0.0217589	0.1117356	0.0296869	0.1338701
Trat 1 (se01)	Mean	6.265221	3.136595	2.472073	4.495903	1.215772	5.395699	1.320850	5.137962
	Std. Deviation	0.0768971	0.0343854	0.0431018	0.0664290	0.0238696	0.0767259	0.0303686	0.0605989
Trat 2 (se02)	Mean	6.107483	3.057952	2.414726	4.397196	1.149353	5.343206	1.313015	5.060668
	Std. Deviation	0.1004064	0.0514033	0.0415742	0.0845120	0.0314661	0.0785693	0.0193445	0.1355010

colony no.		FWL	FWW	RFWL	HWL	HWW	TG3L	TG3W	TG4L
Chanthaburi 1 (se03)	Mean	6.179717	3.089544	2.442444	4.451737	1.185238	5.380751	1.324971	5.074732
	Std. Deviation	0.0786733	0.0448884	0.0394821	0.0800234	0.0264387	0.0988926	0.0276596	0.0793751
Chanthaburi 2 (se04)	Mean	6.179752	3.067101	2.457269	4.386651	1.183198	5.402084	1.309234	5.130265
	Std. Deviation	0.0821997	0.0466495	0.0410473	0.0734516	0.0220140	0.0893079	0.0334750	0.0882727
Chanthaburi 3 (se05)	Mean	6.174887	3.068036	2.458953	4.385262	1.185245	5.391494	1.305243	5.120230
	Std. Deviation	0.0820673	0.0470756	0.0414460	0.0738037	0.0213923	0.0953813	0.0300598	0.0884025
Chanthaburi 4 (se06)	Mean	6.182197	3.090298	2.446956	4.444698	1.189522	5.379438	1.325532	5.073900
	Std. Deviation	0.0803055	0.0462898	0.0398654	0.0797377	0.0275800	0.0986392	0.0272526	0.0789874
Chanthaburi 5 (se07)	Mean	6.114427	3.046076	2.403481	4.341686	1.155163	5.226972	1.281059	4.943251
	Std. Deviation	0.0823665	0.0430513	0.0375521	0.0726769	0.0236916	0.1111268	0.0294409	0.1142248
Chanthaburi 6 (se08)	Mean	6.235413	3.088796	2.472954	4.453891	1.198807	5.403512	1.298927	5.124743
	Std. Deviation	0.0314845	0.0253046	0.0307252	0.0619081	0.0204952	0.0595001	0.0256991	0.0596812
Phetchaburi 1 (sw01)	Mean	6.177642	3.072882	2.463727	4.404881	1.188279	5.379068	1.275412	5.096881
	Std. Deviation	0.0890998	0.0503843	0.0470828	0.0847307	0.0290895	0.0959455	0.0248145	0.0960534
Phetchaburi 2 (sw02)	Mean	6.283610	3.127611	2.463945	4.513279	1.256939	5.499575	1.319929	5.229523
	Std. Deviation	0.1036206	0.0696749	0.0535504	0.0862189	0.0338258	0.1216295	0.0375450	0.1107888
Kanchanaburi 1 (sw04)	Mean	6.179212	3.107394	2.444067	4.410684	1.203270	5.322636	1.330916	5.083199
	Std. Deviation	0.1226827	0.0811478	0.0806106	0.0807433	0.0282303	0.0792792	0.0394038	0.0867920
Kanchanaburi 2 (sw05)	Mean	6.212266	3.116248	2.456951	4.454147	1.215526	5.314263	1.281745	5.083595
	Std. Deviation	0.0882243	0.0571148	0.0429885	0.0893244	0.0231896	0.1304954	0.0386796	0.1225741
Kanchanaburi 3 (sw06)	Mean	6.274057	3.143799	2.483909	4.496407	1.173580	1.312787	5.430396	5.162324
	Std. Deviation	0.0772028	0.0491173	0.0537056	0.0555138	0.0234007	0.0180508	0.0697023	0.0800135
Kanchanaburi 4 (sw07)	Mean	6.090870	3.036728	2.413054	4.366953	1.188905	5.286818	1.291725	5.014607
	Std. Deviation	0.1293646	0.0710603	0.0669483	0.1002306	0.0311932	0.1251154	0.0376778	0.1144876
Kanchanaburi 5 (sw08)	Mean	6.244574	3.118780	2.454357	4.459282	1.225617	5.449813	1.350978	5.190766
	Std. Deviation	0.1096240	0.0624789	0.0430283	0.0795144	0.0263894	0.0747350	0.0236644	0.0734851
Kanchanaburi 7 (sw11)	Mean	6.196568	3.108619	2.458036	4.453082	1.205774	5.312006	1.278271	5.071085
	Std. Deviation	0.1074309	0.0594677	0.0591543	0.0875895	0.0262346	0.0903864	0.0299203	0.0958433
Tenom 2, Sabha, Malaysia (tn02)	Mean	6.225868	3.113010	2.486029	4.450706	1.203118	5.265403	1.291375	5.018665
	Std. Deviation	0.0769357	0.0441270	0.0286263	0.0680829	0.0248038	0.0873097	0.0294022	0.1106196

