ความหลากหลายทางพันธุกรรมของกั้งกระดานสกุล *Thenus* ในประเทศไทย
โดยใช้ยีน ไซโทโครมซี ออกซิเดส หน่วยย่อยที่หนึ่ง

นายอภินันท์ เอี่ยมสุวรรณสุข

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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GENETIC DIVERSITY OF SHOVEL-NOSED LOBSTER OF THE GENUS
THENUS IN THAILAND USING CYTOCHROME C OXIDASE SUBUNIT I
GENE

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อภินันท์ เอี่ยมสุวรรณสุข : ความหลากหลายทางพันธุกรรมของกั้งกระดานสกุล *Thenus* ในประเทศไทยโดยใช้ยีนไซโทโครมซี ออกซิเดส หน่วยย่อยที่หนึ่ง. (GENETIC DIVERSITY OF SHOVEL-NOSED LOBSTER OF THE GENUS *Thenus* IN THAILAND USING CYTOCHROME C OXIDASE SUBUNIT I GENE) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : ผศ. ดร. เจษฎา เด่นดวงบริพันธ์, 135 หน้า.

กั้งกระดานจัดเป็นสัตว์น้ำที่กำลังมีความสำคัญเพิ่มขึ้นของประเทศไทย การศึกษาความหลากหลายทางพันธุกรรมของกั้งกระดานถือว่ามีความจำเป็นเพื่อที่จะทราบว่ากั้งได้เคยมีการแพร่กระจายและพบอาศัยอยู่ทั่วทะเลไทยได้อย่างไร วิทยานิพนธ์นี้ดำเนินการขึ้นเพื่อศึกษาความหลากหลายทางพันธุกรรมของกั้งกระดานในประเทศไทยโดยใช้ยีนไซโทโครมซี ออกซิเดส หน่วยย่อยที่หนึ่งในไมโทคอนเดรีย (CO1) โดยเก็บตัวอย่างกั้งกระดาน 206 ตัวอย่างจาก 10 จังหวัด สกัดจีโนมิกดีเอ็นเอจากส่วนขาเดินและเพิ่มปริมาณตลอดจนอ่านลำดับนิวคลีโอไทด์ของยีน CO1 สร้างแผนภูมิต้นไม้แสดงความสัมพันธ์ทางวิวัฒนาการด้วยวิธีเนฮ์เบอร์จอยนิง วิธีมัธยัสถ์สูงสุด วิธีความเป็นไปได้สูงสุด และวิธีเบย์เซียน จากแผนภูมิต้นไม้สามารถแบ่งตัวอย่างกั้งของไทยออกเป็นสามกลุ่มชัดเจนได้แก่กลุ่ม *T. indicus* 163 ตัว กลุ่ม *T. orientalis* 12 ตัวและกลุ่ม *T. unimaculatus* 31 ตัว ลักษณะของปล้องพรอพอดัสที่มีการเสนอไว้นั้นสามารถใช้ในการจำแนกสปีชีส์กั้งกระดานได้เป็นอย่างดี สำหรับการศึกษาความหลากหลายทางพันธุกรรมของกั้ง *T. indicus* นั้น พบว่ามีอยู่ 87 แฮโพลไทป์ จากการวิเคราะห์ค่าความแปรปรวนเชิงโมเลกุล (AMOVA) แสดงให้เห็นว่ามีความแปรผันทางพันธุกรรมสูงเกิดขึ้นระหว่างประชากรสามกลุ่มคือ อ่าวไทยตอนบน อ่าวไทยตอนล่าง และทะเลอันดามัน เวลาที่ประชากรของกั้งจากฝั่งอ่าวไทยวิวัฒนาการแยกออกจากฝั่งอันดามันมีค่าประมาณ 20,000 ปีมาแล้ว ผลการศึกษาเหล่านี้เป็นข้อมูลสำคัญต่อการวางแผนการอนุรักษ์และการใช้ประโยชน์จากกั้งกระดานต่อไปในอนาคต

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สาขาวิชา........สัตววิทยา.......ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.............................
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APINAN IAMSUWANSUK : GENETIC DIVERSITY OF SHOVEL-NOSED LOBSTER OF THE GENUS *Thenus* IN THAILAND USING CYTOCHROME C OXIDASE SUBUNIT I GENE.
ADVISOR : ASST. PROF. JESSADA DENDUANGBORIPANT, Ph.D.
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Shovel-nosed lobsters (*Thenus* species) or Kang-kradan (in Thai) are an increasingly important fishery in Thailand. Genetic diversity of *Thenus* is needed in order to understand how it has been distributed and currently habits most of Thailand's sea coasts. This thesis was conducted to investigate the genetic diversity of *Thenus* in Thailand using mitochondrial cytochrome c oxidase subunit I (CO1) gene. Two hundred and six adult *Thenus* specimens were sampled from ten provinces. Genomic DNA was extracted from pereiopods and the CO1 gene was amplified and sequenced. Phylogenetic trees were reconstructed using neighbour-joining, maximum parsimony, maximum likelihood, and Bayesian methods. The molecular phylogeny clearly separated the Thai *Thenus* specimens into three clades: 163 individuals of *T. indicus*, 12 of *T. orientalis*, and 31 of *T. unimaculatus*. The recently proposed propodus characteristics can be used to separate Thai *Thenus* species. For the genetic diversity study of *T. indicus*, 87 haplotypes were found. The AMOVA statistical analysis showed the significantly different genetic variation among three geographical groups (upper Gulf of Thailand, lower Gulf of Thailand, and Andaman Sea). The time of divergence between Gulf of Thailand and from Andaman Sea populations was estimated to be about 20,000 years ago. These findings are an important knowledge for establishing a sustainable fisheries and conservation management in the future.

Department : Biology   Student's Signature
Field of Study : Zoology   Advisor's Signature
Academic Year : 2011   Co-advisor's Signature

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

bp   Basepair

BI   Bayesian inference

°C   Degree Celsius

CL   Length of carapace from base of antennal plate sinus to posterior
                margin of carapace on dorsal side

CPUE   Catch per unit effort

CO1   Cytochrome oxidase subunit I gene

cm   Centimetre

CW   Width of widest section: width of carapace with calliper arms sitting
                on left and right postorbital spines

dNTP   Deoxynucleotide triphosphate

DNA   Deoxyribonucleic acid

ESS   Effective sample size

EDTA   Ethylene diamine tetraacetic acid (disodium salt)

EtBr   Ethidium bromide

g   Gram

HPD   Highest posterior density

MP   Maximum parsimony

μg   Microgram

μl   Microlitre

μm	Micrometre
μM	Micromolar
ml	Millilitre
ML3	Length of the 3rd merus: anterior spine to posterior spine
MPT	Most parsimonious tree
MW1(2)	Width of the 1st (and 2nd) merus: widest posterior dimension at right angles to merus length
ng	Nanogram
NJ	Neighbour-joining
PCR	Polymerase Chain Reaction
PL1(2)	Length of the 1st (and 2nd) propodus: anterior internal protrusion to posterior spine
PW1	Width of the 1st propodus: widest posterior dimension at right angles to propodus length
TBE	Tris-Boric-ethylene diamine tetraacetic acid
TL	Length of telson: from mid-anterior margin to posterior margin of the calcified region
Tris	Tris (Hydroxymethyl) aminomethane
TW	Width of telson: from left to right latero-posterior spine
Volt	Voltage

CHAPTER I

INTRODUCTION

Shovel-nosed lobster (genus *Thenus* Leach, 1815) is a marine crustacean in order Decapoda, family Scyllaridae. It is also called "flathead lobster", "locust lobster", "bay lobster", "Moreton bay bug", and "Kang-kradan" (in Thai). The body is divided into two parts: cephalothorax and abdomen. Unlike other common lobsters, shovel-nosed lobster shows no rostrum at its head, and has the flatten body. Its eye orbits are situated on its head which is covered with thick carapace. Its length ranges from 12 to 25 centimeters (Department of Fishery, 1997).

Shovel-nosed lobster is always found in sand or mud from ten to 50 metres depth (Jones, 1993; Jones, 2007). It is fed on the small crustaceans and cephalopods. Unlike other crustaceans, it is lack of giant neurons. Therefore, it buries into and conceals under substratum in order to avoid predators (Espinoza et al., 2006). Its closest relative is the palinurid (spiny lobster) supported by the fossil of *Cancrinos claviger*, aged 100 to 120 million years ago, which is considered to be the ancestor of the scyllarid lobster (Webber and Booth, 2007).

Shovel-nosed lobster is widely distributed in tropical and warm sea, especially in the Indo-Pacific and Australian regions. It is one of the commercially important species in Thailand and partially exported to European countries as frozen meat (Naiyanetr, 1963; Sungthong, 1979, Uraiwan, 1978). Annual global production of shovel-nosed lobster has recently reached 5,000 tonnes per year (FAO, 2010). Most of the production came from the Asian countries.

Recently, there has been an increasing demand of shovel-nosed lobsters in Thailand mainly because of their good taste. In 2003, the price of grade A of shovel-nosed lobsters was 140 baht to 240 baht (four US dollars to seven US dollars) per kilogram (approximately seven to eight individuals). However, the price has been currently raising to 400 baht to 600 baht (11 US dollars to 17 US dollars) per kilogram since a lot of restaurants demand shovel-nosed lobsters. Moreover, there has

been occasionally a shortage of shovel-nosed lobsters in the market, especially in the monsoon season. The high demand of shovel-nosed lobsters is also caused by the decreasing catch of other seafood such as marine shrimp, so that the fishermen have started to capture more and more shovel-nosed lobsters which could give a high profit too. Unfortunately, shovel-nosed lobsters still cannot be cultured because its biological information is limited and its productivity relies only on the natural resource. All of these changing phenomenon lead to the concern of an unsustainable usage of the natural populations of shovel-nosed lobsters in near future (Sungthong, 1979; Uraiwan, 1978).

According to the taxonomic point of view, previous studies recognised only one species, *Thenus orientalis* (Lund, 1793), in Thailand (Naiyanetr, 2007). Nevertheless, a recent revision of the genus *Thenus* (see Burton and Davie, 2007) considering the examination of their external morphology, morphometric ratios, and cytochrome c oxidase subunit I (CO1) nucleotide sequences has suggested that the members of this genus could be recognised as five species: *Thenus indicus* Leach, 1815*, T. orientalis, T. australiensis* Burton and Davie, 2007*, T. unimaculatus* Burton and Davie, 2007, and *T. parindicus* Burton and Davie, 2007. The diagnosis characteristics used to identify these five *Thenus* species are the patterns and colours on their periopods, a spine on the merus, and a dentation on an ischium of the third maxilliped, in combination with the CO1 nucleotide sequences (Burton and Davie, 2007). This newly proposed revision gives a possibility to reclassify the shovel-nosed lobsters in Thailand, possibly into least three species: *T. orientalis, T. indicus* and *T. unimaculatus*.

A genetic diversity study of the shovel-nosed lobster is important for sustainable fisheries and conservation. The data from such study can be used to establish a conservationally management strategy (Palumbi, 2004) by considering the fitness and adaptation of the natural populations (Frankham, 1995). Therefore, in order to obtain a clearer taxonomic status and a better genetic diversity data of the shovel-nosed lobster in Thailand, this M.Sc. thesis study was conducted. Specimens from both Gulf of Thailand and Andaman Sea were collected and identified using

CO1 nucleotide sequences together with externally morphological characteristics. The genetic diversity analyses of *T. indicus*, the most widely distributed *Thenus* species in Thailand, were also performed.

Research objective

To investigate genetic diversity of shovel-nosed lobster of the genus *Thenus* in Thailand using cytochrome c oxidase subunit I gene (CO1).

CHAPTER II

BACKGROUND

2.1 Shovel-nosed lobster

Shovel-nosed lobster is a marine organism classified under phylum Arthropoda, subphylum Crustacea, class Malacostraca, order Decapoda, family Scyllaridae, genus *Thenus* Leach, 1815. There are many common names for this animal such as bay lobster, slipper lobster, shovel-nosed lobster, Moreton Bay bug, sand lobster (in English), Kang hin, and Kang Kradan (in Thai). Following the recent revision of Burton and Davie (2007), this genus contains five species: *Thenus indicus, T. orientalis, T. unimaculatus, T. parindicus, and T. australiensis*. Shovel-nosed lobster is usually found in warm and tropical seas of Asia and Australia. Its unique morphological characteristics are flattened carapace and also flattened second antennae, unlike those of other common lobsters.

2.1.1 Taxonomic description

Figure 2.1 and 2.2 illustrate external morphological characteristics of *Thenus*. Its dorso-ventral body is compacted and the carapace is rather broad than long. The eye orbits are situated on the lateral extremes of the carapace. The stalk eyes are located in the deep orbits covered by several vigorous teeth with largely prolonged upward in the anterolateral borders of the carapace. Fine granules and tubercles among a patchy tomentum are carved in the carapace and abdomen. The elevated median dorsal ridge which has three forwardly and upwardly directed teeth is found on the carapace and the first five abdominal segments. A rostral process is consisted of two small, spiky spines divided by a deep V-shaped sinus. The second and fourth segments of the antennae are widely packed down and expanded with long curving teeth on anterolateral margins. The antennules which rise from beneath the rostral process extend only beyond the anterior margins of the antennae. The pleopods are concealed with downwardly expanded lateral margins positioned on the abdominal segments. The telson is thin and soft distally but calcified and stiff proximally, longer

than broad. The uropods which are expanded beyond the posterior margin of the telson are well developed.

The four pairs of pleopods which decrease in size posteriorly are ventrally situated on the second to the fifth abdominal segments. The endopods are smaller than the exopods. There are five pairs of relatively long and slender periopods and the sharp dactyls which the tips are ensheathed in a keratin sheath equipped on the first four. Two rows of coarse hairs are bear from the dactyls of the three posterior pairs of periopods. It has large and well developed maxillipeds. There are smooth and featureless branchiostegites (Jones, 2007).
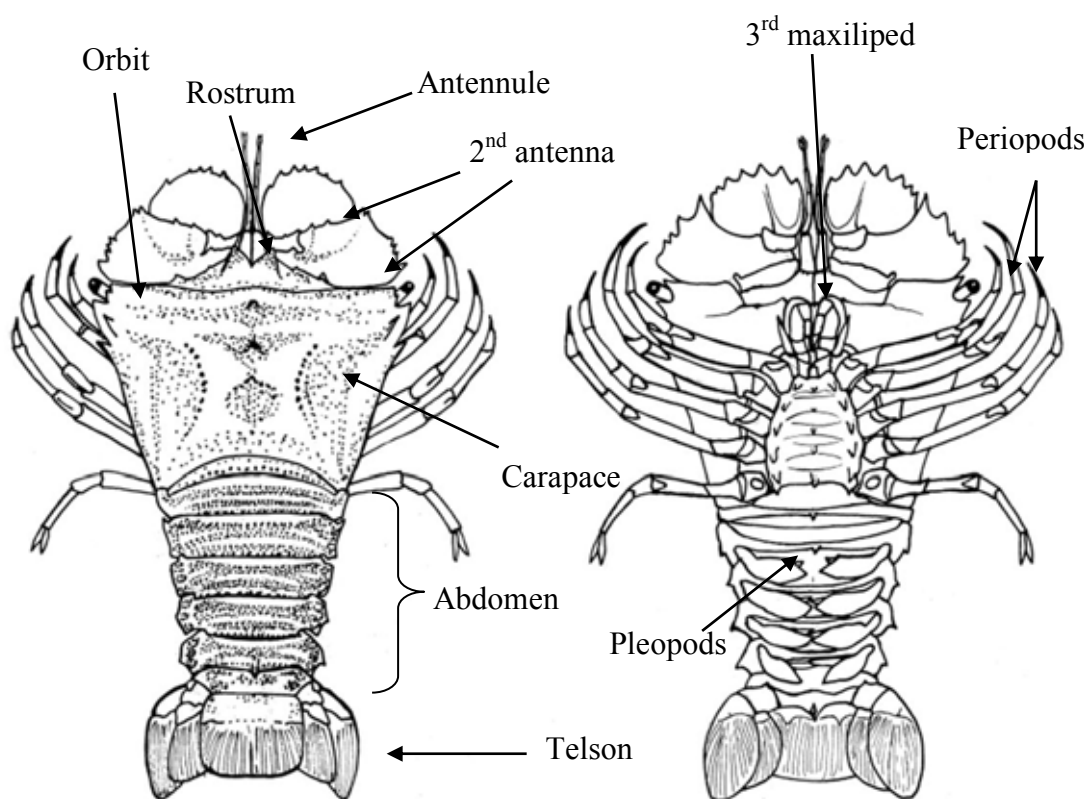
Figure 2.1 External morphological characteristics of *Thenus* (modified from Jones, 2007).
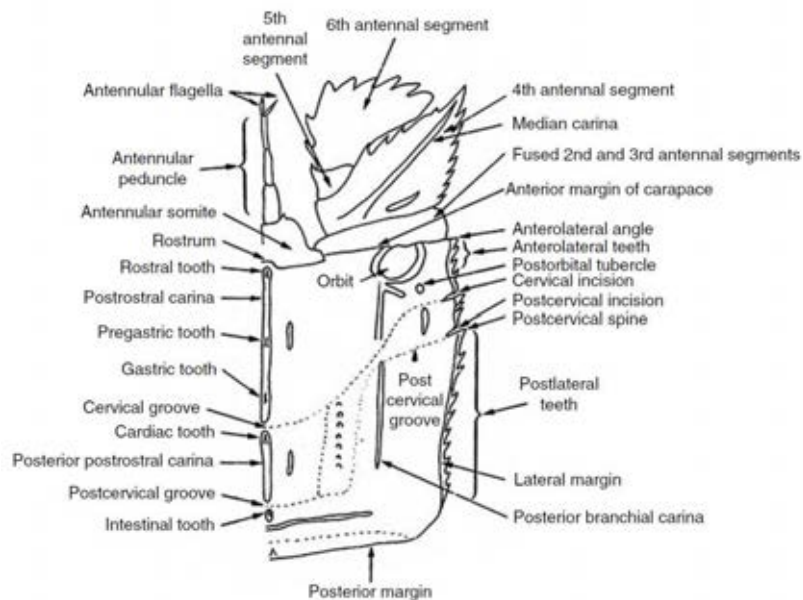
Figure 2.2 Dorsal right view of scyllarid carapace used in taxonomic description (after Holthuis, 1991).

2.1.2 Habitats and ecology

The life history of shovel-nosed lobster is shown in Figure 2.3. After the fertilisation, the egg hatches into a naupliosoma. This larval stage further develops to an early phyllosoma. There are some changes in morphological characters and the early phyllosoma develops to a late phyllosoma. Next, the late phyllosoma develops to a pelagic nisto which looks like an adult. This stage develops to a settling nisto and finally develops to an adult (Lavelli and Spanier, 2007).

Shovel-nosed lobster is always found as patchy or aggregated distribution. The nature of sediment and water depth has an impact on lobster abundance. For instance, there is a strong relationship between the depth of water and catch suggesting that *T. parindicus* is most abundant at depths between 10 and 30 metres whereas *T. australiensis* is most abundant at depths between 40 and 50 metres. A robust relationship between the particle size of sediment and catch was also observed. *Thenus parindicus* prefers particle size less than 0.25 mm while *T. australiensis* preferred particle size between 0.25 mm to 1 mm (Jones, 1993; 2007).