Tenom 4, Sabha, Malaysia (tn02)	Mean	6.092596	3.028905	2.419531	4.356255	1.151847	5.321811	1.307498	5.051450
	Std. Deviation	0.0713507	0.0396398	0.0404329	0.0579577	0.0184627	0.1127598	0.0233780	0.1072543
Tenom 5, Sabha, Malaysia (tn02)	Mean	6.371451	3.176098	2.528321	4.574542	1.202565	5.334238	1.312886	5.035953
	Std. Deviation	0.0828065	0.0347812	0.0330735	0.0636864	0.0234038	0.1276691	0.0218256	0.0949775
Total	Mean	6.182570	3.087302	2.451740	4.430633	1.188402	5.194344	1.441996	5.064787
	Std. Deviation	0.2195731	0.1151080	0.0902296	0.1288875	0.0459592	0.7375370	0.7423781	0.1542789

colony no.		TG4W	ST3W	ST3WL	ST3WW	ST4W	ST4WL	ST4WW	ST6W
Chiang Mai 1 (n01)	Mean	1.249907	1.153050	1.353571	0.675861	1.158147	1.330893	0.712684	1.190320
	Std. Deviation	0.0290053	0.0275296	0.0350517	0.0243887	0.0216869	0.0339181	0.0250528	0.0207585
Chiang Mai 3 (n03)	Mean	1.254383	1.162827	1.357409	0.665887	1.174834	1.324852	0.705637	1.191430
	Std. Deviation	0.0315668	0.0326174	0.0268899	0.0215318	0.0386620	0.0290199	0.0169272	0.0306287
Chiang Mai 4 (n04)	Mean	1.255031	1.170355	1.364969	0.677164	1.169871	1.335770	0.715250	1.188331
	Std. Deviation	0.0278517	0.0253379	0.0232011	0.0241035	0.0260709	0.0240777	0.0255400	0.0248261
Chiang Mai 5 (n05)	Mean	1.297309	1.188208	1.370530	0.650981	1.197711	1.323997	0.699091	1.208707
	Std. Deviation	0.0269181	0.0285164	0.0319081	0.0276388	0.0393719	0.0381970	0.0328887	0.0224007
Chiang Mai 6 (n06)	Mean	1.281578	1.185427	1.355561	0.626980	1.194338	1.337266	0.696975	1.224795
	Std. Deviation	0.0303745	0.0297332	0.0450831	0.0293492	0.0315136	0.0280365	0.0284947	0.0194770
Phuket 1 (s01)	Mean	1.144766	1.054482	1.233627	0.562234	1.059938	1.207622	0.606394	1.069029
	Std. Deviation	0.0215783	0.0041177	0.0264556	0.0154513	0.0094537	0.0377719	0.0233912	0.0404352
Phuket 3 (s03)	Mean	1.217819	1.096124	1.311982	0.618235	1.081971	1.287963	0.654826	1.140242
	Std. Deviation	0.0344485	0.0240199	0.0323834	0.0260171	0.0215429	0.0398419	0.0263874	0.0208370
Phuket 4 (s04)	Mean	1.237806	1.098921	1.320793	0.627082	1.091766	1.292665	0.667939	1.145989
	Std. Deviation	0.0302433	0.0255069	0.0333399	0.0227220	0.0205955	0.0414700	0.0266407	0.0192957
Surat Thani 1 (s05)	Mean	1.250803	1.157039	1.348458	0.629073	1.159635	1.304948	0.669844	1.200591
	Std. Deviation	0.0428590	0.0304668	0.0480544	0.0312406	0.0420897	0.0507764	0.0306004	0.0391737
Surat Thani 2 (s06)	Mean	1.257055	1.129161	1.281181	0.596935	1.127262	1.273087	0.643209	1.165884
	Std. Deviation	0.0345888	0.0254752	0.0413546	0.0316898	0.0197361	0.0502271	0.0313402	0.0201266
Punganga 1 (s07)	Mean	1.234839	1.131456	1.261418	0.622466	1.133631	1.252382	0.660189	1.140144
	Std. Deviation	0.0312409	0.0232791	0.0297219	0.0419157	0.0293102	0.0325144	0.0448816	0.0298023
Trat 1 (se01)	Mean	1.268824	1.156837	1.357760	0.646644	1.147652	1.349106	0.685127	1.175540
	Std. Deviation	0.0355904	0.0300862	0.0319241	0.0273925	0.0339743	0.0290000	0.0228859	0.0165300
Trat 2 (se02)	Mean	1.251608	1.151062	1.321312	0.624532	1.139299	1.290135	0.660099	1.160915
	Std. Deviation	0.0400097	0.0287928	0.0362498	0.0271068	0.0423077	0.0313131	0.0279499	0.0194069
Chanthaburi 1 (se03)	Mean	1.262778	1.153367	1.348328	0.646033	1.148153	1.323245	0.682653	1.164975
	Std. Deviation	0.0258909	0.0321707	0.0316471	0.0193335	0.0336973	0.0306056	0.0223167	0.0263669
Chanthaburi 2 (se04)	Mean	1.251808	1.149763	1.356102	0.611550	1.142988	1.332975	0.656695	1.160250
	Std. Deviation	0.0283244	0.0306293	0.0365458	0.0224787	0.0295315	0.0319431	0.0192210	0.0261893

colony no.		TG4W	ST3W	ST3WL	ST3WW	ST4W	ST4WL	ST4WW	ST6W
Chanthaburi 3 (se05)	Mean	1.248442	1.151306	1.358672	0.611223	1.141333	1.330572	0.657562	1.170405
	Std. Deviation	0.0284854	0.0308622	0.0335383	0.0233080	0.0286991	0.0301351	0.0206913	0.0278868
Chanthaburi 4 (se06)	Mean	1.262891	1.152759	1.345881	0.642382	1.147414	1.325676	0.684114	1.177184
	Std. Deviation	0.0240415	0.0323997	0.0311217	0.0191827	0.0339623	0.0324832	0.0217964	0.0257484
Chanthaburi 5 (se07)	Mean	1.224566	1.111630	1.320450	0.632061	1.104889	1.306359	0.675151	1.132432
	Std. Deviation	0.0273260	0.0312680	0.0300162	0.0203725	0.0249762	0.0269879	0.0171566	0.0229683
Chanthaburi 6 (se08)	Mean	1.239723	1.142762	1.382441	0.644900	1.132046	1.360744	0.682358	1.145718
	Std. Deviation	0.0295337	0.0175780	0.0165900	0.0231247	0.0212500	0.0163123	0.0176192	0.0143912
Phetchaburi 1 (sw01)	Mean	1.223432	1.147126	1.364954	0.631279	1.170455	1.372196	0.692478	1.130612
	Std. Deviation	0.0231328	0.0293100	0.0291352	0.0255939	0.1153818	0.1539671	0.0710822	0.0240329
Phetchaburi 2 (sw02)	Mean	1.260389	1.153002	1.352267	0.645249	1.160155	1.325620	0.681569	1.147522
	Std. Deviation	0.0369274	0.0349173	0.0291490	0.0244642	0.0367270	0.0330906	0.0201045	0.0238996
Kanchanaburi 1 (sw04)	Mean	1.284828	1.171565	1.343459	0.658823	1.160314	1.328442	0.705297	1.193179
	Std. Deviation	0.0394703	0.0273646	0.0258234	0.0231074	0.0259839	0.0166054	0.0210230	0.0200712
Kanchanaburi 2 (sw05)	Mean	1.219854	1.151674	1.346877	0.619969	1.151839	1.330332	0.659858	1.170169
	Std. Deviation	0.0326527	0.0356850	0.0394051	0.0296732	0.0314041	0.0361333	0.0271521	0.0292729
Kanchanaburi 3 (sw06)	Mean	1.252813	1.141995	1.345571	0.616450	1.137428	1.312792	0.671626	1.152308
	Std. Deviation	0.0290676	0.0198006	0.0292529	0.0234589	0.0288114	0.0278864	0.0206100	0.0244874
Kanchanaburi 4 (sw07)	Mean	1.229516	1.126070	1.359153	0.633258	1.113338	1.329793	0.669358	1.141164
	Std. Deviation	0.0367609	0.0425097	0.0349000	0.0307804	0.0390020	0.0311249	0.0279208	0.0312951
Kanchanaburi 5 (sw08)	Mean	1.290628	1.180491	1.354675	0.669183	1.175498	1.330393	0.705988	1.189176
	Std. Deviation	0.0311744	0.0330495	0.0256146	0.0262344	0.0301235	0.0253154	0.0202884	0.0207338
Kanchanaburi 7 (sw11)	Mean	1.218971	1.149152	1.351721	0.624829	1.149376	1.326147	0.665364	1.171260
	Std. Deviation	0.0316011	0.0322152	0.0298296	0.0232568	0.0334514	0.0281418	0.0175301	0.0249642
Tenom 2, Sabha, Malaysia (tn02)	Mean	1.205113	1.157935	1.306847	0.622477	1.156359	1.289636	0.659212	1.133100
	Std. Deviation	0.0264655	0.0217385	0.0241005	0.0242730	0.0297099	0.0241752	0.0201625	0.0210243
Tenom 4, Sabha, Malaysia (tn02)	Mean	1.244764	1.136261	1.304698	0.636553	1.107224	1.289470	0.673551	1.147156
	Std. Deviation	0.0223377	0.0233137	0.0333303	0.0293530	0.0231629	0.0321169	0.0267696	0.0143760
Tenom 5, Sabha, Malaysia (tn02)	Mean	1.237295	1.136524	1.302310	0.609230	1.124824	1.283647	0.636230	1.151935
	Std. Deviation	0.0245767	0.0293036	0.0264290	0.0212803	0.0301288	0.0286986	0.0309270	0.0185460