In Australia, there is an increased abundance of *T. parindicus* occurred in autumn (March/April) and spring (October). This might be resulted from the recruitment pulse of postlarval individuals. In the contrary, *T. australiensis* is more evenly distributed through time (Jones, 2007).
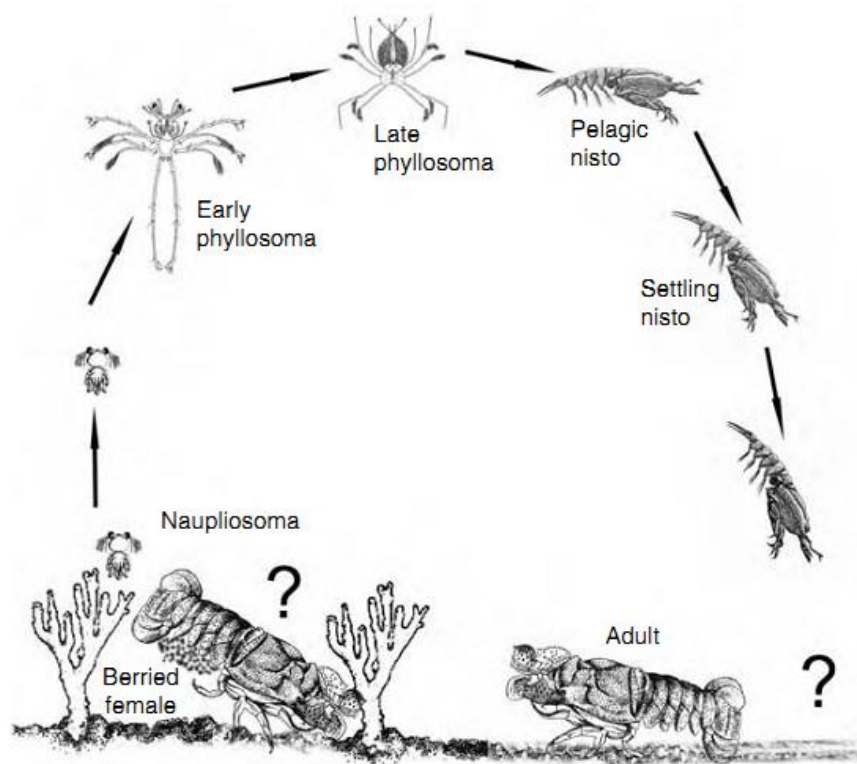
Figure 2.3 Life cycle of shovel-nosed lobster (after Lavelli and Spanier, 2007). Question marks indicate unknown life-history phases.

2.1.3 Geographical distribution

The five shovel-nosed lobster species of the genus *Thenus* are distributed in the Indo-Pacific region. The most common shovel-nosed lobster *T. indicus* is widely distributed from India to Burma, Thailand, Indonesia, Taiwan, and China (see Figure 2.4). Another common shovel-nosed lobster *T. orientalis* is also found in many countries such as United Arab Emirates, Malaysia, Indonesia, Thailand, Taiwan, China, and the Philippines (see Figure 2.5). *Thenus australiensis* is very high endemism as it is only found in Australia (see Figure 2.6). A larger size lobster *T. unimaculatus* is found only in United Arab Emirates, Thailand (Andaman Sea only), and Malaysia (see Figure 2.7). Finally, *T. parindicus* is found in both Pakistan, and Australia (see Figure 2.8)(Burton and Davie, 2007).

In Thailand, shovel-nosed lobster genus *Thenus* can be found from both the Gulf of Thailand and Andaman Sea. Previously, *T. orientalis* was the only species recognised in Thailand and it was reported to be found only in the Gulf of Thailand (Naiyanetr, 1963; 2007). Likewise, our field survey in this study also revealed that the distribution of *T. orientalis* is limited to the Gulf of Thailand whereas *T. indicus* shows a much wider distribution and can be found from both the Gulf of Thailand and Andaman Sea. The third species *T. unimaculatus* were found in Thailand specifically in the Andaman Sea and there is no record that this species has been ever collected in the Gulf of Thailand.
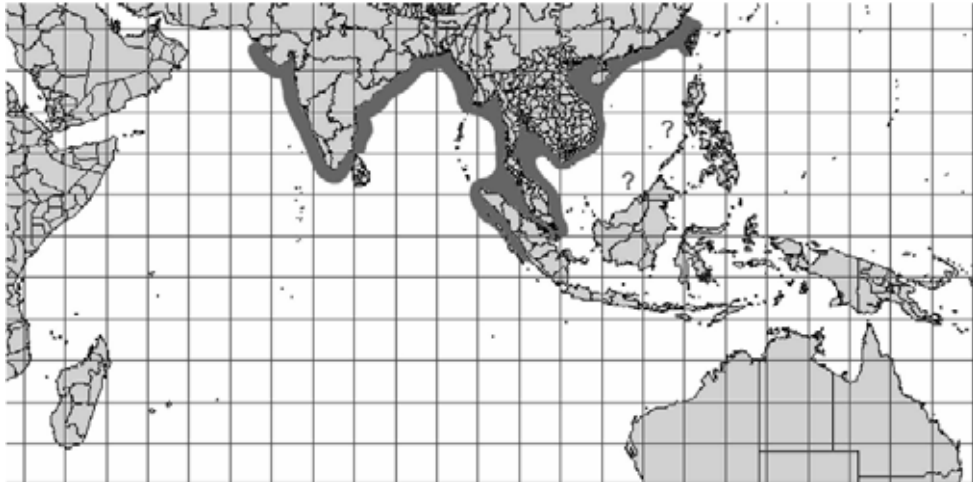
Figure 2.4 The distribution of *T. indicus* (after Burton and Davie, 2007).
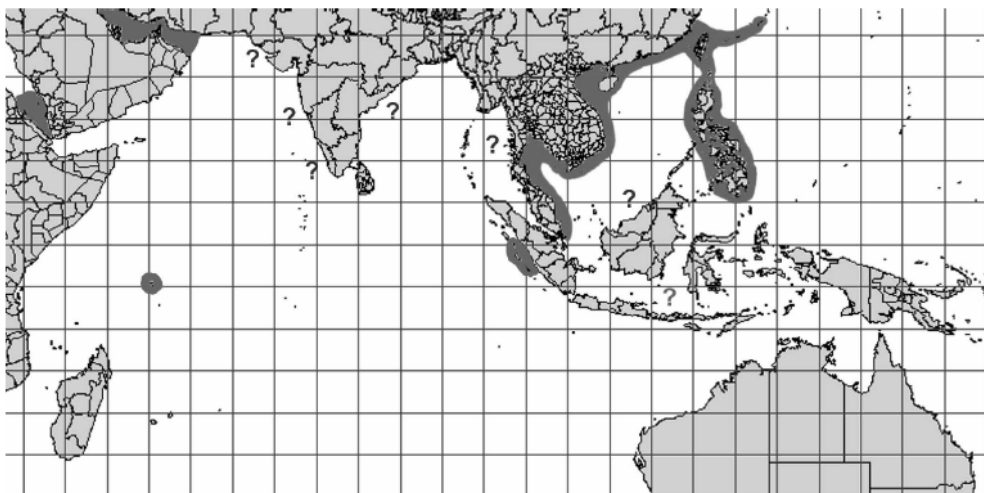
Figure 2.5 The distribution of *T. orientalis* (after Burton and Davie, 2007).
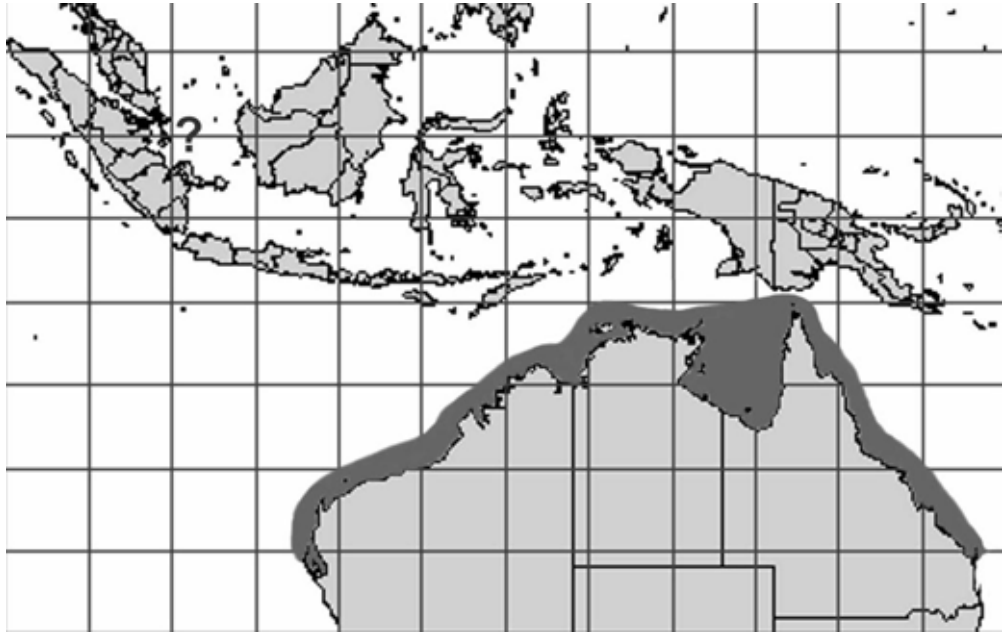
Figure 2.6 The distribution of *T. australiensis* (after Burton and Davie, 2007).
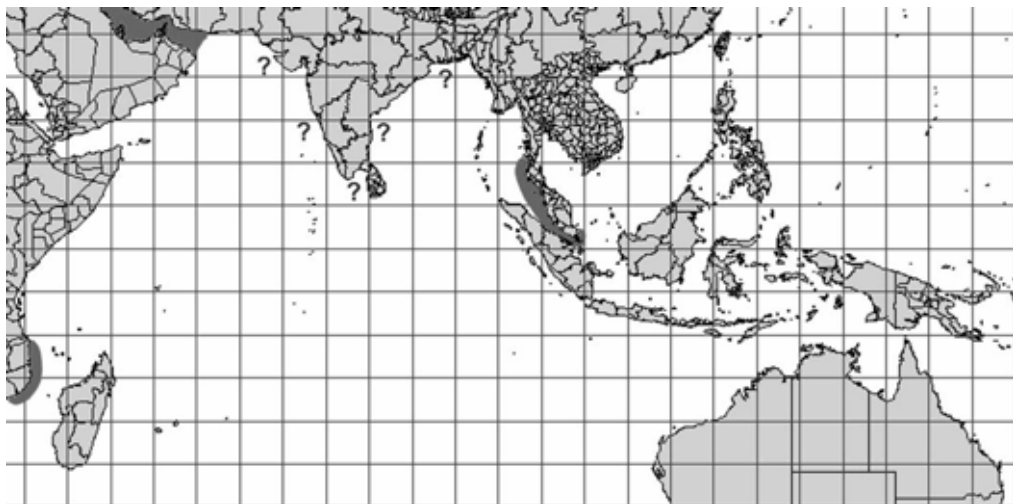
Figure 2.7 The distribution of *T. unimaculatus* (after Burton and Davie, 2007).
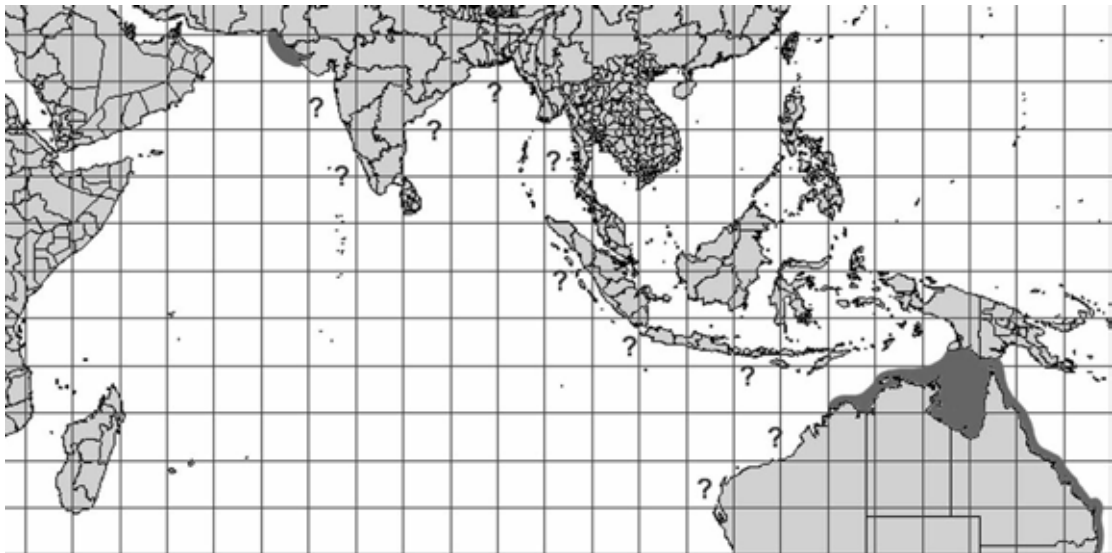
Figure 2.8 The distribution of *T. parindicus* (after Burton and Davie, 2007).

2.1.4 Fisheries importance

Thenus is considered to be one of the most commercially important scyllarid genera in the trawling industry (Jones, 1993). The annual production of all scyllarid genera has reached 5,000 tonnes per year recently. Most of their production came from the species *T. orientalis* (FAO, 2010). This emphasised the significance of *Thenus* to trawling fisheries.

From our field survey and the interviews with several local fishermen, shovel-nosed lobsters are usually available everyday at large fish markets such as Mahachai fish market locating at Muang district of Samut Sakorn province, Ang Sila fish market at Ang Sila district of Chonburi province, and Ranong port (or Ranong fish market) at Muang district of Ranong province. The production of shovel-nosed lobsters in Thailand mostly comes from trawling boats while the minority comes from other types of catching process such as capturing cages of blue swimming crabs (*Portunus* spp.). There are two types of fishery trawling boats catching shovel-nosed lobsters: local fishermen boats and large commercial boats. The small local boats prefer capturing shovel-nosed lobsters near the sea coasts, not more than 30 sea miles, while the larger commercial boats always trawl shovel-nosed lobsters and other marine bottom dwellers in the open seas, far from the sea coast much more than the local boats. The lobsters catched by local fishermen are usually kept alive and the catching trip does not last longer than 7 days. On the other hand, those shovel-nosed lobsters captured by the large commercial boats must be frozen with ice while still in the sea since each catching trip usually last 15 to 30 days.

From the interview of many fishermen, in the past two decades there had been an at least 30% decrease of the amount of natural fishery stocks such as crab, shrimp, fish, and also shovel-nosed lobster due to an overfishing in Thai sea. Although there are still a large member of shovel-nosed lobsters living in the Gulf of Thailand, the fishermen commented that they seem to be rarer and the size are smaller than those catch in the past. This data gives a strong warning that shovel-nosed lobster

populations in Thailand will be threatened in the near future if there is no proper plan for the management of their exploitation.

2.1.5 Literature review

2.1.5.1 Research studies in Thailand

In 1961, Naiyanetr reported that there were only two species of phyllosoma larvae of scyllarid lobsters in the Gulf of Thailand: *Thenus orientalis* larvae and *Scyllarus* sp. larvae. The larval stages II to IX of *T. orientalis* and *Scyllarus* sp. were found. He also stated that *T. orientalis* larvae and *Scyllarus* sp. larvae appear to hatch mainly in the inshore area of the east coast of the Gulf of Thailand and were carried out to the other part of the Gulf by water currents. The research also suggested that the breeding seasons of *T. orientalis* may be in December and January. Two years later, Naiyanetr (1963) revised his previous report that there should be four species of scyllarid lobsters found in the Gulf of Thailand: *T. orientalis, Scyllarus rugosus, S. martensii*, and *S. sorsidus*. Relationships between total length against total weight and number of eggs against meat weight were logarithmic, but between total weight against meat weight were linear. The phyllosoma stages of *Scyllarus martensii* and *Scyllarus* sp. were found nine stages, but those of *T. orientalis* were found only four stages.

Uraiwan (1977) collected *Thenus* samples throughout the Gulf of Thailand by trawling at not excess 50 metre depth and found that *T. orientalis* could be found throughout the Gulf from Nakhon Si Thammarat province to Pattani province. It is most abundant in January and February and the male was found more than female in every month. The male size was ranged from 160 to 170 mm and the female size was 190 to 200 mm. Like the previous study (Naiyanetr, 1961), the relationship between total length and total weight was logarithmic. The fecundity was 9,000 to 30,000 eggs per individual. The egg size was about 374 to 472 μm. The spawning season of this species is throughout the year.

A tagging experiment of sand lobster *T. orientalis* was conducted by Sungthong (1979) in an aquarium. He used an external anchor tag at the first abdominal segment since an internal anchor tag was not effectively for feeding, movement, and molting of the lobsters. From his experiment, the average length of carapace was increased 0.47 cm for the 60 experimental days and the mortality rate was around 31%.

Kowitwatee (1988) conducted a taxonomic study on marine crabs, scyllarid lobsters, and spiny lobsters from the eastern coast of the Gulf of Thailand. Two genera of scyllarid lobsters (*Thenus* and *Scyllarus*) were found and 25 individuals of *T. orientalis* were collected from Chonburi, Rayong, Chanthaburi, and Trat provinces.

Most recently, Bussarawit (1990) performed a trawling survey in Myanmar waters covered Rakhine coast, Delta Area, Tarinthayi coast at 30 to 140 metres depth. One species of scyllarid lobster *T. orientalis* was found.

2.1.5.2 Research in other countries

Many aspects of study concerning the genus *Thenus* have been performed, for instance, taxonomy (Lund, 1793; Leach, 1815; Burton and Davie, 2007), ecology (Radhakrishnan, 2005; Jones, 1993), fishery (Jones, 2007;), larval biology (Raghu Prasad et al., 1975; Mikami et al., 1995, Mikami and Greenwood, 1997), and behavioural biology (Mikami, 2005).

The taxonomy of the genus *Thenus* has been found be problematic since it has several cryptic and sibling species as observed in other crustaceans (Knowlton, 1993). The taxonomic study concerning about *Thenus* was first published around 200 years ago. "Slaegten *Scyllarus*" written by Lund (1793) described the *Thenus* specimens under the name *Scyllarus orientalis*. In 1815, Leach proposed *Thenus* as the name of the genus for the first time and named the species *Thenus indicus* as a monotype species of the genus. From the report of Burton and Davie (2007), the name *Scyllarus orientalis* was later transferred to the genus *Thenus* and the name *T. indicus* was later relegated to be the synonym of *T. orientalis*. Hence, the studies of any *Thenus*

specimens before 1993 were conducted under the name *T. orientalis*. Until 1993, Jones resurrected the name *T. indicus* for the first time because of ecological differences between *T. indicus* and *T. orientalis* in his study of *Thenus* population structure (Jones, 1993; Burton and Davie, 2007). However, a recent revision of the genus *Thenus* by examining its external morphological characteristics, isozyme electrophoresis, and CO1 DNA sequences has proposed five different species: *T. indicus, T. orientalis, T. unimaculatus, T. parindicus* and *T. australiensis* (see Burton and Davie, 2007). Thus, Jones" *T. indicus* was later revised to be *T. parindicus* and his *T. orientalis* was *T. australienesis* (see Burton and Davie, 2007).

Australia two species of shovel-nosed lobster *T. parindicus* (in original paper as *T. indicus*) and *T. australiensis* (originally *T. orientalis*) have mutually limited distribution. Depth and sediment type are correlated with their abundance. *Thenus parindicus* is most abundant in depths of 10 to 30 metres and where the sediments are fine sand and slit. Whereas *T. australiensis* is most abundant in the depth of 40 to 50 metres where the sediments are medium-coarse sands of 0.25 to 1.00 mm particle size (Jones, 1993).

The exploitation of the shovel-nosed lobsters in Australia and throughout most of Southeast Asia is mainly performed by trawlers (Holthuis, 1991). The fishery status of the genus *Thenus* in Australia was also studied (Jones, 2007). More than 90% of Australian catch is from the Queensland water. In 2001, the total production of shovel-nosed lobster in Queensland, Australia, was approximated at 386 metrictonnes, valued at 4.6 million Australian Dollars (2.44 million US Dollars). In 2002, the production has been as high as 755 metrictonnes (Courtney, 2002).