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Total	Mean	1.245318	1.144944	1.336099	0.632651	1.141990	1.313624	0.674544	1.162707
	Std. Deviation	0.0416802	0.0390463	0.0456910	0.0345702	0.0471243	0.0526187	0.0362907	0.0381968

colony no.		ST6WW	AN	PB	TBW	TBL	FML	BSTL	BSTW
Chiang Mai 1 (n01)	Mean	0.798374	2.771268	2.830948	0.651095	2.114026	1.704514	1.545064	0.631244
	Std. Deviation	0.0192185	0.0417326	0.0423534	0.0194561	0.0386821	0.0340658	0.0236689	0.0152206
Chiang Mai 3 (n03)	Mean	0.789335	2.761871	2.822382	0.663798	2.120376	1.715473	1.537577	0.627303
	Std. Deviation	0.0261592	0.0352248	0.0277801	0.0145269	0.0234867	0.0241693	0.0188017	0.0138936
Chiang Mai 4 (n04)	Mean	0.807970	2.743002	2.823310	0.657001	2.105347	1.704877	1.548090	0.638484
	Std. Deviation	0.0165296	0.0361071	0.0303597	0.0205199	0.0339456	0.0272003	0.0202392	0.0155746
Chiang Mai 5 (n05)	Mean	0.794269	2.798131	2.857573	0.653857	2.109430	1.719796	1.547683	0.637395
	Std. Deviation	0.0216325	0.0489947	0.0388974	0.0243411	0.0484822	0.0328259	0.0434686	0.0200260
Chiang Mai 6 (n06)	Mean	0.793265	2.778274	2.843679	0.745111	2.147718	1.747261	1.554503	0.640549
	Std. Deviation	0.0234721	0.0328864	0.0297742	0.0208204	0.0371370	0.0239774	0.0276991	0.0082666
Phuket 1 (s01)	Mean	0.714775	2.610558	2.577344	0.666138	1.962788	1.571797	1.431248	0.591055
	Std. Deviation	0.0235782	0.0332310	0.0181784	0.0156045	0.0405878	0.0199706	0.0347738	0.0113395
Phuket 3 (s03)	Mean	0.745699	2.664621	2.751349	0.706794	2.037766	1.641580	1.470673	0.625209
	Std. Deviation	0.0149292	0.0420623	0.0506433	0.0119660	0.0158518	0.0157140	0.0163471	0.0131778
Phuket 4 (s04)	Mean	0.749467	2.708075	2.766602	0.716930	2.076352	1.667410	1.499592	0.617513
	Std. Deviation	0.0165729	0.0405123	0.0489219	0.0118100	0.0417676	0.0371505	0.0434161	0.0144484
Surat Thani 1 (s05)	Mean	0.775846	2.707115	2.787232	0.702650	2.083144	1.679371	1.504401	0.620667
	Std. Deviation	0.0335266	0.0400697	0.0391139	0.0256422	0.0396976	0.0307093	0.0256532	0.0255692
Surat Thani 2 (s06)	Mean	0.750434	2.693867	2.758726	0.701297	2.081181	1.674310	1.486027	0.612127
	Std. Deviation	0.0296491	0.0367660	0.0276512	0.0168677	0.0290395	0.0272237	0.0145806	0.0165949
Pung-nga 1 (s07)	Mean	0.723861	2.737458	2.836629	0.702604	2.057309	1.664242	1.485775	0.615948
	Std. Deviation	0.0295533	0.0497480	0.3326779	0.0157610	0.0230833	0.0269558	0.0252323	0.0128276
Trat 1 (se01)	Mean	0.768002	2.733216	2.813367	0.664996	2.124619	1.700384	1.514453	0.639298
	Std. Deviation	0.0180331	0.0416275	0.0494987	0.0153468	0.0345830	0.0257625	0.0184548	0.0176183
Trat 2 (se02)	Mean	0.767784	2.714215	2.758174	0.651020	2.056626	1.679676	1.514973	0.630609
	Std. Deviation	0.0187367	0.0326100	0.0344130	0.0257027	0.0467079	0.0302726	0.0287185	0.0153830
Chanthaburi 1 (se03)	Mean	0.776938	2.717290	2.798011	0.682161	2.105362	1.678730	1.528703	0.646222
	Std. Deviation	0.0200002	0.0379010	0.0334802	0.0220181	0.0275099	0.0242873	0.0247575	0.0135168
Chanthaburi 2 (se04)	Mean	0.772737	2.719054	2.770252	0.665885	2.098868	1.677415	1.502880	0.628200
	Std. Deviation	0.0198647	0.0441330	0.0380651	0.0100294	0.0370082	0.0257672	0.0267962	0.0147598