The population parameters such as catch per unit effort (CPUE) and mean size of *Thenus* spp. living in the Gulf of Carpentaria between 1963 (before the establishment of a commercially shrimp trawl fishery) and 1983 were reported by Jones (1988). There were significant changes resulted from commercial fishing activities. The CPUE of lobsters was 44% (1.8 individuals per hectare) in 1983, which was less than in 1963/64 (3.2 individuals per hectare). Moreover, the mean size of

lobsters was also decreased for 15% between the periods of time. Interestingly, the study of exploited standing stock of *Thenus* spp. in the Gulf of Carpentaria showed that the stock was reduced by 65% over a 20-year period from 1963 to 1983 (Jones, 2007). Jones (2007) later assumed that a long-term commercial exploitation would also cause a reduction in mean size of lobsters in natural populations, as has been documented for lobster fisheries in India (Radhakrishnan et al., 2005). Currently, the fishery of shovel-nosed lobster in Australia is therefore managed and regulated. Taking egg-bearing female lobsters and those with carapace width lower than 75 mm is prohibited at anytime. The recent CPUE of lobsters remained stable over the past two decades, even though the total catch was declined because of the decreased fishing effort (Courtney, 2002). Unlike in India where the *Thenus* populations are significantly overexploited (Radhakrishnan et al., 2005), the *Thenus* fishery in northern Australia nowadays seems to be sustainable (Jones, 2007).

Interestingly, the studies of larval biology of shovel-nosed lobster may pave the way to its aquaculture. Raghu Prasad et al. (1975) studied the phyllsoma larvae collected from the Indian Ocean. Twelve species of scyllarid phyllosoma larvae in the genera *Scyllarus*, *Parribacus*, *Scyllarides*, *Thenus*, and *Evibacus* were found and reported. Mikami et al. (1995) studied the relationship between initial starvation and duration of feeding period on survival, intermoult period, and growth of newly hatched phyllosomas of *Panulirus japonicus* and *Thenus* sp. under the laboratory condition. Their results showed that period of time-to-first-feeding (TFF) correlated positively with the intermoult period of first instar. They also found that palinurid phyllosomas could survive longer periods than scyllarid phyllosomas could. Mikami and Greenwood (1997) also reported the rearing of phyllosoma larvae of *T. orientalis* and *Thenus* sp. to the juvenile stage in the laboratory.

The Australian Fresh Research and Development Corporation Pty Ltd (AFR & DC) in association with the Department of Primary Industries and Fisheries, Bribie Island Aquaculture Research Centre (DPIF, BIARC) has continued researching and developing the aquaculture potential of *Thenus* spp. since 1995. The attributes of *Thenus*, including the rapid growth to commercially acceptable sizes within 13

months from hatching, ability to tolerate crowding, and the high market prices attract the preposition of commercial aquaculture. This research group is the first one in the world who can successfully rear the larvae through to the juvenile stage and to the mature stage. The research on a pilot scale production has been conducted. Subsequently, the pilot system has been operating successfully due to better understanding of animal biology. Australian Bay Lobster Producers (ABLP) has developed the aquaculture facility for the production of the *Thenu*s spp. in northern New South Wales, Australia. However, the major obstacle in the commercialisation of *Thenus* aquaculture is still the difficulty in maintaining the phyllosoma stage (Mikami, 2007).

2.2 Molecular phylogenetics

In a phylogenetic study, most data used to identify an evolutionary relationship among groups of organisms is molecular data, either DNA sequences or DNA polymorphic fragments. DNA is a carrier of heredity genetic information. It is in a shape of double helix with two antiparallel polynucleotide strands. The basic unit of DNA called "nucleotide", which can be classified as four different types: thymine (T), cytosine (C), adenine (A), and guanine (G). In the coding region of any gene, these nucleotides are sorted into an order of triplet codons called a genetic code. There are 64 genetic codes which encode for 20 different amino acids. When DNA is in a duplication process, sometimes DNA polymerase enzyme incorrectly incorporates non-complement nucleotides into the newly synthesised strand, resulting in a phenomenon called "point mutation". Such mutation may or may not cause any change in the translated amino acid sequence. The mutation that does not cause an amino acid change is called "synonymous mutation" and the one causing changes to the protein sequence called "non-synonymous mutation". If the errors in an incorporation of non-complementary nucleotides happen within purine (A or G) or pyrimidine (C or T) groups, these nucleotide substitutions are called "transition". On the other hand, if the substitution errors occur between purine and pyrimidine are called "transversion". Sometimes errors in the duplication can be occurred as an insertion and a deletion of nucleotides (also known as an indel). There are some other

events driving the evolution at a molecular level such as a DNA recombination, a horizontal gene transfer, and a gene duplication. The DNA recombination is a genetic exchange, which happening during the prophase of meiosis, when some parts of two different DNA strands are synapsed. This recombination process always occurs between two homologous chromosomes. The horizontal or lateral gene transfers are genetic exchanges between different groups of organisms and usually happen in bacteria. For instance, the transfer of plasmid DNA via sex pili between two bacterial cells is one of the horizontal-gene-transfer cases. Lastly, a polyploidisation which is mostly occurred in plant is an example of the gene duplication and usually results in an acquisition of new genes and sometimes new functions (Vandamme, 2009).

DNA sequences are currently the most preferable genetic data used to analyse the evolutionary or phylogenetic relationship among groups of organisms. In order to get the phylogenetic relationship, the suitable region of DNA must be carefully chosen. For instance, cytochrome c oxidase subunit I (CO1) gene was used as a representative DNA region in many research concerning animals (Hebert, et al, 2003). Sometimes, a whole genomic DNA is analysed through a DNA profiling techniques such as RFLP (restriction fragment length polymorphism), AFLP (Amplified fragment length polymorphism), and RAPD (Random amplified polymorphic DNA). The profiles or the patterns of polymorphisms are used to reconstruct a tree diagram to infer phylogeny. This phylogenetic tree could be interpreted and used to clarify the evolution of the interested organisms (Vandamme, 2009).

There are four main methods to infer and reconstruct phylogenetic trees: distance method, parsimony method, likelihood method, and Bayesian method (Vandamme, 2009). The reconstructed tree is then evaluated for the confidence of each topology mostly using bootstrap statistical analysis.

2.2.1 Distance method

This method is considered to be the simplest method for estimating the divergence between two aligned DNA sequences. The proportions of two different homologous sites are called an observed distance (or *p*-distance) and expressed as the

number of nucleotide differences per site. However, p-distance always underestimates the actual substitutions per site or true genetic distance, d because of multiple hits. In phylogenetic analysis, this p-distance is needed to be corrected up to the substitution model used. There are two common methods used to infer phylogeny: UPGMA and neighbour-joining (Vandamme, 2009).

UPGMA (unweighted-pair group method with arithmetic means) is one of the two cluster analyses commonly used for inferring phylogenies. The phylogenetic trees are reconstructed by clustering the smallest value in the pairwise distance matrix on the assumption that the evolutionary rate is the same in all branches. The newly formed cluster replaces the operational taxonomic unit (OTUs) and the new genetic distance is calculated. This process is repeated until all OTUs are clustered (Vandamme, 2009).

The other clustering method is Neighbour-joining (NJ) which constructs a phylogenetic tree by finding pairs of OTUs connected by a single node. The tree starts with star-like formation and then connected every possible pair of the OTUs in order to obtain the shortest tree (Vandamme, 2009).

2.2.2 Parsimony method

The maximum parsimony (MP) uses an algorithm which infers the minimum number of character changes along the branches to clarify the observed character states at the terminal nodes. The MP method tried to finds the tree topology of the set of aligned sequences. The selected smallest tree is called the most parsimonious tree (Vandamme, 2009).

2.2.3 Likelihood method

The algorithms of maximum likelihood (ML) find the maximum probability of observing character states to give the tree topology and model of evolution. This method is like the MP methods that it determines different tree topologies and evaluates the support by summing over all sequence positions. However, finding the most likelihood trees are very time-consuming (Vandamme, 2009).

2.2.4 Bayesian method

Bayesian method is a character-state method that uses an optimality criterion like MP and ML. However, it does not only search for the single best tree, but also search for a set of plausible trees for the data by targeting a posterior probability distribution of the trees depending on the model parameters. These posterior probabilities can be obtained by searching for the tree space using the sampling technique called Markov chain Monte Carlo (MCMC). The sampling starts by simulation of a random set of parameters and a new state proposed. The likelihood and prior ratios for each step will be calculated. The parameters will be accepted and a next step is proposed if the case of the combined products is better. But if it is worse, the state will be rejected. All particular trees from MCMC will be calculated for a consensus or maximum posterior tree (Vandamme, 2009).

2.2.5 Bootstrap statistical analysis

Bootstrap analysis is the sampling method which is generally used to estimate the statistical error with assumption that the original sampling distribution is either unknown or difficult to obtain analytically (Efron and Gong, 1983). This technique is useful to approximate the underlying sampling distribution by resampling from the original data set. It was firstly applied to estimate the confidence intervals for phylogenies inferred from sequence data by Felsenstein (1985). The nucleotide sequence data obtained from the new alignment resulted from the original with randomly choosing columns from it with replacements are firstly bootstrapped. A new set of sequence, a bootstrap replicate which has the same length as original one is constructed by selection of each column in the alignment more than once or not at all. Thus, there are some characters not included at all in a given bootstrap replicate and others will be included once, twice, or more in this resampling technique. After that, the tree is constructed from each reproduced (artificial) data set and the proportion of each clade among all the bootstrap replicates (which is taken as the statistical confidence supporting the monophyly of the subset) is calculated (Van de Peer, 2009).

2.3 Genetic diversity

Genetic diversity refers to total genetic characteristics in the genetic makeup of the species (http://en.wikipedia.org/wiki /Genetic_ diversity). Genetic diversity serves as a way for populations to adapt to ever-changing environments. The more genetic variations, the higher chance that there is an organism possesses an allele which is proper for new environments. (http://www.nbii.gov/portal/server.pt?512&obj ID=405&PageID=0&cached=true& mode =2&userID=2).

The knowledge in population genetics enables us to study and measure the genetic diversity. There are many theories and hypotheses regarding genetic diversity, for example, the neutral theory of evolution and the selection (http://en.wikipedia.org/ wiki/Genetic_diversity). There are several ways to measure genetic diversity and assess the demographic events using genetic data such as molecular diversity indices, Analysis of Molecular Variance (AMOVA) test, population comparison F_{st}, mismatch distribution analysis, coalescent-based Bayesian skyline plot, and the time to recent common ancestor (t_{mrca}).

2.3.1 Molecular diversity indices

Molecular diversity indices include number of alleles or haplotypes, nucleotide diversity and haplotype diversity. Number of alleles or haplotypes refers to the total count of alleles in the population. Nucleotide diversity (or average gene diversity over L loci ($\hat{\pi}_n$)) is the probability that two randomly chosen homologous

nucleotide are different. This index can be calculated from: $\hat{\pi}_n = \dfrac{\sum\limits_{i=1}^{k}\sum\limits_{j<i} p_i p_j \hat{d}_{ij}}{L}$,

where $\hat{d}_{ij}$ is an estimate of the number of mutations having occurred since the divergence of haplotypes i and j, k is the number of haplotypes, p_i is the frequency of haplotype i, p_j is the frequency of haplotype j.

Haplotype diversity ($\hat{H}$) is defined as the probability that two haplotypes selected by random in the sample are different. It can be estimated from:

$\hat{H} = \dfrac{n}{n-1}(1 - \sum\limits_{i=1}^{k} p_i^2)$, where n is the number of gene copies in the sample, k is the number of haplotypes, and p_i is the sample frequency of the i-th haplotype.

2.3.2 AMOVA test

The Analysis of Molecular Variance (AMOVA) approach is the analysis of variance of gene frequencies which are also take the number between molecular haplotypes in account (Excoffier et al., 1992; Excoffier and Lischer, 2010). The groups of populations must be firstly defined before tests. A hierarchical analysis of variance partitions the total variance into covariance components due to intra-individual differences, inter-individual differences, and/or inter-population differences. The covariance components (σ_i^2) are used to compute the fixation indices in terms of inbreeding coefficients (Wright, 1965) or coalescent time by Slatkin (1991).

The significance of fixation indices is tested using a non-parametric permutation approach (Excoffier et al., 1992) consisting in permuting haplotypes, individuals, populations, or groups of populations. The AMOVA test for haplotypic data for several groups of populations is shown below.

Source of variation	Degrees of freedom	Sum of squared (SSD)	Expected mean squared
Among groups	G-1	SSD (AG)	$n''\sigma_a^2 + n'\sigma_b^2 + \sigma_c^2$
Among populations / within groups	P-G	SSD (AP/WG)	$n\sigma_b^2 + \sigma_c^2$
Within populations	N-P	SSD (WP)	σ_c^2
Total:	N-1	SSD (T)	σ_T^2

Where SSD (*T*) is to the total sum of squared deviations, SSD *(AG)* is the sum of squared deviations among groups of populations, SSD *(WP)* is the sum of squared deviations within populations, SSD *(AP/WG)* is the sum of squared deviations among groups of populations within groups, *G* is the number of groups in the structure, *P* is the total number of populations, *N* is the total number of gene copies for haplotypic data, N_p is the total number of gene copies in population *p* for haplotypic data, and N_g is the number of gene copies in group *g* for haplotypic data. The *n*"s and the *F*-statistics are defined by:

$$S_G = \sum_{g \in G} \sum_{p \in g} \frac{N_p^2}{N_g} \,,\; n = \frac{N - S_G}{P - G} \,,\; n' = \frac{S_G - \sum_{p \in P} \frac{N_p^2}{N}}{G - 1} \,,\; n'' = \frac{N - \sum_{g \in G} \frac{N_g^2}{N}}{G - 1} \,,$$

$$F_{CT} = \frac{\sigma_a^2}{\sigma_T^2} \,,\; F_{SC} = \frac{\sigma_b^2}{\sigma_b^2 + \sigma_c^2} \,,\; \text{and } F_{ST} = \frac{\sigma_a^2 + \sigma_b^2}{\sigma_T^2} \;\; \text{(Excoffier and Lischer, 2010)}$$

- σ_c^2 and F_{ST} were tested by permuting haplotypes among populations among groups.

- σ_b^2 and F_{SC} were tested by permuting haplotypes among populations within groups.

- σ_a^2 and F_{CT} were tested by permuting haplotypes populations among groups.

2.3.3 Population pairwise comparison (*F_{st}*)

The pairwise F_{ST} can be used as short-term genetic distances between populations, with the application of a slight transformation to linearise the distance with population divergence time (Reynolds et al., 1983; Slatkin, 1995). The pairwise F_{ST} values are given in the form of matrix. The null distribution of pairwise F_{ST} values under the hypothesis of no difference between the populations is obtained by permuting haplotypes between populations. The *p*-value of the test is the proportion

of permutations leading to a F_{ST} value larger or equal to the observed one. The *p*-value are also given in matrix form (Excoffier and Lischer, 2010).

2.3.4 Neutrality tests

There are two statistics to test for neutrality such as Tajima"s *D* and Fu"s F_s tests. First, Tajima"s *D* test is based on the infinite-site model without recombination. This test is appropriate for short DNA sequences or RFLP haplotypes. It compares two estimators of the mutation parameter theta ($\theta = 2M\mu$, with $M = 2N$ in diploid populations or $M = N$ in haploid populations of effective size *N*).The statistic *D* test is then defined as $D = \dfrac{\hat{\theta}_{\pi} - \hat{\theta}_S}{\sqrt{Var(\hat{\theta}_{\pi} - \hat{\theta}_S)'}}$ Where $\hat{\theta}_{\pi} = \hat{\pi}$ and $\hat{\theta}_S = S / \sum_{i=0}^{n-1}(1/i)$ and *S* is the number of segregating sites in the sample (Tajima, 1989).

The significance of the *D* statistic is tested by generating random samples under the hypothesis of selective neutrality and population equilibrium, using a coalescent simulation algorithm adapted from Hudson (1990). The *p*-value of the *D* statistic is then obtained as the propotion of random F_S statistics less or equal to the observation. A parametric approximation of the *p*-value assuming a beta-distribution limited by minimum and maximum possible *D* values was conducted. Note that significant *D* values can be due to many factors other than selective effects, for example, population expansion, bottleneck, or heterogeneity of mutation rates (Tajima, 1993; 1996).

The other neutrality test is Fu"s F_s test, which is also based on the infinite-site model without recombination like Tajima"s *D* test. Thus, it is also appropriate for short DNA sequences or RFLP haplotypes. The probability of observing a random neutral sample with a number of alleles similar or smaller than the observed value given the observed number of pairwise differences, taken as estimator of θ was evaluated. In more details, Fu (1997) first called this probability $S' = \Pr(K \geq k_{obs} | \theta = \hat{\theta}_{\pi})$ and defined the F_s statistic as the logit of *S'* $F_s = \ln(\dfrac{S'}{1 - S'})$.

Fu (1997) also noticed that the F_s statistic was very sensitive to demographic expansion, which generally leads to large negative F_s values.

The significance of the F_s statistic is tested by generating random samples under the hypothesis of selective neutrality and population equilibrium, using a coalescent simulation algorithm adapted from Hudson (1990). The p-value of the F_s statistic is then obtained as the proportion of random F_s statistics less or equal to the observation. Using simulations, Fu (1997) noticed that the 2% percentile of the distribution corresponded to the 5% cut-off value (i.e. the critical value of the test at the 5% significance level).

2.3.5 Mismatch distribution analysis

The mismatch distribution is the distribution of the observed number of differences between pairs of haplotypes. At demographic equilibrium, this distribution is usually multimodal in samples drawn from populations, since it reflects the highly stochastic shape of gene trees, but it is usually unimodal in populations having passed through a recent demographic expansion (Rogers and Harpending, 1992). For pure demographic expansion, on assumption that a stationary haploid population at equilibrium has suddenly passed τ generations ago from a population size of N_0 to N_1, then the probability of observing S differences between two randomly chosen non-recombining haplotypes is therefore given by

$$F_S(\tau,\theta_0,\theta_1) = F_S(\theta_1) + \exp(-\tau\frac{\theta_1+1}{\theta_1})\sum_{j=0}^{S}\frac{\tau^j}{j!}[F_S-_j(\theta_0) - F_S-_j(\theta_1)] \quad (\text{Li, 1977); where}$$

$F_S(\theta) = \dfrac{\theta^S}{(\theta+1)^{S+1}}$, which is the probability of observing two random haplotypes with

S differences in a stationary population (Watterson, 1975), $\theta_0 = 2\mu N_0$, $\theta_0 = 2\mu N_1$,
$\tau = 2\mu t$ and μ is the mutation rate for the whole haplotype.

The above equation was simplified by Rogers (1995), by assuming that $\theta_1 \to \infty$, implying that there were no coalescent after the expansion, which was only reasonable if the expansion size was large. With this simplifying assumption, it is

possible to derive the moment estimators of the time to the expansion τ and the mutation parameter θ_0, as

$$\hat{\theta}_0 = \sqrt{v - m}$$

$$\hat{\tau}_0 = m - \hat{\theta}_0 \text{ (Rogers, 1995)}$$

where m and v are the mean and the variance of the observed mismatch distribution, respectively. These estimators can then be used to plot $F_S(\tau, \theta_0, \infty)$ values. Note that this estimation cannot be done if the variance of this mismatch is smaller than the mean.

However, Schneider and Excoffier (1999) found that this moment estimator often led to an underestimation of the age of the expansion (τ). They rather proposed to estimate the parameters of the demographic expansion τ, θ_0, and θ_1 by a generalised non-linear least-square approach. Approximate confidence intervals for those parameters are obtained by a parametric bootstrap approach. The principle is the following: approximate confidence intervals for the estimated parameters $\hat{\theta}_1$, $\hat{\theta}_0$, and $\hat{\tau}$ were computed using a parametric bootstrap approach (Schneider and Excoffier, 1999) generating percentile confidence intervals.

A large number of random samples (B) was generated according to the estimated demography, using a coalescent algorithm modified from Hudson (1990). For each of the B simulated data sets, τ, θ_0, and θ_1 were re-estimated to get B bootstrapped value θ_0^*, θ_1^* and τ^*. For a given confidence level α, the approximate limits of the confidence interval were obtained as the $\alpha/2$ and $1-\alpha/2$ percentile values.