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colony no.		ST6WW	AN	PB	TBW	TBL	FML	BSTL	BSTW
Chanthaburi 3 (se05)	Mean	0.768667	2.722890	2.771509	0.663542	2.100661	1.679248	1.502218	0.632784
	Std. Deviation	0.0220478	0.0385199	0.0398131	0.0111873	0.0353366	0.0265218	0.0271791	0.0150160
Chanthaburi 4 (se06)	Mean	0.773224	2.717236	2.800364	0.680797	2.108252	1.677936	1.527904	0.650642
	Std. Deviation	0.0215025	0.0320318	0.0335466	0.0226760	0.0298760	0.0255216	0.0261216	0.0124612
Chanthaburi 5 (se07)	Mean	0.746409	2.642964	2.736866	0.675118	2.059377	1.671870	1.487708	0.643116
	Std. Deviation	0.0205562	0.0353834	0.0422821	0.0245760	0.0677020	0.0496160	0.0258753	0.0145012
Chanthaburi 6 (se08)	Mean	0.774487	2.706976	2.792551	0.670627	2.078562	1.685290	1.512078	0.627874
	Std. Deviation	0.0125563	0.0418900	0.0193188	0.0165843	0.0279612	0.0209077	0.0209465	0.0146060
Phetchaburi 1 (sw01)	Mean	0.774047	2.720713	2.761402	0.639765	2.088637	1.676362	1.509139	0.611764
	Std. Deviation	0.0146663	0.0346342	0.0327351	0.0246236	0.0466930	0.0232719	0.0388287	0.0150912
Phetchaburi 2 (sw02)	Mean	0.779258	2.687845	2.797791	0.666038	2.082028	1.688678	1.482044	0.638320
	Std. Deviation	0.0181290	0.0515149	0.0425221	0.0149496	0.0319640	0.0226504	0.0357862	0.0144804
Kanchanaburi 1 (sw04)	Mean	0.790501	2.753831	2.771230	0.648875	2.083420	1.703035	1.505078	0.632621
	Std. Deviation	0.0213857	0.0492986	0.0324129	0.0203902	0.0341764	0.0271182	0.0326124	0.0167570
Kanchanaburi 2 (sw05)	Mean	0.784807	2.712283	2.764565	0.657935	2.086702	1.685161	1.498374	0.638612
	Std. Deviation	0.0158760	0.0460962	0.0487678	0.0208671	0.0391898	0.0271773	0.0299263	0.0144434
Kanchanaburi 3 (sw06)	Mean	0.764957	2.657819	2.753555	0.668474	2.076923	1.692273	1.518258	0.633568
	Std. Deviation	0.0181425	0.0364242	0.0342891	0.0221075	0.0366454	0.0228697	0.0242161	0.0139385
Kanchanaburi 4 (sw07)	Mean	0.765595	2.688836	2.743801	0.652450	2.045976	1.665094	1.492255	0.634508
	Std. Deviation	0.0201560	0.0470901	0.0517041	0.0196554	0.0590537	0.0446712	0.0414526	0.0147884
Kanchanaburi 5 (sw08)	Mean	0.787075	2.740537	2.819129	0.665870	2.111023	1.718067	1.531468	0.624073
	Std. Deviation	0.0179956	0.0333402	0.0281933	0.0120357	0.0277473	0.0312207	0.0257495	0.0165891
Kanchanaburi 7 (sw11)	Mean	0.781889	2.705209	2.772882	0.657391	2.075892	1.685101	1.493819	0.628528
	Std. Deviation	0.0156288	0.0416365	0.0428371	0.0209422	0.0422701	0.0260807	0.0290964	0.0178288
Tenom 2, Sabha, Malaysia (tn02)	Mean	0.750388	2.750668	2.789684	0.675718	2.100209	1.704749	1.521319	0.619700
	Std. Deviation	0.0223300	0.0359705	0.0273557	0.0153954	0.0349980	0.0204335	0.0218791	0.0119190
Tenom 4, Sabha, Malaysia (tn02)	Mean	0.757989	2.722903	2.768903	0.635943	2.063503	1.674632	1.479770	0.638257
	Std. Deviation	0.0177950	0.0537474	0.0279612	0.0168453	0.0307164	0.0167774	0.0280229	0.0192754
Tenom 5, Sabha, Malaysia (tn02)	Mean	0.744631	2.722961	2.802453	0.668916	2.078171	1.700022	1.522469	0.635776
	Std. Deviation	0.0238354	0.0346609	0.0386343	0.0159739	0.0370773	0.0282267	0.0272546	0.0163984

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Total	Mean	0.769089	2.717056	2.781409	0.671960	2.084008	1.684478	1.508518	0.629732
	Std. Deviation	0.0294261	0.0556092	0.0847948	0.0300825	0.0496464	0.0401719	0.0381625	0.0192661

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APPENDIX III

Factor analysis 1

Descriptive Statistics

	Mean	Std. Deviation	Analysis N
FWL	6.182268	.2196320	599
FWW	3.087170	.1151586	599
RFWL	2.451635	.0902682	599
HWL	4.430398	.1288661	599
HWW	1.188251	.0458485	599
TG3L	5.193853	.7380554	599
TG3W	1.442242	.7429741	599
TG4L	5.064228	.1537990	599
TG4W	1.245167	.0415503	599
ST3W	1.144829	.0389769	599
ST3WL	1.336025	.0456932	599
ST3WW	.632582	.0345582	599
ST4W	1.141922	.0471347	599
ST4WL	1.313554	.0526350	599
ST4WW	.674502	.0363062	599
ST6W	1.162707	.0381968	599
ST6WW	.769023	.0294052	599
AN	2.717241	.0554697	599
PB	2.781431	.0848640	599
TBW	.671943	.0301048	599
TBL	2.083990	.0496859	599
FML	1.684505	.0402001	599
BSTL	1.508590	.0381535	599
BSTW	.629732	.0192822	599

KMO and Bartlett's Test

Kaiser-Meyer-Olkin Measurement of Sampling Adequacy.		.863
Bartlett's Test of Sphericity	Approx. Chi-Square	11980.183
	df	276
	Sig.	.000

Component Matrix (a)

	Component				
	1	2	3	4	5
FWL	.599	.647	.350	-.241	.055
FWW	.611	.653	.345	-.228	.051
RFWL	.537	.669	.392	-.227	.112
HWL	.769	.141	.007	.125	-.141
HWW	.749	-.053	.037	-.107	-.218
TG3L	.161	-.600	.752	-.112	-.032
TG3W	.026	.593	-.780	.114	.003
TG4L	.780	.025	-.173	.049	-.186
TG4W	.643	-.029	-.099	.155	-.192
ST3W	.730	-.134	-.070	-.058	-.001
ST3WL	.624	-.165	-.250	-.302	-.088
ST3WW	.583	-.272	-.110	-.267	-.104
ST4W	.662	-.195	-.136	-.074	.443
ST4WL	.563	-.223	-.248	-.303	.374
ST4WW	.632	-.247	-.213	-.254	.368
ST6W	.713	-.088	.010	.134	-.020
ST6WW	.658	-.128	-.106	-.213	-.097
AN	.607	-.193	.219	.141	-.064
PB	.524	-.108	.079	.205	-.110
TBW	.014	.133	.208	.682	.368
TBL	.670	-.061	.044	.471	.166
FML	.739	-.001	.002	.411	.097
BSTL	.689	-.028	-.074	.266	-.031
BSTW	.415	.037	-.060	.178	-.462

Extraction Method: Principal Component Analysis. a 5 components extracted.

Rotated Component Matrix(a)

	Component				
	1	2	3	4	5
FWL	.403	.893	-.039	-.006	.001
FWW	.416	.893	-.049	.003	-.006
RFWL	.330	.918	-.020	.047	.022
HWL	.757	.234	-.079	.057	-.100
HWW	.743	.162	.086	-.194	-.039
TG3L	.160	-.034	.968	-.017	.015
TG3W	.028	.055	-.984	-.003	-.008
TG4L	.799	.082	-.151	-.086	-.042
TG4W	.684	.007	-.074	.010	-.111
ST3W	.721	.056	.035	-.084	.166
ST3WL	.620	-.009	-.063	-.375	.219
ST3WW	.591	-.048	.106	-.336	.180
ST4W	.602	.026	.013	.092	.570
ST4WL	.500	-.006	-.033	-.162	.618
ST4WW	.575	-.008	.004	-.119	.601
ST6W	.720	.059	.049	.092	.044
ST6WW	.648	.066	.023	-.267	.141
AN	.621	.044	.278	.111	-.031
PB	.558	-.004	.106	.122	-.096
TBW	.013	.025	.004	.812	-.027
TBL	.690	-.002	.016	.474	.051
FML	.754	.052	-.042	.389	.020
BSTL	.717	.014	-.070	.185	-.017
BSTW	.495	-.022	-.084	-.082	-.404

Extraction Method: Principal Component Analysis. Rotation Method: Quartimax with Kaiser Normalization. a Rotation converged in 5 iterations.