The validity of the estimated stepwise expansion model is also tested using the parametric bootstrap approach. The sum of square (*SSD*) between the observed and the expected mismatch was used as a test statistic. Its distribution was obtained under the hypothesis that the estimated parameters are true by simulating B samples around

these parameters. As before, each time new parameters θ_0^*, θ_1^* and τ^* were re-estimated and their associated sums of squares SSD_{sim} were computed. The p-value of the test is therefore approximately by

$$p = \frac{\text{number of } SSD_{sim} \text{ larger or equal to } SSD_{obs}}{B}$$

For convenience, the raggedness index of the observed distribution defined by Harpending (1994) could be computed as $r = \sum_{i=1}^{d+1}(x_i - x_{i-1})^2$; where d is the maximum number of observed differences between haplotypes and the x"s are the observed relative frequencies of the mismatch classes. This index takes larger values for multimodal distributions commonly found in a stationary population than for unimodal and smoother distributions typical of expanding populations. Its significance is tested similarly to that of SSD.

2.3.6 Coalescent-based Bayesian skyline plot

The coalescent-based Bayesian skyline plot is the method for estimating past population dynamics through time from a sample of molecular sequences without dependence on a prespecified parametric model of demographic history (Drummond et al., 2005). The Bayesian skyline plot model uses standard Markov chain Monte Carlo (MCMC) sampling approaches to estimate a posterior distribution of effective population size through time straightforwardly from a sample of gene sequences, given any specified nucleotide-substitution model (Drummond et al., 2005).

2.3.7 Time to recent common ancestor (t_{mrca})

Time to recent common ancestor (t_{mrca}) or divergence time is the time that distantly two taxa or two populations separated from their last common ancestor. It can be calculated from $E(T_{ij}) = \dfrac{E(K_{ij})}{2 \times E(v)}$, where T_{ij} is the divergence time, K_{ij} is the number of substitution site per site between two taxa, and v is the number of substitutions per site per year which can be estimated by using references such as

fossil data (Haubold and Wiehe, 2001). The t_{mrca} estimation also based on molecular clock assumption, i.e. the universal molecular clock for mitochondrial DNA is 2% sequence divergence per million year (Arbogast et al., 2002).

2.4 Cytochrome c oxidase subunit I (CO1)

2.4.1 Structure and function

Cytochrome c oxidase is one of a key enzyme in aerobic metabolism which catalyses the reduction of oxygen to water. Thus, it is an essential enzyme for aerobic metabolism (Castresana et al., 1994). Its structure is composed of three subunits: CO1 or Cox 1, Cox 2, and Cox 3. These regions consist of the amino and carboxyl terminals, six external loops which extend into the cellular cytoplasm, five internal loops, and 12 alpha-helix segments which transverse the inner membrane of the mitochondrion. The amino acid of CO1 protein ranges from 510 to 530 amino acids in length among different animal species and can be divided into 25 structural regions. The coding region of CO1 gene is about 1500 bp to 2000 bp in length which varies among animals. Although CO1 protein is conserved across all life because of its crucial metabolic function, there are amino acid variations especially at the carboxyl terminal (http://www.dnabarcoding.ca/primer/COIProtein.html).

2.4.2 Usage in genetic diversity and phylogenetic analyses

The rate of evolution of CO1 gene is three times greater than the former barcoding genes such as 12S and 16S ribosomal DNA genes, and appears to be rapid enough to allow the discrimination between closely related species. In addition, like many other functionally coding genes, the CO1 always contains nucleotide substitutions at the third position of a codon when compared between species. Therefore, it possesses a greater range of phylogenetic signal than the other barcoding genes and can even reveal phylogeographic relationship within a species (Cox and Hebert, 2001; Wares and Cunningham, 2001; Hebert et al., 2003). For examples, CO1 was used to clarify the molecular systematics of the freshwater prawn genus *Macrobrachium* (see Liu et al., 2007), taxonomy of the land-locked freshwater prawn

genus *Neocaridina* (see Shih and Cai, 2007), the phylogeography of mud carb (*Scylla serrata*) in the Indo-West Pacific region (He et al., 2011), and the phylogegraphic relationship within European spiny lobster (*Palinurus elephas*)(Palero et al., 2008). Hebert et al. (2003) have assumed that the threshold for discrimination between animal species using CO1 sequence differences is about 3%.

The mitochondrial DNA is extensively used in the studies of population structure, phylogeography, and phylogenetic relationship since it is maternally inherited and absent in the intermolecular genetic recombination (Avise, 2000). It is especially useful in clarification of intraspecific of genetic structure among populations of many crustaceans (Liu et al., 2009), for instance, CO1 DNA was used to investigate the genetic differentiation between populations of swimming crab *Portunus trituberculatus* within East China Sea (Liu et al., 2009), study population genetics of mud crab *Scylla paramamosain* in Hainan Island, China (Ma et al., 2011) investigate genetic structure of mud crab *Scylla serrata* in Indo-West Pacific region (Gopurenko, 2002), and assess the genetic variation and population structure of hair crab *Erimacrus isenbeckii* in Japan (Azuma et al., 2008).

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Animal specimens

A total of 206 individuals of shovel-nosed lobster genus *Thenus* were sampled from the Gulf of Thailand and Andaman Sea for this study. The specimens were collected from fish markets and ports located along the coasts of these following provinces: Chanthaburi, Chonburi, and Rayong provinces on the eastern side of the upper Gulf of Thailand; Phetchaburi and Prachuab Khiri Khan provinces on the western side of the upper Gulf of Thailand; Chumporn and Songkhla provinces on the lower Gulf of Thailand; and Phuket, Ranong, and Satun provinces along Andaman Sea (see Figure 3.1 and Table 3.1 for more details). Only adult specimens (with length more than 13 centimetres) were sampled. The specimens could be either frozen or still alive. However, the live samples were preferred since we can confidently assumed that they have been captured around 55 kilometres (not excess 30 sea miles) near the coast by small fishing boats, ensuring that the specimens should have lived in that area. The collected specimens were labelled, photographed, examined their external morphology, and then preserved in 95 percent of ethanol solution before proceeded to the genomic DNA extraction step.
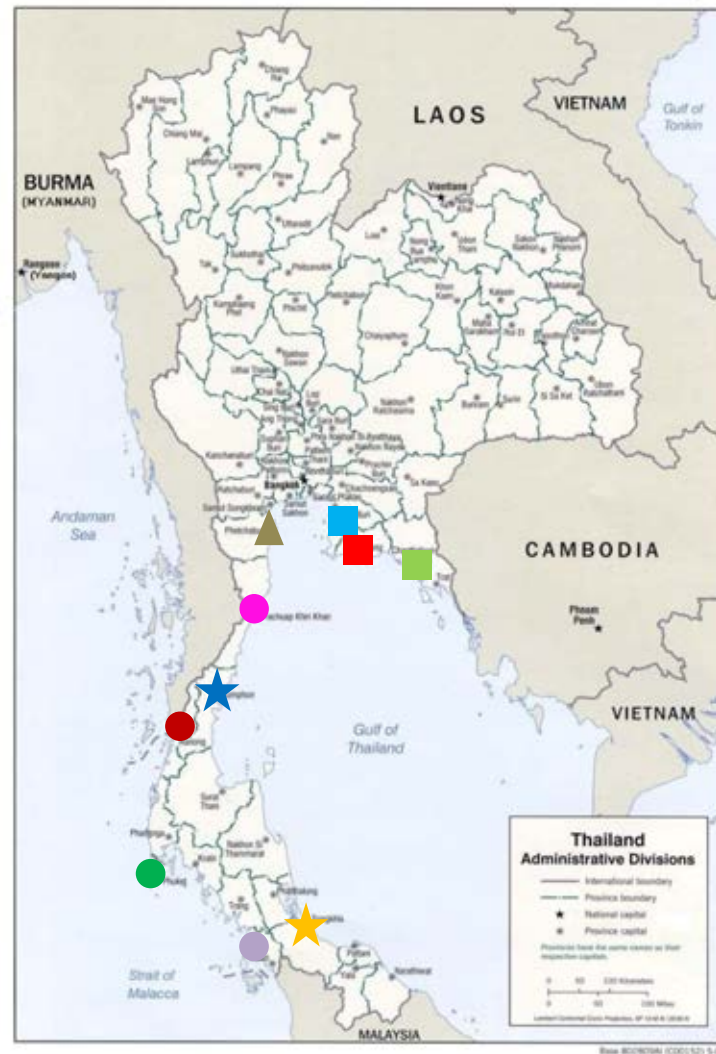
Figure 3.1 Sample collecting sites on ten provinces: Chanthaburi (green square symbol), Rayong (red square), Chonburi (blue square), Phetchaburi (tan triangle), Prachuap Khiri Khan (pink circle), Chumporn (blue star), Songkhla (yellow star), Satun (purple circle), Phuket (green circle), and Ranong (red circle).

Table 3.1 Shovel-nosed lobster specimens used in this study.

Sample names	Locality	Live/ frozen	Amount (individuals)	Collecting date
Chon01 to Chon24	Sri Racha port, Bang Lamung district, Chonburi province	Live	24	2/2010
Chon25 to Chon35	Samaesan district, Chonburi province	Frozen	11	8/1/2011
Chan01 to Chan24	Laem Sing, Laem Sing district, Chanthaburi province	Frozen	24	25/9/2010
Rayo01 to Rayo25	Mae Rumpheung beach, Muang district, Rayong province	Live	25	25/9/2010
Phet01 to Phet10	Cha-am fish market, Cha-am district, Phetchaburi province	Frozen	10	29/4/2010
Prac01 to Prac09	Noi bay, Muang district, Prachuab Khiri Khan province	Frozen	9	5/2/2011
Chum01 to Chum25	Chumporn port, Muang district, Chumporn province	Frozen	25	22/10/2010
Song01 to Song26	Songkhla port, Muang district, Songkhla province	Frozen	26	23/10/2010
Satu01 to Satu02	Satun port (Tam Malang), Muang district, Satun province	Frozen	2	23/10/2010
Phuk01 to Phuk28	Phuket port, Muang district, Phuket province	Frozen	28	24/10/2010
Rano01 to Rano22	Ranong port, Muang district, Ranong province	Frozen	22	25/10/2010

3.1.2 Equipments

- AC/DC power supply: model EC570-90 LVD CE (E-C Apparatus Corporation, USA)

- Autoclave: model SX-700 (Tomy Tech, Inc., USA)

- Automatic micropipette: (P10, P20, P200, and P1000) (Gilson, France)

- Centrifuge/vortex mixer: model centrifuge FVL-2400 (Biosan, Latvia)

- Electronic UV transilluminator: model M-20V (UVP, UK)

- Electrophoresis chamber set: model Mupid (Advance Co., Ltd., Japan)

- Microcentrifuge tubes: (1.5 ml) (Axygen Scientific, Inc., USA)

- Microcentrifuge: model centrifuge Sorvall pico D-37520 Osterode (Kendro Laboratory Products, Germany)

- Microwave oven: model Sharp Carousel R7456 (Sharp, Thailand)

- PCR machine: model GeneAmp$^{®}$ PCR system 9700 (Applied Biosystem, Singapore)

- Pipette tips: (10, 200, and 1,000 μl) (Axygen Scientific, Inc., USA)

- Plates: (Pyrex$^{®}$, USA)

- Scissors

- Vortex mixer: model MS I Minishaker (IKA-Works, Inc., USA)

3.1.3 Chemicals

- Absolute Ethanol

- 95% Ethanol

- Agarose gel (Research Organics, USA)

- Boric acid (Research Organics, USA)

- Bromophenol blue ($C_{19}H_{10} Br_4O_5S$), M.W. = 670 (Research Organics, USA)

- 100 bp DNA Ladder Marker (SibEnzyme, Russia)

- EDTA (Ethylene diamine tetra-acetic acid) ($C_{10}H_{14}N_2O_8Na_2H_2O$), M.W. = 372.24 (Bio Basic, Inc., USA)

- Ethidium bromide, M.W. = 934.32 (Bio Basic, Inc., USA)

- Dynazyme Taq DNA polymerase (Finnzyme, Finland)

- Tris-base (Research Organics, USA)

- 6x loading dye (glycerol 4 ml, bromophenol blue 25 mg and 1x TBE buffer upto 100 ml)

- 10x TBE buffer (Tris-base 108 g, boric acid 55 g, 0.5 M EDTA (pH 8.0) 80 ml and distilled water added upto 1 litre)

3.2 Methods

3.2.1 Specimen identification

The specimens were brought back to the laboratory to identify their putative scientific names following these morphological characteristics (Burton and Davie, 2007): the pattern of spot or colour on a propodus of periopods, a spine on a merus, and a dentation on an ischium of the third maxilliped. *Thenus indicus* does not have any spot on the propodus of the periopods, but shows a spine on the merus and a dentation on the ischium of the third maxiliped (see Figure 3.2A-C). The morphometric ratios applied for *T. indicus*: are as follows: the ML3 to CL ratio is more than 0.45 and the MW1 to CL ratio is less than 0.07. *Thenus orientalis* has brown spots on the propodus of the periopods (see Figure 3.2D-F). There is a spine on the merus and a dentation on the ischium of the third maxiliped. The MW2 to CL ratio is less than 0.079 and the TL to TW ratio is more than 0.31. *Thenus unimaculatus* has purple blotches on the propodus of the periopods, also with a spine on the merus and a dentation on the ischium of the third maxiliped (see Figure 3.2G-I). The CW to CL ratio is more than 1.29, the PL1 to CL ratio is less than 0.23, the PL2 to CL ratio is more than 0.39, and the PW1 to PL1 ratio is more than 0.35.
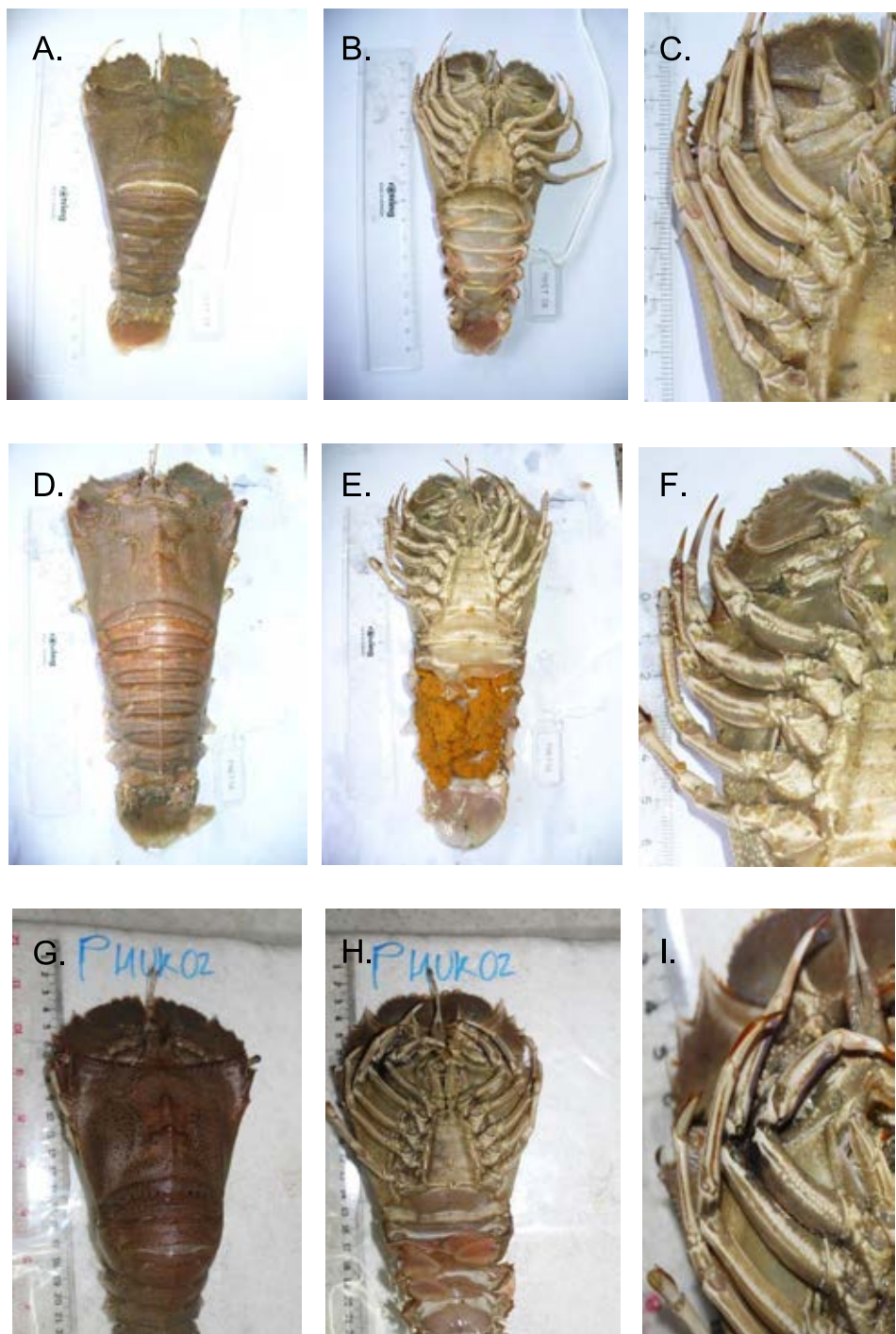
Figure 3.2 Externally morphological characteristics of three *Thenus* species of Thailand. (A.-C.) The specimen Phet08 as a representative of *T. indicus*. [(A) dorsal view, (B) ventral view, (C) non-spotted periopods], (D.-F.) Phet06 sample as *T. orientalis*. [(D) dorsal view, (E) ventral view, (F) brown spots and dots on periopods], and (G.-I.) Phuk02 sample as *T. unimaculatus* [(G) dorsal view, (H) ventral view, (I) purple blotched on periopods].

3.2.2 Genomic DNA extraction

Total genomic DNA was extracted from the periopod or abdominal tissues using innuPREP DNA Mini Kit (Analytik Jena, Germany) following manufacturer"s protocols.

Firstly, 50 mg of the tissue was cut into small pieces and placed into a 1.5 ml microcentrifuge tube. Four hundred micolitres of Lysis solution TLS and 25 µl of Proteinase K were added and the solution was mixed thoroughly by pulsed vortexing for five seconds. The solution was incubated at 50°C approximately one to two hours. After that, the sample was centrifuged in order to spin down unlysed material for one minute at 10,000 g (12,000 rpm) and then the supernatant was transferred into a new 1.5 ml microcentrifuge tube. Four hundred micolitres of TBS Binding solution was added to the supernatant and mixed by briefly vortexing for 15 seconds. Next, the sample was applied to the spin filter (blue) situated in a 2.0 ml receiver tube. The cap of the spin filter was closed and centrifuged at 10,000 g (12,000 rpm) for two minutes. The receiver tube with the filtrate was discarded. At this step, DNA was immobilised on silica gel of the spin filter. After that, the spin filter was placed into a new 2.0 ml receiver tube and 500 µl of HS washing solution was added and then centrifuged at 10,000 g (12,000 rpm) for one minute. The receiver tube with the filtrate was discarded and the spin filter was placed into a new 2.0 ml receiver tube. Next, 750 µl of MS washing solution was added and then centrifuged at 10,000 g (12,000 rpm) for one minute. The receiver tube with the filtrate was discarded and the spin filter was placed into a new 2.0 ml receiver tube. After that, the centrifugation at max speed for two minutes was performed in order to remove all traces of ethanol. The receiver tube with the filtrate was discarded. Then, the spin filter was placed into a 1.5 ml elution tube and then 100 µl of elution buffer was added. Next, the sample was incubated at room temperature for one minute. The second elution step was performed in order to increase the yield of extracted DNA. The extracted DNA was stored in -20°C for long term usage. Subsequently, DNA was electrophoresed to examine quality and estimate concentration of the extracted DNA compared with the DNA marker.

3.2.3 Agarose gel electrophoresis

The samples of genomic DNA or PCR products were mixed with 6x loading dye (0.15% bromophenol blue) in ratio 5 to 1 and then transferred to the wells of an agarose gel chamber. The concentration of agarose gel solution was 1% agarose in TBE buffer (Tris/Borate/EDTA buffer) for genomic DNA and 1.8% agarose for PCR products. The DNA samples were run under 80 Volt for 30 minutes for genomic DNA and 45 minutes for PCR products. Afterward, the gel was stained in 1.27 µM ethidium bromide (EtBr) solution for 5 minutes and then destained for 15 minutes to remove an excess amount of EtBr. The gel was visualised under UV light and photographed using UV transilluminator.

3.2.4 Polymerase chain reaction (PCR)

The mitochondrial CO1 gene was amplified using a PCR technique. Forward and reverse primers for the CO1 gene of crustaceans were suggested by Folmer et al. (1994). The total 50 µl PCR mixture contained 5 µl of 10x *Taq* polymerase buffer, 1.2 µl of 10 mM dNTP mix solution (equally to 2.5 mM dNTP each), 5 µl each of 10 µM CO1-1490 forward primer (5"-GGT CAA CAA ATC ATA AAG ATA TTG G-3") and 10 µM CO1-2198 reverse primer (5"-TAA ACT TCA GGG TGA CCA AAA AAT CA-3"), 0.5 µl of 2 U/µl Dynazyme II *Taq* DNA polymerase (Finnzyme, Finland), 1 µl of the extracted genomic DNA with at least 25 ng/µl concentration, and sterile distilled water added to final 50 µl volume. The condition of PCR was adapted from Folmer et al. (1994) as following: an initial denaturation at 95°C for 5 minutes, 35 cycles of denaturation step at 95°C for 1 minute, an annealing step at 48 to 52°C for 1 minute, and an extension step at 72°C for 1 minute 30 seconds and the final extension step at 72°C for 5 minutes. The PCR products were checked for size and quantity by agarose gel electrophoresis (see Section 3.2.3).

3.2.5 DNA sequencing

The PCR products were purified with innuPREP PCRpure kit (Analytik Jena, Germany) following the manufacturer"s protocols before sending to Macrogen Inc.

(Korea) for sequencing. The spin filter of the purification kit was firstly placed into a 2.0 ml receiver tube. Then, 50 µl of the PCR product was well mixed with 500 µl of binding buffer by vortexing or pipetting in a separate 1.5 ml microcentrifuge tube. After this, the mixed sample was completely transferred onto a spin filter. Next, the centrifugation was performed at 10,000 g (12,000 rpm) and the receiver tube containing filtrate was discarded. The spin filter was placed on a new elution tube and 50 µl of elution buffer was pipetted directly onto the centre of the spin filter. An incubation was performed for 1 minute at room temperature. Then, the centrifugation was performed at 6,000 g (8,000 rpm) and the elution tube now contained the purified PCR fragments.

The sequencing reactions was conducted using BigDye® Terminator chemicals and run on ABI3730XL automated sequencer (Applied Biosystems, USA) as the service provided by Macrogen Inc. (Korea). The sequence output came as a chromatogram file (with extension .ab1).

3.2.6 Molecular phylogenetic analyses

The chromatogram files (.ab1) were analysed in Chromas Lite program version 2.01 (Technelysium Pty Ltd) and exported as FASTA format for further analyses. If there were a lot of noise signals found in the chromatogram, the sequencing reactions had to be repeated. If the repeated sequencing cannot decrease the noise, the PCR reactions were optimised by increasing the annealing temperature to at least 2°C and the purified PCR products were sequenced again. The CO1 sequences from all specimens were combined into one single FASTA file and aligned using ClustalW algorithm (Larkin et al., 2005) as implemented in MEGA (Molecular Evolutionary Genetic Analysis) program version 5 (Tamura et al., 2011). The aligned DNA sequence matrix was used to reconstructed phylogenetic trees with these approaches: neighbour-joining (NJ) and maximum likelihood (ML) methods implemented in MEGA5, maximum parsimony (MP) method implemented in PAUP* (Phylogenetic Analysis Using Parsimony and other methods) program version 4.10b (Swofford, 2002), and Bayesian inference (BI) method implemented in MrBayes

program version 3.12 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). When using NJ approach analysis, the sequence dissimilarity values were calculated using genetic distance corrected by Kimura-2 parameters and the NJ tree was evaluated with 1,000 replicates of bootstrap analysis. For MP approach, the parsimony searching strategy was set following these options: heuristic search, branch swapping algorithm used tree-bisection-reconnection (TBR), random addition, and steepest descent. The MP search results were also tested with 1,000 replicates of bootstrap analysis. To reconstruct a ML tree, the nucleotide sequence data was subjected to find the best fit evolutionary model with lowest AIC (Akaike information criterion) value (Akaike, 1974) (AIC = 3712.5217) using jmodeltest program version 0.1.1 (Posada, 2008). The best model suggested was Tamura and Nei parameter model (TrN+I+G) with invariant site and gamma distribution. The following options were set: ln L (natural logarithm of likelihood) = -1811.2609, f(A) (frequency of A) = 0.2568, f(T) = 0.2171, f(C) = 0.1824, f(G) = 0.3419, A=>T (rate of change from A to T) = 1.0000, A=>C = 1.0000, A=>G = 18.5871, T=>A = 1.0000, T=>C = 8.2002, C=>T = 8.2002, T=>G = 1.0000, C=>A = 1.0000, C=>G = 1.0000, G=>A = 18.5871, G=>T = 1.0000 and G=>C = 1.0000, assumed proportion of invariable sites = 0.607, distribution of rate at variable sites = gamma, shape parameter (alpha) = 0.745, number of rate categories = 4. The searching strategy for ML appoarch was heuristic search, branch swapping algorithm used tree-bisection-reconnection (TBR), random addition, steepest descent, and stepwise addition option at additional sequence by random 50 replicates. The ML search results were also tested with 1,000 replicates of bootstrap analysis. For BI method, GTR model was used to reconstruct a BI tree using MrBayes. The MCMC (Monte Carlo Markov Chain) process was used to sample trees at 8,000,000 generations and the sample frequency was set to 1,000. The sampling process was conducted until the standard deviation fell below 0.05. Twenty five percentage of the samples were discarded as a burn-in. Then, the BI trees were summarised and viewed with FigTree program version 1.3.1.

The CO1 DNA sequences were prepared into three separated data sets: data set I contained the aligned 390 bp data matrix of total 206 Thai *Thenus* sequences

from this study with 5 reference *Thenus* sequences (HM015421 *T. parindicus*, HM015433. *T. australiensis*, HM015440 *T. orientalis*, HM015445 *T. indicus*, and HM015449 *T. unimaculatus*) and use HM015458 *Ibacus peronii* as an outgroup for species identification of Thai *Thenus* samples, data set II composed of the aligned 667 bp data matrix of selected 20 *Thenus* sequences (*T. indicus*: Chan06, Chon19, Chum09, Chum16, Prac06, Rayo03, Rayo04, Rayo14, Rano01, Rano09, Rano12, and Rano22, *T. orientalis*: Phet03, Phet04, Song01, and Song15, *T. unimaculatus*: Phuk01, Phuk06, Phuk11, and Phuk16) for clarifying evolutionary relationship between three *Thenus* spp., and data set III contained the aligned 669 bp data matrix of 163 *T. indicus* sequences and use Song04 (*T. orientalis*) and Satu02 (*T. unimaculatus*) as outgroups for clarifying genetic relationship within *T. indicus* samples form Thailand. The data set I will be analysed by NJ approach and data set II will be analysed by MP and ML approaches while the data set III will be analysed by BI approach.

3.2.7 Genetic diversity analyses

The FASTA file of the aligned CO1 sequences was loaded into DnaSP (DNA Sequence Polymorphism) program version 5.10.01 (Librado and Rozas, 2009) and the genetic code option was set for invertebrate mitochondrial DNA and haploid genome. Ten different FASTA files were prepared following the provincially geographical distributions of the shovel-nosed lobster species: Overall.fas which contained every samples in this study (163 sequences), Gulf of Thailand.fas which contained the samples from the Gulf of Thailand (142 sequences), Chonburi province.fas (35 sequences), Rayong.fas (25 sequences), Chanthaburi.fas (24 sequences), Phetchaburi.fas (3 sequences), Phachuap Khiri Khan.fas (9 sequences), Chumporn (25 sequences), Songkhla (21 sequences), and Ranong (21 sequences). The data files were also exported as NEXUS and ARLEQUIN formats. The molecular diversity indices, i.e. a number of alleles or haplotypes, nucleotide diversity, and haplotype diversity (Nei, 1987), were estimated with ARLEQUIN program version 3.5.1.2 (Excoffier and Lischer, 2010).

The populations of *T. indicus* in Thailand were assigned into three groups; the upper Gulf of Thailand, which was composed of Chonburi, Chanthaburi, Rayong, Phetchaburi, Prachuap Khiri Khan, and Chumporn samples, the lower Gulf of Thailand, which was composed of only Songkhla samples; and the group of Andaman Sea, which was composed of Ranong samples. The analysis of molecular variance (AMOVA) was performed using two separate data sets. The first AMOVA test was performed using the data set containing three population groups (the upper Gulf of Thailand, the lower Gulf of Thailand, and the Andaman Sea). The second AMOVA test was analysed using the data set containing the upper Gulf of Thailand and the lower Gulf of Thailand groups. The AMOVA analysis was conducted using ARLEQUIN program with Tamura-Nei parameter (TN93) model and the number of permutations was set at 10,000.

The F_{st} population comparisons were performed with 10,000 permutations equal to, also using TN93 model at the 0.05 significant level. The mismatch distribution analysis was conducted by estimating the expected number of mismatch differences, Tau ($\tau = 2\mu t$), Theta0 ($\theta_0 = 2N_o\mu$), and Theta1 ($\theta_1 = 2N_1\mu$) from, , and with the assumption that initial effective population size of N_0 grows rapidly to a new of population size N_1 at a time before the present t generations, and μ is the probability that a mutation occurs at a particular nucleotide per generation (Roger and Harpending, 1992). Tajima''s D and Fu''s F_s neutrality tests were conducted with 10,000 simulated samples. The CO1 data sequences of *T. indicus* also exported as a ROEHL data file and further used to construct a haplotype network by Median-Joining network algorithm as implemented in Network version 4.6 program (Bandelt et al., 1999; http://www.fluxus-engineering.com).

The NEXUS file exported from DnaSP was further imported into BEAUti (Bayesian Evolutionary Analysis Utility) version 1.6.1 program (Drummond and Rambaut, 2007). The ingroup was every sample in this study. Tip dates were guessed using "guess dates" option. The substitution model was set to HKY (Hasegawa, Kishino and Yano''s model). The strict molecular clock was set at 1.15% divergence

per lineage per million year (suggested by Brower, 1994). The tree prior was set to "Coalescent: Bayesian Skyline" with ten groups. The prior distribution of "skyline.popsize" parameter was set to normal with default values; and the length of chain of MCMC was set at 8,000,000. The BEAST"s XML file was created and used in BEAST (Bayesian Evolutionary Analysis by Sampling Trees) program version 1.6.1 (Drummond and Rambaut, 2007). The log of output file was opened with Tracer program version 1.5.1 (Drummond and Rambaut, 2007) and then the Bayesian Skyline plot was constructed. For the time to recent common ancestor (t_{mrca}), the preparation of BEAST"s XML file was performed as described above, except for that the tree prior was set to "Coaslescent: Constant Size". The analysis was conducted on BEAST and viewed the t_{mrca} result in Tracer program.

CHAPTER IV

RESULTS

4.1 Shovel-nosed lobsters fishery of Thailand

A total of six field trips for sample collection was conducted in this study. The first field survey was on February 2010 at a Thai restaurant located at Bang Lamung district, Chonburi Province. A lot of live lobsters were found. These lobsters were transported from Sri Racha port, Sri Racha district, Chonburi province and the price was about 600 baht per kilogram. The restaurant owner informed that every seafood restaurant there would rather prefer selling live lobsters than the frozen ones because the taste was better. All of the live lobsters there were found to be *Thenus indicus* due to a lack of spots on their propodus of periopods. Ten specimens were sampled.

The second trip was on 24th April 2010 to a fish market at Cha-am district in Phetchaburi province. There were both live and frozen lobsters for sale at the price of 420 baht per kilogram and 450 baht per kilogram, respectively. The live lobsters were caught by small local fishing boats while the frozen ones were thrown by much larger commercially fishery boats. The shovel-nosed lobsters then were captured by a single or a pair of trawl nets and they could live for only two or three days after the capture. The selling of live lobsters might be only in March to April and their selling on the other period of the year would be available only as frozen. Ten frozen specimens were randomly collected and three of them (Phet01, Phet07, and Phet08) were identified as *T. indicus* (see Figure 3.1, A.-C.) while the remaining seven (Phet02 to Phet06, Phet09, and Phet10) were identified as *T. orientalis* since they had brown spots on their propodus of periopods (see Figure 3.1, D.-F.).

Figure 4.1 Frozen (A) and live (B) shovel-nosed lobsters for sale in Phetchaburi province.

The third survey was on 25th September 2010 in rainy season. Five provinces (Samut Prakarn, Rayong, Chanthaburi, Trat, and Chonburi) were visited and totally 49 shovel-nosed lobster specimens were collected. Firstly, Pak Nam fish market, Muang district, Samut Prakarn province, was visited but no shovel-nosed lobster was sold there because no trawling boat arrived on that day. However, some interesting information on the fishery of shovel-nosed lobster was obtained such as only small amount of shovel-nosed lobsters could be captured (only 20 to 30 kilograms per a small boat) in the Gulf of Thailand while their price was around 150 baht per kilogram. Any shovel-nosed lobsters in large size (0.4 to 0.5 kilogram per individual) sold in many famous seafood restaurants in Thailand might come from Indonesian waters. Such open sea region is much deeper than the Gulf of Thailand and then there is still a lot of shovel-nosed lobsters, usually captured in a huge amount (over 100 kilograms per boat with even bigger in size).

From the survey at Mae Rumpheung beach, Muang district, Rayong province, small live lobsters were available there (Figure 4.2) and the price was around 580 baht per kilogram (equally to 7 to 8 individuals). The lobsters were locally caught either with crab traps or trawl nets around Rayong water. Some frozen shovel-nosed lobsters captured by the large commercial boats were also available. The fishermen also reported that the number of shovel-nosed lobsters captured there was usually under the influence of rainy monsoon. There would be a lower number of the lobsters if no monsoon. Twenty-five periopod samples were collected and identified as *T. indicus*.

Figure 4.2 The seafood stalls in Rayong province (A) storing both live and frozen shovel-nosed lobsters. Live shovel-nosed lobsters (B) for sale were aerated in the bucket.

The following province being surveyed in the third trip was Laem Sing fish market, Laem Sing district, Chanthaburi. Although no shovel-nosed lobster could be found at the market, some was available at local seafood restaurants. The price over there was 250 baht per kilogram for frozen lobsters and 300 baht per kilogram for live lobsters. The local seller also said that the period of catching time for one sailing trip of local fishing boats was one to three days. Twenty-four frozen lobster samples were collected and all of them were identified as *T. indicus*.

Laem Ngob district of Trat province was later visited in the third trip. Unfortunately, none of any shovel-nosed lobster was not be found there. The fishermen in that area informed that only a little number of shovel-nosed lobsters were caught each year with the price was around 200 to 300 baht. They also confirmed that the lobsters were found mostly in pebble habitat of Thai sea floors and the lobsters were abundant in rainy season with strong monsoon, usually from September to October, and low in number in January to March. The last collecting place in that trip was Bang Lamung district, Chonburi province (the same place as in the first trip) and 14 live specimens of *T. indicus* were collected.

The fourth trip was on 22nd to 25th October 2010 to five southern provinces: Chumporn, Songkhla, Satun, Phuket, and Ranong, and totally 103 specimens were collected. The first sampling location was Chumporn port, Muang district, Chumporn province on 22nd October 2010 where there were many local boats with trawl nets. Their information suggested that shovel-nosed lobsters could be found and captured at sand dune area under water of 15 to 18 metres depth. However, the number of capturing was rather low in November. The price over there was around 200 baht per kilogram and the 25 frozen specimens, which were identified as *T. indicus*, were collected.

On 23rd October 2010 Tha Sa-an port (or so-called Songkhla port), Muang district, Songkhla province was surveyed and 26 frozen specimens were collected. Five of them were identified as *T. orientalis* and the other 21 samples were *T. indicus*. One local fish seller informed that recently there had been a quite lower number of

shovel-nosed lobsters in the Gulf of Thailand (40 to 50 kilograms per catch) compared to the past. They were captured by medium-sized trawling boats at the 30 metres depth and the price was around 130 baht per kilogram. Some of the lobsters sold there were captured from Sarawak region, Malaysia, where they could be caught in larger amount of over 100 kilogram per catch.

Next sampling location was Tummalung port, Muang district, Satun province. Only two frozen specimens were found and identified as *T. unimaculatus* (with purple blotches on propodus of periopods). The fishermen informed that the lobsters usually inhabited coral reef or rocky shore, at 20 metres depth, and could be found at only three sea miles far from the shore. The lobsters were usually found in a rather low number (not exceed 20 individuals per catch). Therefore, they would not sell their lobsters, but rather keep them for their own food during the long 10-to-15 days sailing trip.

The survey was then to Phuket Island, at Phuket port, Muang district, on 24th October 2010. Some local fishermen informed that the shovel-nosed lobsters over there were usually caught by fishing boats with a pair of trawl nets throughout the year. Twenty-eight frozen specimens were collected and all of them were identified as *T. unimaculatus*. This species lived in sandy and muddy habitats at 80 to 100 metres depth. The fishermen informed me that the catching amount of shovel-nosed lobsters in Phuket had decreased recently and the lobsters were sold by three sizes: number one (250 baht per kilogram) of a large lobster which weights more than 0.2 kilogram per individual, number two (220 baht per kilogram) weighting 0.1 to 0.2 kilogram per individual, and number three (150 baht per kilogram) weighting up to 0.1 kilogram per individual.

On the last day of this fourth trip, next sampling location was Ranong port, Muang district, Ranong province. Twenty one individuals of frozen *T. indicus* and other one specimen of *T. unimaculatus* were collected. The local fish sellers informed that the lobsters sold there did not only come from Ranong but also from Myanmar waters.

The last two surveys were on 8th January and 5th Febuary 2011, respectively. First, 12 frozen specimens which were identified as *T. indicus* were collected from a small seafood market in Chong Samae San, Sattahip district, Chonburi province. Then, nine frozen *T. indicus* were collected from a fish market at Noi bay, Muang district, Prachuap Khiri Khan province. Some local fishermen interviewed in this last trip suggested that shovel-nosed lobsters could be captured from sandy and muddy sea floors about ten sea miles from the seashore. Unfortunately, a little amount of shovel-nosed lobster had been recently found throughout the year.

4.2 Morphometric ratio results

The number of individuals, the sex ratios, and the average morphological measurement of total 106 *Thenus* specimens are shown in Table 4.1 (see Appendix for detailed measurements). The average sex ratios (male : female) of *T. indicus, T. orientalis*, and *T. unimaculatus* are 1:1.44, 1:4.00, and 1:1.81, respectively. The average total lengths of *T. orientalis* was found to be biggest among all three species with the total length of approximately 22 cm. The size of *T. unimaculatus* was close to that of *T. orientalis* with the average length of 19 cm. However, one of the samples was as big as 23.6 cm (Satu01). *Thenus indicus* was found to be the smallest species with the mean length around 17 cm. Notably, *T. indicus* specimens sampled from the Andaman Sea, were approximately 3 cm longer than those from the Gulf of Thailand. Their size is similar to that of *T. unimaculatus*, which also live in the Andaman Sea.

Table 4.1 Morphological measurements of totally 106 *Thenus* specimens in this study.