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	7.798	38.988	38.988	7.798	38.988	38.988	7.337	36.684	36.684
2	2.290	11.449	50.437	2.290	11.449	50.437	2.570	12.852	49.537
3	1.890	9.452	59.889	1.890	9.452	59.889	2.059	10.297	59.833
4	1.516	7.582	67.470	1.516	7.582	67.470	1.527	7.637	67.470
5	.905	4.523	71.993						
6	.849	4.246	76.238						
7	.737	3.687	79.926						
8	.704	3.518	83.443						
9	.591	2.953	86.397						
10	.545	2.725	89.121						
11	.527	2.637	91.758						
12	.462	2.312	94.070						
13	.298	1.492	95.562						
14	.244	1.222	96.784						
15	.214	1.069	97.853						
16	.186	.931	98.785						
17	.145	.725	99.510						
18	.058	.290	99.800						
19	.029	.147	99.946						
20	.011	.054	100.000						

Extraction Method: Principal Component Analysis.

APPENDIX IV

Factor analysis 2

Descriptive Statistics

	Mean	Std. Deviation	Analysis N
FWL	6.182268	.2196320	599
FWW	3.087170	.1151586	599
RFWL	2.451635	.0902682	599
HWL	4.430398	.1288661	599
HWW	1.188251	.0458485	599
TG3L	5.193853	.7380554	599
TG3W	1.442242	.7429741	599
TG4L	5.064228	.1537990	599
TG4W	1.245167	.0415503	599
ST3W	1.144829	.0389769	599
ST3WL	1.336025	.0456932	599
ST4W	1.141922	.0471347	599
ST4WL	1.313554	.0526350	599
ST6W	1.162707	.0381968	599
ST6WW	.769023	.0294052	599
AN	2.717241	.0554697	599
TBW	.671943	.0301048	599
TBL	2.083990	.0496859	599
FML	1.684505	.0402001	599
BSTL	1.508590	.0381535	599

KMO and Bartlett's Test

Kaiser-Meyer-Olkin Measurement of Sampling Adequacy.		.847
Bartlett's Test of Sphericity	Approx. Chi-Square	10755.129
	df	190
	Sig.	.000

Component Matrix (a)

	Component			
	1	2	3	4
FWL	.634	.592	.441	-.107
FWW	.644	.600	.435	-.096
RFWL	.577	.611	.484	-.072
HWL	.783	.081	-.034	.070
HWW	.749	-.093	.015	-.185
TG3L	.146	-.686	.689	-.065
TG3W	.039	.673	-.724	.051
TG4L	.784	-.008	-.204	-.029
TG4W	.643	-.063	-.145	.123
ST3W	.723	-.146	-.103	-.088
ST3WL	.606	-.138	-.229	-.389
ST4W	.652	-.198	-.151	-.087
ST4WL	.541	-.189	-.213	-.350
ST6W	.720	-.146	-.051	.103
ST6WW	.656	-.143	-.107	-.281
AN	.608	-.262	.144	.118
TBW	.029	.055	.113	.779
TBL	.680	-.145	-.062	.471
FML	.748	-.076	-.088	.402
BSTL	.687	-.080	-.135	.212

Extraction Method: Principal Component Analysis. a 4 components extracted.

Rotated Component Matrix (a)

	Component			
	1	2	3	4
FWL	.398	.895	-.035	-.002
FWW	.407	.898	-.045	.008
RFWL	.331	.914	-.018	.039
HWL	.742	.251	-.071	.082
HWW	.730	.178	.096	-.174
TG3L	.146	-.030	.974	-.009
TG3W	.042	.053	-.988	-.007
TG4L	.793	.096	-.134	-.042
TG4W	.660	.032	-.066	.110
ST3W	.742	.047	.034	-.097
ST3WL	.645	-.018	-.049	-.412
ST4W	.694	-.040	.031	-.107
ST4WL	.592	-.069	-.008	-.376
ST6W	.732	.053	.062	.099
ST6WW	.674	.053	.041	-.289
AN	.612	.054	.281	.131
TBW	.011	.017	.000	.790
TBL	.704	-.012	.028	.461
FML	.758	.051	-.031	.395
BSTL	.707	.026	-.053	.199

Extraction Method: Principal Component Analysis. Rotation Method: Quartimax with Kaiser Normalization. a Rotation converged in 4 iterations.

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	7.798	38.988	38.988	7.798	38.988	38.988	7.337	36.684	36.684
2	2.290	11.449	50.437	2.290	11.449	50.437	2.570	12.852	49.537
3	1.890	9.452	59.889	1.890	9.452	59.889	2.059	10.297	59.833
4	1.516	7.582	67.470	1.516	7.582	67.470	1.527	7.637	67.470
5	.905	4.523	71.993						
6	.849	4.246	76.238						
7	.737	3.687	79.926						
8	.704	3.518	83.443						
9	.591	2.953	86.397						
10	.545	2.725	89.121						
11	.527	2.637	91.758						
12	.462	2.312	94.070						
13	.298	1.492	95.562						
14	.244	1.222	96.784						
15	.214	1.069	97.853						
16	.186	.931	98.785						
17	.145	.725	99.510						
18	.058	.290	99.800						
19	.029	.147	99.946						
20	.011	.054	100.000						

Extraction Method: Principal Component Analysis.

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APPENDIX V

Mean of factor scores

colony no.	REGR factor score 1 for analysis 1	REGR factor score 2 for analysis 1	REGR factor score 3 for analysis 1	REGR factor score 4 for analysis 1
n01	.7901486	.4912336	.3147471	-.3612460
n03	.9924785	-1.1113372	.2050679	.0113150
n04	.8668384	.2499932	.2192735	-.5262388
n05	1.3463805	.3765633	.2684753	-.1910327
n06	1.4437190	.6563601	.2182196	1.8474238
s01	-3.3270039	-.4407604	.1615301	-.2250985
s03	-1.3227059	-.5362242	.0635068	.5424396
s04	-.7233123	.5027513	.1780801	1.0774555
s05	.1113983	-.9823683	.0966849	.5142701
s06	-.5792349	.2047515	.1499753	1.0978312
s07	-.9502184	-.0092295	.2769424	1.3202157
se01	.5678868	.1417838	.1661173	-.0678915
se02	-.2028193	-.2891698	.1431509	-.2336990
se03	.2695015	-.1751006	.1358193	.1854657
se04	.1301114	-.1436962	.1835827	-.3293594
se05	.1469230	-.1465889	.2010736	-.3137510
se06	.2902073	-.1605654	.1491391	.2073576
se07	-.9074163	-.0994619	.0285666	.0385225
se08	.1788704	.1002374	.1319490	-.6445474
sw01	.1247908	-.1132173	.2080487	-1.2014428
sw02	.3192301	.3192024	.1835135	-.6552399
sw04	.5509125	-.2068321	.2163770	-.5074589
sw05	.1614656	.1439118	.2196292	-.6416267
sw06	.0461517	.2751908	.3210686	-.0477211
sw07	-.4286385	-.2686204	.1294246	-.9644454
sw08	.9439244	-.1858662	.1570787	-.0439238
sw11	.0662657	.1358842	.2030805	-.6938303
tn02	-.1206113	.3758850	.2893016	.6030118
tn04	-.5622607	-.2065295	.2181750	-.3967579
tn05	-.2070216	1.1177796	.2137142	.5672406

APPENDIX VI

A. Reagent preparation

Agarose gel electrophoresis

1) 1% (w/v) agarose gel

- agarose	0.3	g
- 1x TBE buffer	30	ml

2) 1x Tris Boric EDTA buffer (TBE buffer), pH 8.0

- Tris aminomethane (50 mM)	108	g
- Boric acid (50 mM)	50.4	g
- EDTA (0.65 mM)	7.44	g

Adjust pH to be 8.0 and quantitate volume to be 1,000 ml.

Polyacrylamide gel electrophoresis (PAGE)

1) 8% (v/v) polyacrylamide gel

- 30% acrylamide solution (29.2% Bio-rad® acrylamide monomer: 0.8% bis-acrylamide)	4.8	ml
- 10x TBE buffer (1x)	1.2	ml
- 10% APS [$(NH_4)_2S_2O_8$] (3%)	240	µl
- TEMED (0.2%)	15	µl
- d-H_2O	17.7	ml

2) 5x loading dye

- 1 M Tris-Hcl, pH 6.8 (0.312 M)	0.6 ml
- Glycerol (50% v/v)	5.0 ml

- 10% (w/v) SDS 2.0 ml

- 2-Mercaptoethanol 0.5 ml

- 1% Bromophenol blue 0.1 g

- d-H_2O 0.9 ml

One part of sample buffer was added to four parts of sample. The mixture was heated for 5 min in boiling water before loading to the gel.

3) Silver staining

1. Fix a gel in 40% (v/v) methanol and 10% (v/v) acetic acid for 12 min or until loading dye is disappeared.

2. Rinse a gel with d-H_2O.

3. Soak a gel 1 M nitric acid for 5 min and discard solution.

4. Soak a gel in d-H_2O for 4 min and discard solution.

5. Soak a gel in 0.2% (w/v) fresh prepared silver nitrate solution for 16 min.

6. Rinse a gel shortly with d-H_2O.

7. Soak a gel in developer solution [3% (w/v) Sodium carbonate and 40%(v/v) Formaldehyde] until products are visible. Then, discard the solution.

8. Soak a gel in stop solution (0.1 M citric acid or 20% (v/v) acetic acid for 3 min. Then, discard solution.

9. Soak a gel in d-H_2O for 5 min.

10. Wrap a gel with cellophane, air dry overnight, and kept at RT.

BIOGRAPHY

Mr. Atsalek Rattanawannee was born on December 29, 1979 in Kalasin province, Thailand. He finished his secondary school level from Baukhoaw School in 1998, Kalasin province. After that, he got a Bachelor's Degree in Biology from Department of Biology, Faculty of Science, Chulalongkorn University in 2001. At present, he is a graduate candidate in Master's Degree in Zoology, Department of Biology, Faculty of Science, Chulalongkorn University.

Presentation:

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