	Thenus specimens				
		T. indicus		*T. orientalis*	*T. unimaculatus*
	Total	**Gulf of Thailand**	**Andaman Sea**		
numbers of individuals	83	73	10	11	12
Sex ratios (male : female)	1:1.44	1:1.53	1:1.33	1:4	1:1.81
Average total length (cm)	16.67 ± 3.05	16.17 ± 2.96	19.12 ± 2.20	21.72 ± 2.98	19.29 ± 2.23
Carapace					
CL1 (cm)	5.22 ± 0.85	5.10 ± 0.80	6.08 ± 0.69	6.90 ± 0.77	6.61 ± 0.78
CW1 (cm)	6.49 ± 1.00	6.41 ± 0.97	7.07 ± 1.08	8.24 ± 0.92	8.28 ± 1.04
CW2 (cm)	4.70 ± 0.72	4.62 ± 0.72	5.24 ± 0.47	6.15 ± 0.68	5.97 ± 0.79
Antenna I					
A1L (cm)	1.39 ± 0.27	1.37 ± 0.27	1.58 ± 0.15	1.87 ± 0.30	1.64 ± 0.27
A1W (cm)	2.10 ± 0.39	2.05 ± 0.36	2.51 ± 0.31	2.72 ± 0.45	2.46 ± 0.37
Antenna II					
A2L (cm)	1.92 ± 0.42	1.89 ± 0.42	2.14 ± 0.28	2.56 ± 0.45	2.18 ± 0.28
A2W (cm)	2.50 ± 0.45	2.48 ± 0.47	2.65 ± 0.28	3.27 ± 0.40	2.75 ± 0.31
1st Abdomen segment					
AL1 (cm)	1.08 ± 0.20	1.05 ± 0.19	1.25 ± 0.16	1.35 ± 0.19	1.43 ± 0.16
AW1 (cm)	4.27 ± 0.74	4.17 ± 0.73	4.97 ± 0.47	5.92 ± 0.71	5.57 ± 0.74
Periopod 1					
PL1 (cm)	1.29 ± 0.27	1.26 ± 0.27	1.51 ± 0.20	1.76 ± 0.21	1.63 ± 0.19
PW1 (cm)	0.43 ± 0.10	0.41 ± 0.10	0.53 ± 0.08	0.59 ± 0.12	0.62 ± 0.09
ML1 (cm)	1.55 ± 0.30	1.53 ± 0.31	1.69 ± 0.17	2.23 ± 0.31	1.81 ± 0.26
MW1 (cm)	0.56 ± 0.11	0.54 ± 0.10	0.68 ± 0.10	0.70 ± 0.13	0.77 ± 0.12
Periopod 2					
PL2 (cm)	1.67 ± 0.38	1.63 ± 0.37	2.01 ± 0.25	2.23 ± 0.29	2.10 ± 0.31
PW2 (cm)	0.41 ± 0.11	0.40 ± 0.10	0.53 ± 0.08	0.55 ± 0.15	0.62 ± 0.09
ML2 (cm)	2.11 ± 0.41	2.07 ± 0.41	2.38 ± 0.22	2.82 ± 0.34	2.48 ± 0.35
MW2 (cm)	0.51 ± 0.11	0.49 ± 0.10	0.63 ± 0.10	0.65 ± 0.13	0.68 ± 0.11
Periopod 3					
PL3 (cm)	1.42 ± 0.29	1.39 ± 0.29	1.63 ± 0.19	2.12 ± 0.38	1.88 ± 0.26
PW3 (cm)	0.35 ± 0.10	0.34 ± 0.09	0.46 ± 0.08	0.47 ± 0.13	0.53 ± 0.09
ML3 (cm)	2.18 ± 0.41	2.14 ± 0.41	2.46 ± 0.26	3.00 ± 0.32	2.55 ± 0.33
MW3 (cm)	0.49 ± 0.16	0.48 ± 0.16	0.57 ± 0.08	0.59 ± 0.13	0.65 ± 0.09
6th abdominal seg.					
AL2 (cm)	0.92 ± 0.19	0.92 ± 0.19	0.98 ± 0.14	1.11 ± 0.21	0.92 ± 0.21
AW2 (cm)	2.80 ± 0.48	2.75 ± 0.47	3.18 ± 0.36	3.71 ± 0.48	3.49 ± 0.53
Telson					
TL (cm)	0.76 ± 0.15	0.75 ± 0.15	0.83 ± 0.10	0.99 ± 0.15	1.00 ± 0.17
TW (cm)	2.34 ± 0.40	2.29 ± 0.39	2.66 ± 0.33	3.12 ± 0.44	2.91 ± 0.45

The detailed results of eight morphometric ratios measured from 106 shovel-nosed lobster specimens are shown in Table 4.2. The summary of mean and standard deviation values of each province is shown in Table 4.3. The morphometric ratios of total 106 samples revealed only 11.3% (12 samples, i.e. eight samples of *T. indicus*, three *T. orientalis*, and one *T. unimaculatus*) was correctly identified following the species identification criterion of Burton and Davie (2007). Forty-three samples (38 samples of *T. indicus*, four *T. orientalis*, and one *T. unimaculatus*) (= 40.6%) were identified to a single specific name using the morphometric ratios, but the given names are not consist with those suggested by propodus morphology. Furthermore, 42 samples equivalent to 39.6% (30 samples of *T. indicus*, three *T. orientalis*, and nine *T. unimaculatus*) were ambiguously given two or more specific names. In the worst case, the rest of the samples left (seven samples of *T. indicus* and one *T. orientalis*) (7.6%) cannot be identified using the morphometric ratios because the ratios were not in any range of the criterion. The comparison between the three *Thenus* species revealed that *T. indicus* was the most problematic one to be identified using the morphometric measurement ratios since 45.8% of them were incorrectly identified.

Table 4.2 Morphometric ratios of 106 *Thenus* samples in this study. The bold numbers indicated the in-ranged ratios, which can suggest a scientific name following Burton and Davie's criterion (2007). Suggested specific names were compared between morphological ratios and morphological characteristics. Abbreviations of the suggested specific names are: ind for *T. indicus*, ori for *T. orientalis*, and uni for *T. unimaculatus*.

Sample names	Morphological measurement ratios								Species identified by measurement ratios	Species identified by morphological characteristics
	CW1/CL	ML3/CL	MW1/CL	MW2/CL	PL1/CL	PL2/CL	PW1/PL1	TL/TW		
Chon01 (live)	1.29	**0.46**	-[a]	0.088	-[a]	0.27	-[a]	0.27	ind	ind
Chon02 (live)	1.29	**0.51**	0.09	**0.077**	0.28	-[a]	0.19	0.25	ori, ind	ind
Chon03 (live)	1.24	**0.47**	0.1	0.083	0.27	0.33	0.27	**0.34**	ori, ind	ind
Chon04 (live)	1.29	**0.45**	0.1	0.083	0.27	0.35	0.3	0.25	ind	ind
Chon05 (live)	1.23	**0.48**	0.1	0.083	0.26	0.34	0.27	**0.33**	ori, ind	ind
Chon06 (live)	1.27	**0.49**	0.09	**0.078**	0.25	0.34	0.28	0.29	ori, ind	ind
Chon07 (live)	**1.43**	**0.54**	0.1	0.088	0.28	**0.51**	0.28	0.27	uni, ind	ind
Chon08 (live)	1.2	**0.47**	0.09	0.087	0.25	0.36	0.29	0.25	ind	ind
Chon09 (live)	1.24	**0.47**	0.1	0.084	0.26	0.35	0.3	0.29	ind	ind
Chon10 (live)	1.2	**0.49**	0.27	0.081	0.27	-[a]	0.27	0.3	ind	ind
Chon25 (frozen)	1.11	0.41	0.1	0.1	0.23	0.29	0.32	0.28	-[b]	ind
Chon26 (frozen)	1.05	0.37	0.1	0.091	**0.22**	0.26	0.32	0.27	uni	ind
Chon27 (frozen)	1.06	0.39	0.09	0.081	**0.22**	0.26	0.33	0.38	uni	ind
Chon28 (frozen)	1.04	0.45	0.09	0.087	**0.22**	0.28	0.32	0.33	uni	ind
Chon29 (frozen)	1.06	0.37	0.09	0.093	**0.21**	0.28	0.35	0.29	uni	ind
Chon30 (frozen)	1.08	0.4	0.09	0.081	**0.21**	0.27	0.33	0.27	uni	ind
Chon31 (frozen)	1.04	0.4	0.1	0.096	**0.22**	0.29	0.29	0.28	uni	ind
Chon32 (frozen)	1.06	0.39	0.1	0.086	0.24	0.28	0.26	0.29	-[b]	ind
Chon33 (frozen)	1.04	0.36	0.09	0.083	**0.19**	0.25	**0.37**	0.28	uni	ind
Chon34 (frozen)	1.09	0.39	0.09	0.084	**0.22**	0.31	0.32	0.42	uni	ind
Chon35 (frozen)	1.06	0.4	0.1	0.087	**0.21**	0.26	0.34	0.3	uni	ind

[a]Some morphological ratios could not be calculated because of the loss of the first and the second periopods.
[b]These specimens cannot be given a specific name by the ratio method.

Table 4.2 (continued).

Sample names	Morphological measurement ratios								Species identified by measurement ratios	Species identified by morphological characteristics
	CW1/CL	ML3/CL	MW1/CL	MW2/CL	PL1/CL	PL2/CL	PW1/PL1	TL/TW		
Chan01 (frozen)	1.21	**0.41**	0.1	0.095	0.26	0.32	0.36	0.37	ori, uni	ind
Chan02 (frozen)	1.26	**0.41**	0.11	**0.095**	0.26	0.33	0.33	0.34	ori	ind
Chan03 (frozen)	1.26	**0.42**	0.1	0.102	0.25	0.32	0.37	**0.32**	ori, uni	ind
Chan04 (frozen)	1.35	**0.38**	0.12	0.112	0.27	-^{a}	0.35	0.28	uni	ind
Chan05 (frozen)	1.18	**0.38**	0.1	0.098	0.24	0.3	0.34	**0.34**	ori	ind
Chan06 (frozen)	1.33	**0.41**	0.11	**0.11**	0.24	0.33	0.36	0.4	ori, uni	ind
Chan07 (frozen)	**1.24**	**0.3**	0.12	0.121	0.25	**0.32**	0.33	0.34	ori	ind
Chan08 (frozen)	1.3	**0.49**	0.12	0.116	0.29	0.37	0.33	0.39	ind, ori, uni	ind
Chan09 (frozen)	1.31	**0.42**	0.12	0.13	0.26	0.33	0.36	0.34	ori, uni	ind
Chan10 (frozen)	1.26	**0.41**	0.11	0.104	0.26	0.34	0.32	0.34	ori	ind
Chan11 (frozen)	1.26	0.41	0.12	0.099	0.23	0.32	0.39	0.37	ori, uni	ind
Chan12 (frozen)	1.27	0.41	0.13	0.12	**0.26**	0.33	0.33	0.4	ori	ind
Chan13 (frozen)	1.2	0.41	0.12	0.1	**0.26**	0.34	0.35	0.3	-^{b}	ind
Chan14 (frozen)	1.25	0.41	0.12	0.109	**0.25**	0.31	0.37	0.3	uni	ind
Chan15 (frozen)	1.24	0.4	0.11	0.102	**0.24**	0.32	0.38	0.31	uni	ind
Chan16 (frozen)	1.25	0.41	0.11	0.106	**0.26**	0.33	0.34	0.31	ori	ind
Chan17 (frozen)	1.25	0.4	0.12	0.102	**0.22**	0.33	0.42	0.31	ori, uni	ind
Chan18 (frozen)	1.22	0.41	0.11	0.103	0.25	0.34	0.33	0.42	ori	ind
Chan19 (frozen)	1.29	0.41	0.11	0.105	**0.29**	0.31	**0.31**	0.38	ori	ind
Chan20 (frozen)	1.27	0.4	0.11	0.109	**0.25**	0.33	0.38	0.41	ori, uni	ind
Chan21 (frozen)	1.26	0.4	0.12	0.101	**0.25**	0.32	0.3	0.41	ori	ind
Chan22 (frozen)	1.25	0.41	0.11	0.11	0.24	0.33	0.34	0.32	ori	ind
Chan23 (frozen)	1.41	0.43	0.14	0.126	0.3	0.33	0.29	0.32	ori, uni	ind
Chan24 (frozen)	1.2	0.38	0.1	0.092	0.23	0.29	0.32	0.4	ori	ind

Table 4.2 (continued).

Sample names	Morphological measurement ratios								Species identified by measurement ratios	Species identified by morphological characteristics
	CW1/CL	ML3/CL	MW1/CL	MW2/CL	PL1/CL	PL2/CL	PW1/PL1	TL/TW		
Phet01 (frozen)	1.25	**0.56**	0.09	0.08	0.29	0.36	0.21	0.26	ind	ind
Phet02 (frozen)	1.24	**0.48**	0.1	**0.093**	0.27	0.33	0.3	0.34	ori, ind	ori
Phet03 (frozen)	1.24	**0.44**	0.1	0.091	0.24	0.31	0.34	**0.33**	ori	ori
Phet04 (frozen)	1.22	**0.46**	0.1	0.089	0.26	0.31	0.3	0.29	ind	ori
Phet05 (frozen)	1.21	**0.46**	0.09	0.084	0.26	0.35	0.31	**0.32**	ori, ind	ori
Phet06 (frozen)	1.21	**0.45**	0.09	**0.085**	0.26	0.32	0.33	0.29	ind	ori
Phet07 (frozen)	**1.28**	**0.53**	0.09	0.088	0.27	**0.32**	0.26	0.29	ind	ind
Phet08 (frozen)	1.27	**0.52**	0.1	0.08	0.29	0.38	0.27	0.27	ind	ind
Phet09 (frozen)	1.22	**0.44**	0.09	0.08	0.25	0.31	0.3	0.28	-[b]	ori
Phet10 (frozen)	1.24	**0.46**	0.09	0.086	0.26	0.32	0.29	0.31	ind	ori
Prac01 (frozen)	1.11	0.39	0.08	0.067	0.21	0.27	0.22	0.38	ori	ind
Prac02 (frozen)	1.27	0.53	0.12	0.103	**0.25**	0.34	0.33	0.29	ind	ind
Prac03 (frozen)	1.08	0.38	0.09	0.081	**0.16**	0.24	0.37	0.4	ori, uni	ind
Prac04 (frozen)	1.1	0.37	0.1	0.083	**0.21**	0.27	0.35	0.27	-[b]	ind
Prac05 (frozen)	1.06	0.38	0.09	0.085	**0.22**	0.26	0.3	0.35	ori	ind
Prac06 (frozen)	1.27	0.43	0.1	0.093	**0.21**	0.26	0.34	0.27	-[b]	ind
Prac07 (frozen)	1.1	0.47	0.1	0.092	**0.24**	0.32	0.29	0.31	ind, ori	ind
Prac08 (frozen)	1.13	0.4	0.1	0.095	0.21	0.29	0.35	0.33	ori, uni	ind
Prac09 (frozen)	1.11	0.4	0.09	0.067	**0.21**	0.23	**0.25**	0.31	ori	ind

Table 4.2 (continued).

Sample names	Morphological measurement ratios								Species identified by measurement ratios	Species identified by morphological characteristics
	CW1/CL	ML3/CL	MW1/CL	MW2/CL	PL1/CL	PL2/CL	PW1/PL1	TL/TW		
Chum01 (frozen)	1.26	**0.4**	0.11	0.099	0.25	0.33	0.37	0.36	ori, uni	ind
Chum02 (frozen)	1.23	**0.39**	0.1	**0.095**	0.22	0.15	0.35	0.35	ori, uni	ind
Chum03 (frozen)	1.29	**0.38**	0.12	0.095	0.24	0.29	0.36	**0.37**	ori, uni	ind
Chum04 (frozen)	1.26	**0.38**	0.12	0.092	0.26	0.32	0.33	0.34	ori	ind
Chum05 (frozen)	1.23	**0.38**	0.11	0.103	0.23	0.31	0.38	**0.43**	ori, uni	ind
Chum06 (frozen)	1.18	**0.39**	0.11	**0.103**	0.25	0.32	0.36	0.4	ori, uni	ind
Chum07 (frozen)	**1.29**	0.37	0.11	0.108	0.25	**0.3**	0.34	0.36	ori	ind
Chum08 (frozen)	1.26	**0.38**	0.12	0.093	0.25	0.28	0.36	0.35	ori, uni	ind
Chum09 (frozen)	1.28	**0.39**	0.11	0.109	0.23	0.31	0.37	0.35	ori, uni	ind
Chum10 (frozen)	1.26	**0.39**	0.11	0.104	0.23	0.3	0.38	0.33	ori, uni	ind
Song01 (frozen)	1.12	0.39	0.1	0.109	0.24	0.31	0.39	0.34	ori, uni	ori
Song02 (frozen)	1.19	0.41	0.11	0.103	**0.27**	0.36	0.36	0.32	ori, uni	ind
Song03 (frozen)	1.14	0.39	0.11	0.102	**0.25**	0.33	0.41	0.3	uni	ori
Song04 (frozen)	1.18	0.43	0.12	0.114	**0.27**	0.34	0.36	0.34	ori, uni	ori
Song05 (frozen)	1.12	0.39	0.1	0.099	**0.26**	0.3	0.34	0.35	ori	ori
Song06 (frozen)	1.19	0.44	0.11	0.103	**0.27**	0.36	0.37	0.3	uni	ind
Song07 (frozen)	1.19	0.45	0.11	0.104	**0.27**	0.37	0.34	0.36	ori	ind
Song08 (frozen)	1.28	0.44	0.12	0.106	0.27	0.39	0.36	0.33	ori, uni	ind
Song09 (frozen)	1.19	0.42	0.1	0.096	**0.24**	0.34	**0.33**	0.31	- [b]	ind
Song10 (frozen)	1.19	0.42	0.11	0.102	**0.27**	0.35	0.34	0.31	ori	ind

Table 4.2 (continued).

Sample names	Morphological measurement ratios								Species identified by measurement ratios	Species identified by morphological characteristics
	CW1/CL	ML3/CL	MW1/CL	MW2/CL	PL1/CL	PL2/CL	PW1/PL1	TL/TW		
Satu01 (frozen)	1.3	**0.4**	0.12	0.103	0.25	0.34	0.42	0.37	ori, uni	uni
Phuk01 (frozen)	1.34	**0.43**	0.13	**0.11**	0.28	0.35	0.34	0.28	uni	uni
Phuk02 (frozen)	1.27	**0.38**	0.12	0.115	0.24	0.33	0.38	**0.34**	ori, uni	uni
Phuk03 (frozen)	1.27	**0.4**	0.12	0.099	0.25	0.3	0.36	0.37	ori, uni	uni
Phuk04 (frozen)	1.22	**0.38**	0.11	0.1	0.24	0.31	0.35	**0.35**	ori, uni	uni
Phuk05 (frozen)	1.23	**0.38**	0.11	**0.097**	0.23	0.29	0.42	0.36	ori, uni	uni
Phuk06 (frozen)	**1.21**	**0.36**	0.1	0.098	0.23	**0.3**	0.38	0.32	ori, uni	uni
Phuk07 (frozen)	1.26	**0.38**	0.11	0.097	0.24	0.3	0.37	0.36	ori, uni	uni
Phuk08 (frozen)	1.23	**0.4**	0.12	0.1	0.27	0.32	0.33	0.34	ori	uni
Phuk09 (frozen)	1.2	**0.35**	0.12	0.109	0.22	0.29	0.41	0.32	ori, uni	uni
Phuk10 (frozen)	1.24	0.36	0.11	0.097	0.25	0.31	0.37	0.36	ori, uni	uni
Phuk11 (frozen)	1.24	0.39	0.11	0.102	**0.25**	0.34	0.39	0.33	ori, uni	uni
Rano01 (frozen)	1.18	0.42	0.11	0.102	**0.25**	0.34	0.36	0.33	ori, uni	ind
Rano02 (frozen)	1.14	0.4	0.11	0.093	**0.25**	0.32	0.34	0.32	ori	ind
Rano03 (frozen)	1.06	0.41	0.11	0.098	**0.24**	0.32	0.38	0.31	ori, uni	ind
Rano04 (frozen)	1.19	0.4	0.11	0.101	**0.23**	0.3	0.36	0.3	uni	ind
Rano05 (frozen)	1.18	0.38	0.11	0.101	**0.26**	0.34	0.34	0.33	ori	ind
Rano06 (frozen)	1.22	0.39	0.11	0.099	0.25	0.32	0.34	0.33	ori	ind
Rano07 (frozen)	1.07	0.42	0.11	0.096	**0.26**	0.34	**0.32**	0.33	ori	ind
Rano08 (frozen)	1.12	0.41	0.13	0.12	**0.25**	0.34	0.37	0.3	uni	ind
Rano09 (frozen)	1.21	0.42	0.11	0.104	**0.25**	0.34	0.32	0.28	-[b]	ind
Rano10 (frozen)	1.23	0.39	0.12	0.113	0.25	0.34	0.37	0.3	uni	ind

When comparing between only *T. indicus* samples from each province, the highest average CW1/CL (carapace width to carapace length) ratio of all was from the three samples of Phetchaburi province (1.27 ± 0.02), and the lowest CW1/CL was from 35 samples of Chonburi (1.16 ± 0.12) and Ranong (1.16 ± 0.05) provinces. For the ML3/CL (merus length of third periopod to carapace length) ratios, the highest mean value was from those lobsters of Phetchaburi province (0.54 ± 0.02), and the lowest one was from Chumporn samples (0.39 ± 0.01). The MW1/CL (merus width of first periopod to carapace length) ratios were equally high in the Chanthaburi, Chumporn, Songkhla, and Ranong samples (0.11 ± 0.01), while the lowest ratio was from Phetchaburi samples (0.09 ± 0.01). Chanthaburi samples showed the highest MW2/CL (merus width of second periopod to carapace length) ratio (0.11 ± 0.01), while Phetchaburi samples showed the lowest ratio (0.08 ± 0.01). The highest PL1/CL (propodus length of first periopod to carapace length) ratio was from Phetchaburi specimens (0.28 ± 0.01) while the average ratio was lowest in the case of Prachuap Khiri Khan samples (0.21 ± 0.02). The samples from Songkhla province showed the highest PL2/CL (propodus length of second periopod to carapace length) ratio (0.36 ± 0.02) while the lowest ratio was from those of Prachuap Khiri Khan (0.28 ± 0.03). The PW1/PL1 (propodus width of first periopod to propodus length of first periopod) ratio was highest in Songkhla samples (0.36 ± 0.02) and lowest in Phetchaburi (0.25 ± 0.03). The TL/TW (telson length to telson width) average ratios was highest in the specimens from Chumporn province (0.36 ± 0.03), and lowest in those from Phetchaburi province (0.27 ± 0.02). The comparison between these ratios of each province is illustrated in Figure 4.3.
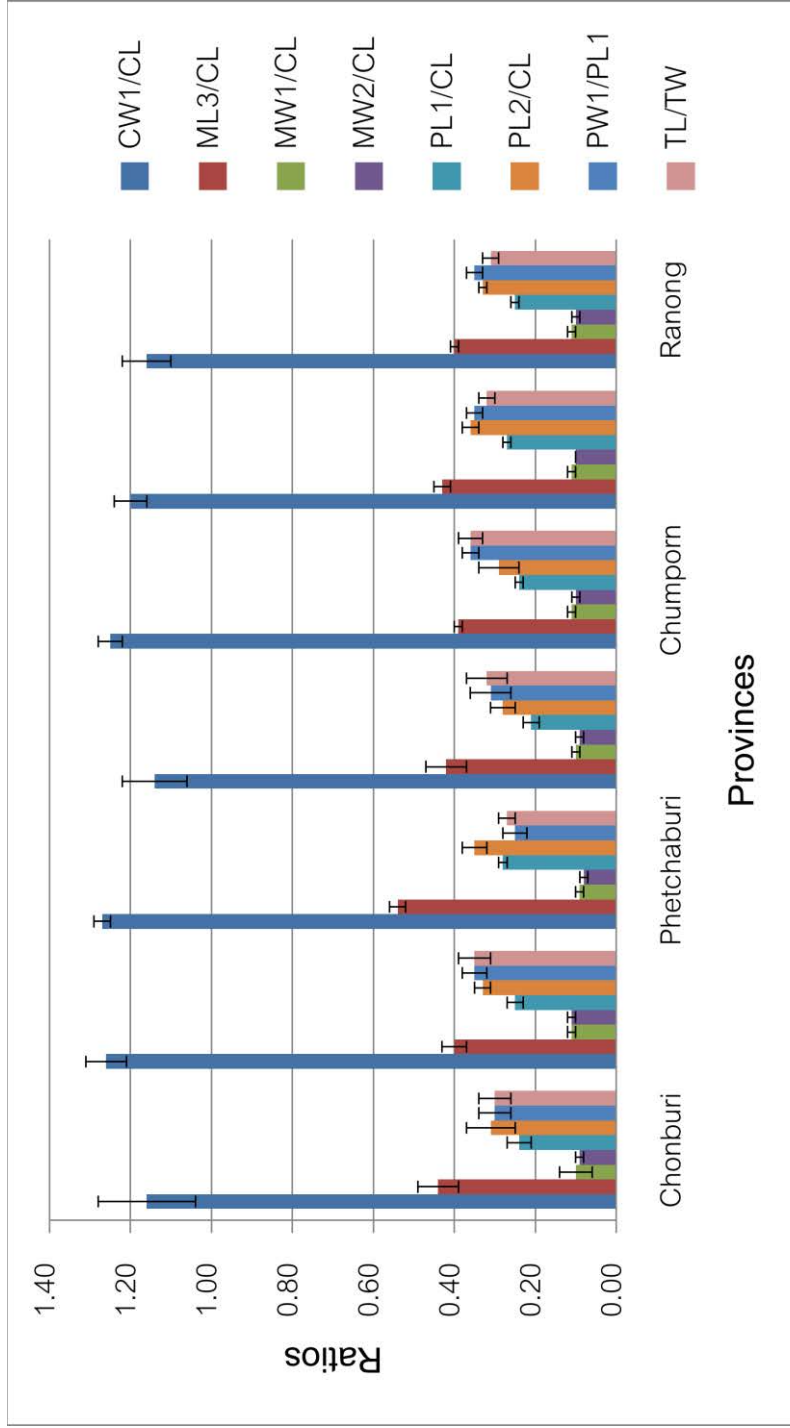
Figure 4.3 Mean values of CW1/CL (dark blue), ML3/CL (red), MW1/CL (green), MW2/CL (purple), PL1/CL (green blue), PL2/CL (orange), PW1/PL1 (blue), and TL/TW (pink) with standard deviation values as error bars.

Table 4.3 Eight morphometric ratios of *T. indicus*.

Provinces	Ratios						
	CW1/CL	ML3/CL	MW1/CL	MW2/CL	PL1/CL	PL2/CL	PW1/PL1
Chonburi	1.16 ± 0.11	0.44 ± 0.05	0.10 ± 0.04	0.09 ± 0.01	0.24 ± 0.03	0.31 ± 0.06	0.30 ± 0.04
Chanthaburi	1.26 ± 0.05	0.40 ± 0.03	0.11 ± 0.01	0.11 ± 0.01	0.25 ± 0.02	0.33 ± 0.02	0.35 ± 0.03
Phetchaburi	1.27 ± 0.02	0.54 ± 0.02	0.09 ± 0.01	0.08 ± 0.01	0.28 ± 0.01	0.35 ± 0.03	0.25 ± 0.03
Prachuap Khiri Khan	1.14 ± 0.08	0.42 ± 0.05	0.10 ± 0.01	0.09 ± 0.01	0.21 ± 0.02	0.28 ± 0.03	0.31 ± 0.05
Chumporn	1.25 ± 0.03	0.39 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.24 ± 0.01	0.29 ± 0.05	0.36 ± 0.02
Songkhla	1.20 ± 0.04	0.43 ± 0.02	0.11 ± 0.01	0.10 ± 0.00	0.27 ± 0.01	0.36 ± 0.02	0.35 ± 0.02
Ranong	1.16 ± 0.06	0.40 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.25 ± 0.01	0.33 ± 0.01	0.35 ± 0.02

According to Figure 4.3, almost all of the average values of the morphometric ratios of these *T. indicus* samples from seven provinces were found to be similar, and the provincial grouping cannot be clearly distinguished considering only the highest and the lowest values of such ratios. Even the samples from Andaman Sea (Ranong province) seemingly gave the morphometric ratios which are very similar to those of the samples from the Gulf of Thailand.

4.3 Molecular phylogenetic analyses

The NJ phylogenetic tree resulted from the nucleotide data set of totally 206 Thai *Thenus* CO1 sequences is shown in Figure 4.4. Five reference *Thenus* sequences and HM015458 *Ibacus peroinii* sequence from GenBank were also incorporated with this analysis as an outgroup. The 206 Thai samples can be divided into three clusters: Cluster A contains all *T. indicus* samples and strongly grouped together with 100% bootstrap support; Cluster B contains all specimens of *T. orientalis* and highly supported with 98% bootstrap statistic value, and the other 31 *T. unimaculatus* samples are grouped as Cluster C with 99% bootstrap support. Considering the subgrouping within Cluster A, the cluster can be further divided into two subgroups: Subgroup A1 (Gulf of Thailand) and Subgroup A2 (Andaman Sea). The Andaman Sea subgroup A2 contained 20 *T. indicus* samples from Ranong province and moderately grouped together with 76% bootstrap support.
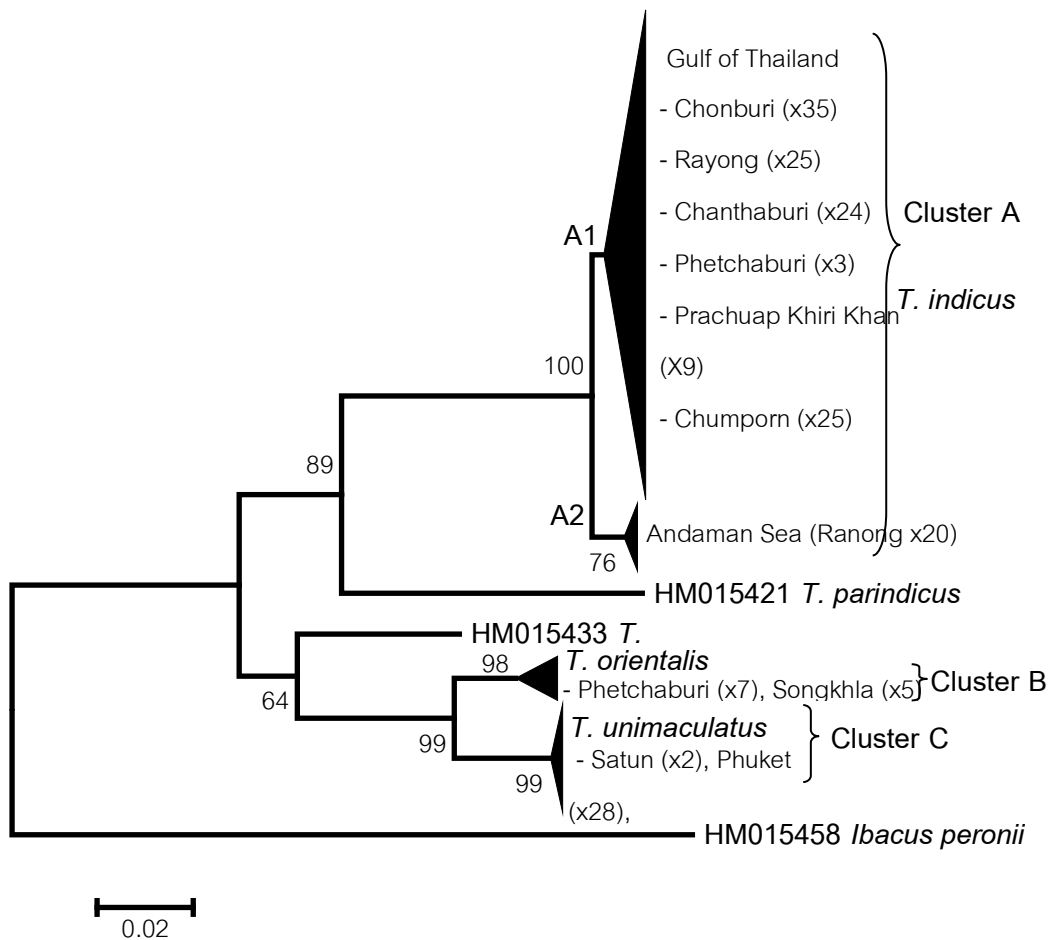
Figure 4.4 NJ tree of the 390 bp aligned nucleotide data set of CO1 gene sequences from 206 *Thenus* samples. *Ibacus peroinii* sequence was used as an outgroup for the analysis. The numbers under branches indicate the percentages of the bootstrap supporting values with 1,000 replicates.

The genetic distances with Kimura-2 parameter (K2P) correction within each cluster were estimated as follow: Cluster A of *T. indicus* had the widest range of the K2P genetic distance from 0.000 to 0.034 with an average of 0.010 ± 0.009; Cluster B of *T. orientalis* ranged from 0.000 to 0.018 with an average of 0.005 ± 0.005; and the K2P distance of Cluster C of *T. unimaculatus* was found to be narrowest, ranging from 0.000 to 0.015 (with an average of 0.005 ± 0.004). When considering the genetic distances between the three *Thenus* species and their closely related species, the dissimilar percentages between *T. indicus* and *T. parindicus* CO1 sequences ranged from 11.2% to 13.7% with an average of 12.9 ± 0.5% while that of the sister pair of *T. orientalis* and *T. unimaculatus* ranged from 3.5% to 6.2% with the mean and standard deviation values of 4.6 ± 0.4%. Interestingly, the genetic distances between *T. indicus* samples from the Gulf of Thailand and those from Andaman Sea were rather high ranging from 0.001 to 0.034 with an average of 0.023 ± 0.005.

Twenty Thai *Thenus* CO1 sequences were selected for reconstruction of maximum parsimony (MP) analysis. One of 11 equally most parsimonious trees (MPTs) is shown in Figure 4.5, which was resulted from the 667 bp CO1 aligned data matrix with 108 bp parsimony informative sites. The best tree length is 176 evolutionary step changes with consistency index = 0.8239, retention index = 0.9524, and rescaled consistency index = 0.7846. The strict consensus tree of all 11 MPTs is shown in Figure 4.6. A semi-strict consensus tree was also reconstructed and resulted as an identical topology of the strict consensus tree. There are three strongly-supported clades recognised on this MP tree: Clade A contained *T. indicus* samples both from Andaman Sea (also formed a subclade with 99% bootstrap support) and other eight *T. indicus* from the Gulf of Thailand (100% bootstrap support), Clade B contained only *T. orientalis* samples (99% bootstrap support), and Clade C contained only *T. unimaculatus* samples (96% bootstrap support).

When considering the numbers of evolutionary step changes within the three clades, the evolutionary differences between the members of Clade B of *T. orientalis* ranged from four step changes between Phet04 and Song15 samples to 14 step changes of Phet03 and Phet04. Within Clade B (*T. unimaculatus*) the evolutionary

changes had a narrower range than those of Clade A, from six (Phuk01 and Phuk06) to ten step changes (Phuk11 and Phuk16). The highest COI sequence variation was found in Clade C of *T. indicus* which ranged from only two step changes of the members of Andaman Sea Clade (Rano01 and Rano12) to 26 step changes between sample Rayo04 from the Gulf of Thailand and Rano09 of Andaman Sea. The sequence differences within Clade A of Andaman Sea specimens ranged from two (Rano01 and Rano12) to five step changes (Rano09 and Rano22). From the branch lengths of the phylogram on Figure 4.5, Clade B of *T. orientalis* and Clade C of *T. unimaculatus* are closely paired together with 26 evolutionary step changes, approximately two times higher than the highest range of sequence variation within each clade (14 and ten changes, respectively). The minimum evolutionary step change between the pair of Clade B and C and Clade A (*T. indicus*) is as many as 68 changes or around five times higher than the numbers of nucleotide support on each clade (14 and 12 basepairs from Clade B and Clade C, respectively). The members of Clade B (*T. orientalis*) are distinct from those of *T. indicus* samples at least 90 (Song01 and Rayo04) to 111 step changes (Phet03 to Rano09). Clade C specimens differed from Clade A of Andaman Sea specimens at least 86 (Phuk16 and Rayo04) to 110 step changes (Phuk11 and Rano09).
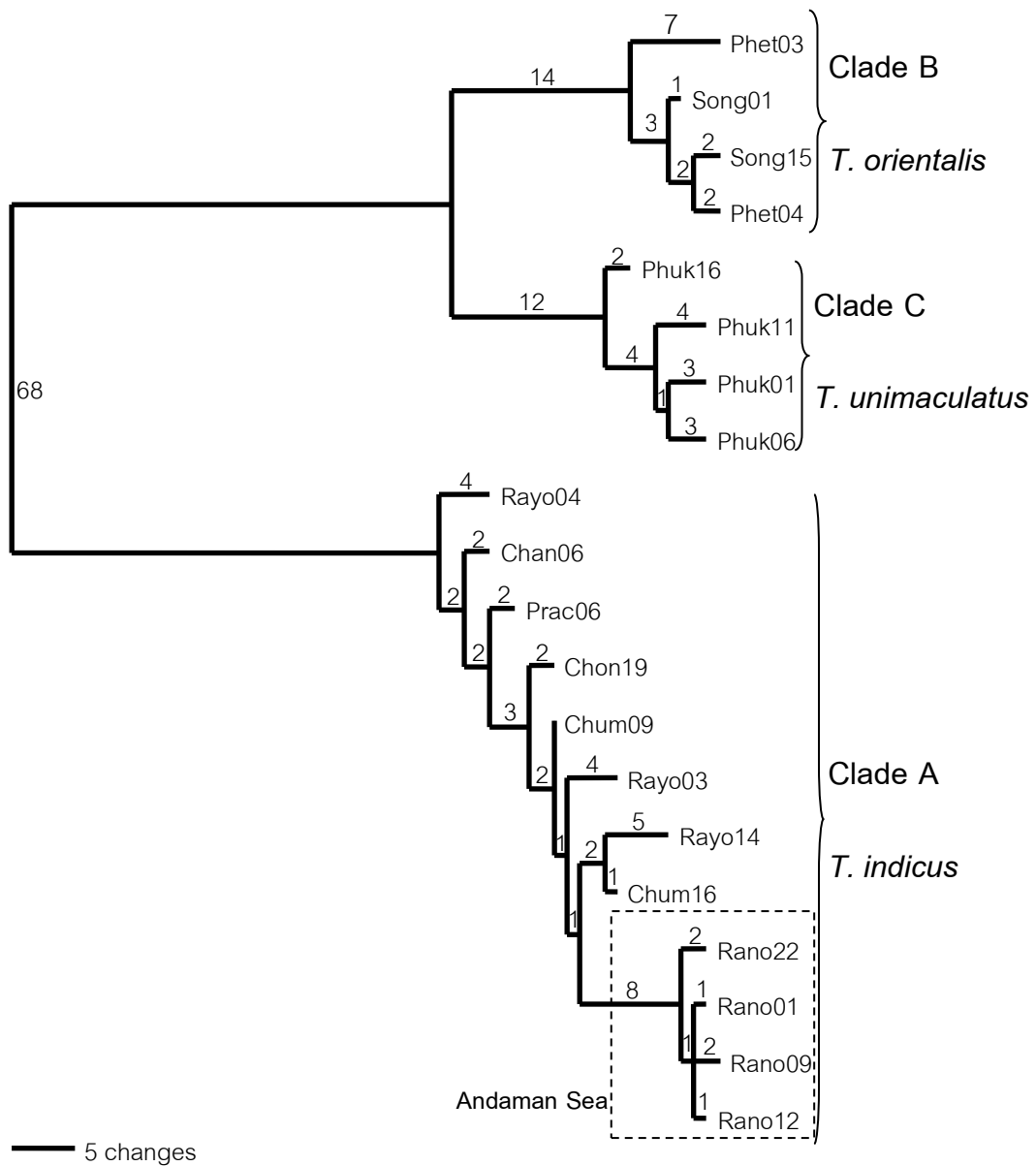
Figure 4.5 The phylogram of one of 11 equally most parsimonious trees of CO1 nucleotide sequences of 20 Thai *Thenus* specimens. The MPTs were reconstructed using heuristic search strategy and the numbers along branches indicate evolutionary step changes.
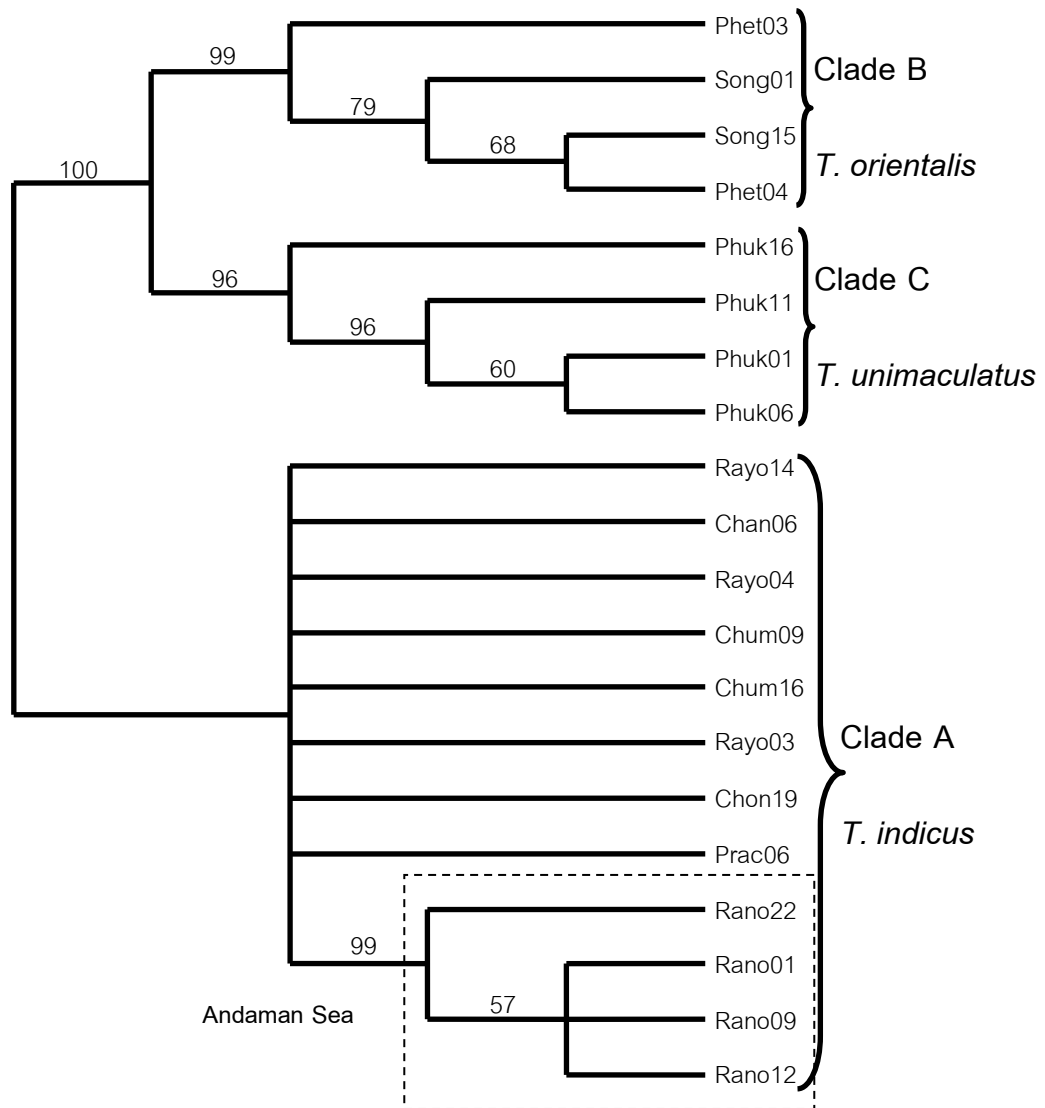
Figure 4.6 Strict consensus tree of 11 equally most parsimonious trees of
CO1 nucleotide sequences of 20 Thai *Thenus* specimens. The numbers
above branches indicate the percentages of bootstrap support (with 1,000
replicates).

A maximum likelihood (ML) tree was also reconstructed from the same data set as the MP analysis (Figure 4.5 for example) and the ML phylogeny is shown in Figure 4.7. Three major clades found from MP analysis are also clearly shown in ML tree: Clade A of *T. indicus* samples strongly grouped together with 100% bootstrap support, Clade B with 92% bootstrap support, and Clade C with 79% bootstrap support. There were also some subgroupings found in ML tree consisting with those in MP tree (Figure 4.6) such as the subclade of Song01/Song15/Phet04 within Clade B (79% bootstrap for MP and 71% for ML) and the subclade of Phuk01/Phuk06/Phuk11 within Clade C (96% bootstrap for MP and 84% for ML).
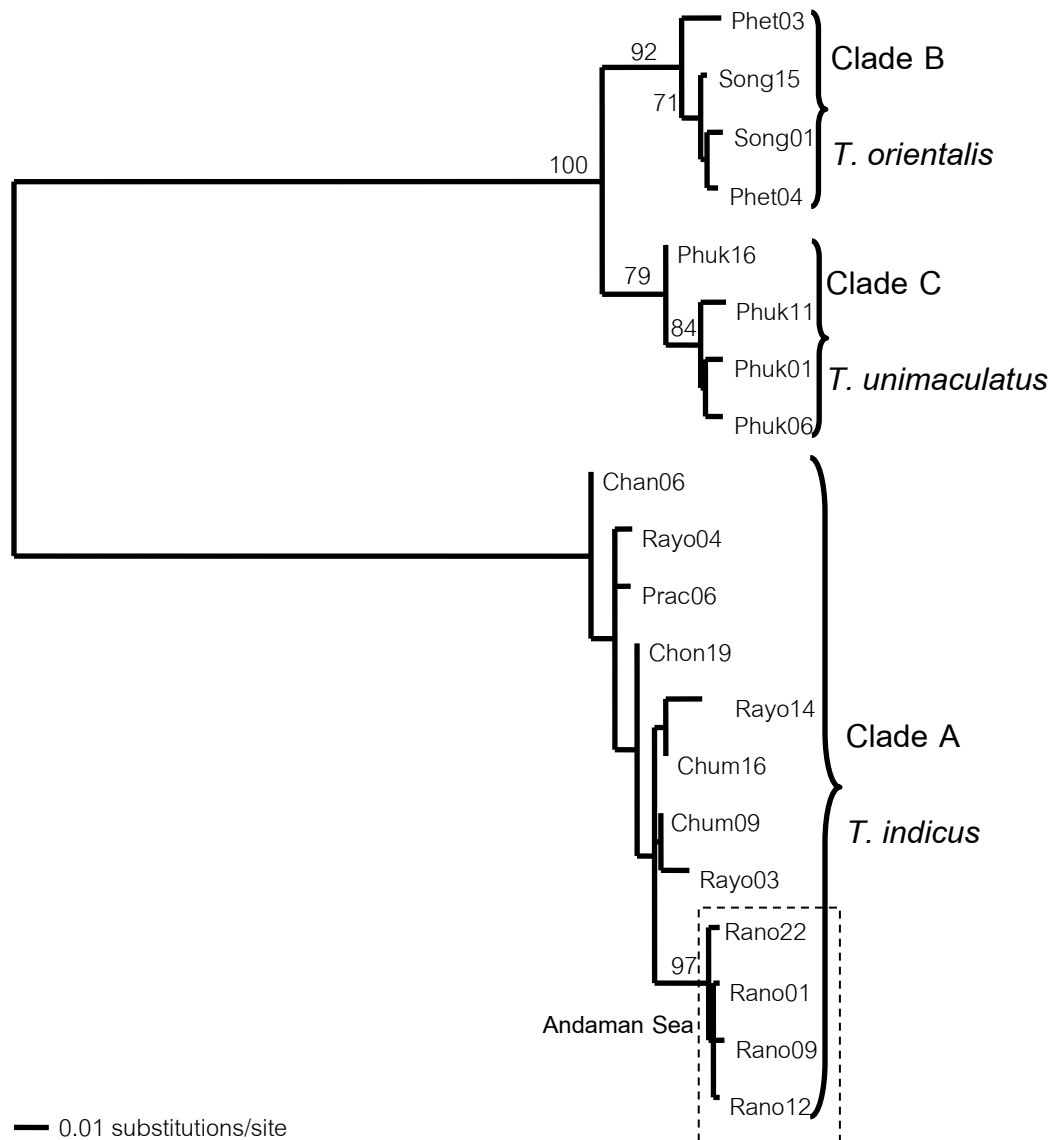
Figure 4.7 Maximum likelihood tree of CO1 nucleotide sequences of 20 Thai *Thenus* specimens. The Numbers above branches indicate the percentages of bootstrap support (with 1,000 replicates).

The Bayesian tree inferred from the 669 bp aligned CO1 data matrix of 163 *T. indicus* specimens is shown in Figure 4.8. Every *T. indicus* was strongly grouped together with posterior probability equal to 1.00. Twenty *T. indicus* specimens from Ranong province (Andaman Sea) were also strongly grouped together with the posterior probability equal to 1.00. Only Chan09/Prac04 and Chan11/Rayo14 samples were strongly paired together supported by the posterior probability more than 0.95. Interestingly, there is no provincially subgrouping of *T. indicus* samples from the Gulf of Thailand. Notably, Rano17 from Ranong was not placed within the Andaman group but mixed with other *T. indicus* samples from the Gulf of Thailand.

Chon01
Chon03
Chon04
Chon05
Chon06
Chon07
Chon09
Chon10
Chon11
Chon12
Chon13
Chon15
Chon16
Chon18
Chon19
Chon20
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Rayo10
Rayo11
Rayo13
Rayo17
Rayo18
Rayo19
Rayo20
Rayo21
Rayo22
Rayo23
Rayo24

1.00

Figure 4.8 Bayesian tree inferred from 669 aligned CO1 DNA data matrix of 163 *T. indicus* specimens. The numbers along branches indicate the posterior probabilities of the nodes. *Thenus unimaculatus* (Satu02) and *T. orientalis* (Song04) were used as outgroups.

Rano17 sample (a Ranong sample is grouped with other *T. indicus* from the Gulf of Thailand).

Subclade

T. indicus from Andaman Sea
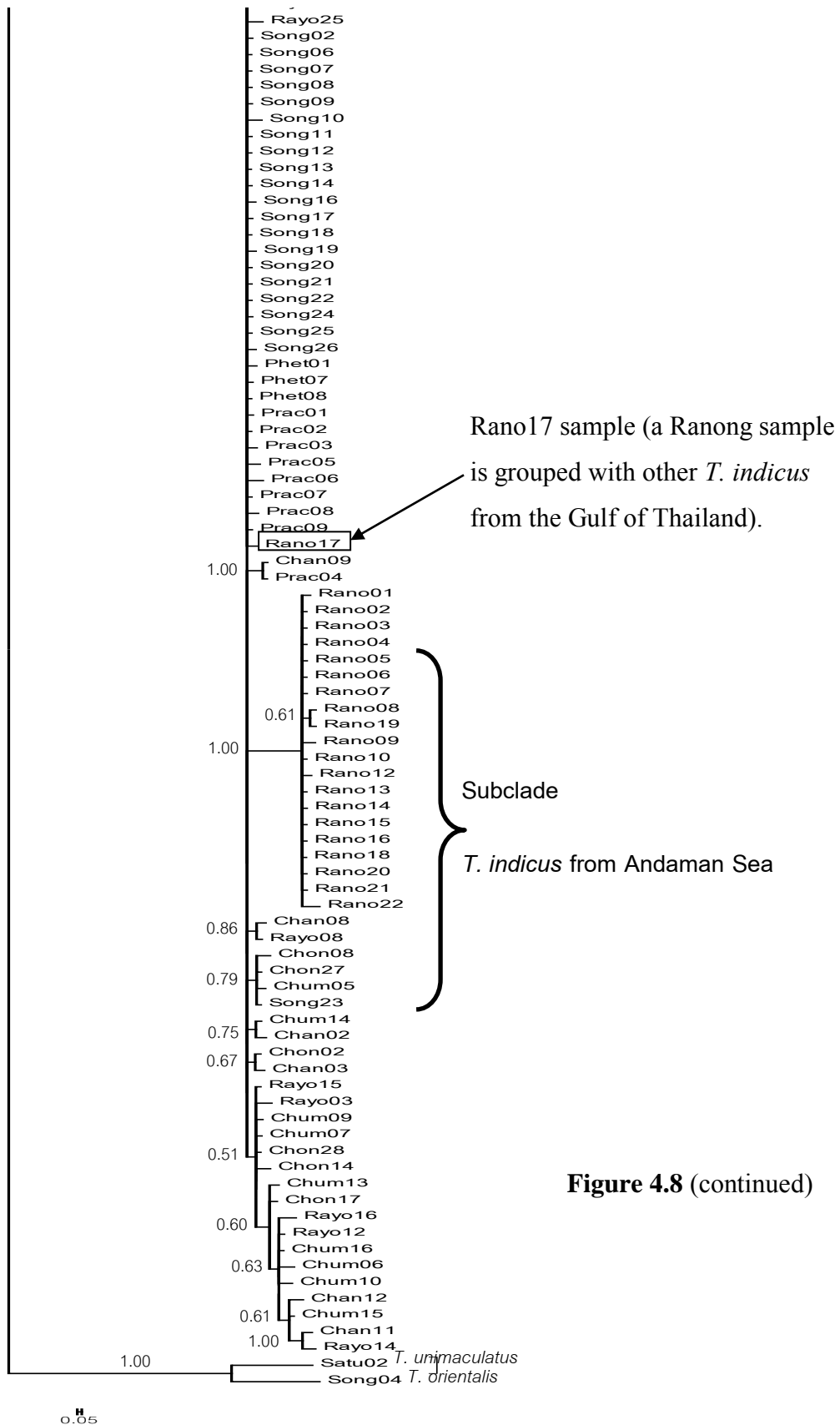
Figure 4.8 (continued)

4.4 Genetic diversity analyses

4.4.1 Molecular diversity indices

The results of molecular diversity index analyses are shown in Table 4.4. Totally 87 haplotypes were found in this study. Eighty haplotypes belonged to the samples collected from the Gulf of Thailand and seven haplotypes were from Andaman Sea. For each collecting locality, the numbers of haplotypes ranged from the lowest number of three haplotypes in Phetchaburi province to the highest 23 haplotypes in Chonburi province. The haplotype diversity of the total 163 Thai *T. indicus* was 0.945 ± 0.013 and the haplotype diversity of those in the Gulf of Thailand was 0.937 ± 0.016. The haplotype diversity values of each province ranged from as low as 0.562 in Ranong province to as very high as 1.000 in Phetchaburi province. Almost all of the collecting locations (Chonburi, Chumporn, Chanthaburi, Rayong, Phetchaburi, and Prachuap Khiri Khan) had high haplotype diversity index with the values greater than 0.9, except for Songkhla and Ranong provinces. The overall nucleotide diversity value of every sample was 0.0102 ± 0.0053, but that of the specimens in the Gulf of Thailand was only 0.0064 ± 0.0035. The nucleotide diversity value of each collecting location ranged from 0.0018 in Songkhla province to 0.0081 in Rayong province. Notably, *T. indicus* populations in Chonburi, Chumporn, Chanthaburi, and Rayong provinces have high nucleotide diversity values (higher than 0.0050). The mean numbers of pairwise differences between CO1 sequences of the eight populations is 6.77 ± 3.21, and of the populations in the Gulf of Thailand is 4.26 ± 2.12. The minimum number of pairwise differences is 1.17 ± 0.78 within Phetchaburi specimens and the highest number is 5.15 ± 2.58 of the samples from Chumporn province. Only Chumporn, Chanthaburi, and Rayong samples have the mean numbers of pairwise differences greater than 4.00.

Considering the substitution events (transitions and transversions) within CO1 sequences of Thai *T. indicus* samples, the total number of substitutions of CO1 sequences is 76 and 64 are found in the samples from the Gulf of Thailand. The numbers of substitutions of CO1 sequences of the eight populations ranged from four

to 32. The comparison between the numbers of transitions and transversions revealed that transitions occured much more frequently than transversions (approximately eight times higher). The numbers of transitions of all eight populations and the populations from the Gulf of Thailand are 68 and 57, respectively. The numbers of transitions ranged from two in Phetchaburi population to 27 in Chanthaburi population. The total numbers of transversions of all populations and those of the Gulf of Thailand are eight and seven, respectively. The numbers of transversions of each population ranged from one to five.

Table 4.4 Molecular diversity indices of 163 *T. indicus* specimens.

Samples	Number of samples	Number of haplotypes	Haplotype diversity	Nucleotide diversity	Mean number of pairwise differences	Number of substitutions	Number of transitions	Number of transversions
Total	163	87	0.945 ± 0.013	0.0102 ± 0.0053	6.77 ± 3.21	76	68	8
Gulf of Thailand	142	80	0.937 ± 0.016	0.0064 ± 0.0035	4.26 ± 2.12	64	57	7
- Chonburi	35	23	0.929 ± 0.034	0.0056 ± 0.0032	3.71 ± 1.92	23	20	3
- Chumporn	25	20	0.977 ± 0.019	0.0077 ± 0.0043	5.15 ± 2.58	20	17	3
- Chanthaburi	24	19	0.946 ± 0.040	0.0067 ± 0.0038	4.46 ± 2.28	32	27	5
- Rayong	25	22	0.987 ± 0.017	0.0081 ± 0.0045	5.37 ± 2.68	30	26	4
- Songkhla	21	9	0.724 ± 0.101	0.0018 ± 0.0013	1.17 ± 0.78	10	8	2
- Phetchaburi	3	3	1.000 ± 0.272	0.0035 ± 0.0032	2.34 ± 1.72	4	2	2
- Prachuap Khiri Khan	9	8	0.972 ± 0.064	0.0048 ± 0.0031	3.22 ± 1.83	13	12	1
Andaman Sea								
- Ranong	21	7	0.562 ± 0.126	0.0034 ± 0.0022	2.29 ± 1.31	22	20	2

4.4.2 Haplotype network

The haplotype network result of total 163 Thai *T. indicus* specimens is shown in Figure 4.9. Eighty seven haplotypes can be divided into two groups: Group A represents seven haplotypes collected from Andaman Sea, and Group B represents the other 80 haplotypes sampled from the Gulf of Thailand. From the haplotype network, none of a star-like grouping pattern referring to a rapid expansion of population is found. Additionally, Group B does not show any geographically subgrouping corresponding to any specific province.

Figure 4.9 Haplotype network of the 87 haplotypes found from 163 Thai *Thenus indicus* specimens. The size of the circle is corresponding to the haplotype frequency.

4.4.3 Neutrality tests

The results of neutrality tests of 163 Thai *T. indicus* samples are shown in Table 4.5. Overall population sample of Thai *T. indicus* shows a negative Tajima's D (-1.294). In addition, almost all of the population samples shows negative Tajima' D, except for the Chumporn and the Phetchaburi samples which show the D values of 0.250 and zero, respectively. Only the Tajima's D values of *Thenus* populations from Chanthaburi, Songkhla, Ranong provinces, and from the overall populations of the Gulf of Thailand were significantly accepted with p-value of 0.043, 0.017, 0.002, and 0.004, respectively.

The other neutrality test performed in this study was Fu's F_s. The F_s values of all populations were found to be negative, indicating that these populations deviated from neutral evolution and might have experienced population expansion. To test the confidence of Fu's F_s analysis, the p-values were also calculated. Almost all of the p-values, for except that of Phetchaburi and Ranong provinces, are less than 0.05, indicating that these populations might have undergone population expansion. The p-value of Fu's F_s values of Phetchaburi (= 0.145) and Ranong (= 0.359) provinces were larger than 0.05 indicated that these population did not significantly deviate from neutral evolution.

Comparison between the finding from Tajima's D analysis and those of Fu's F_s test revealed that only the results of the Songkhla and Chathaburi populations and also the overall populations of the Gulf of Thailand were agreeable as these populations significantly deviated from neutral evolution and possibly have been expanded.

Table 4.5 Neutrality tests of 163 Thai *T. indicus* samples.

Samples	Tajima's *D*				Fu's *F*$_s$			
	Segregating sites (S)	Mean number of pairwise differences (Pi)	Tajima's *D*	Tajima's *D* *p*-value	Theta (pi)	Expected number of alleles	Fu's *F*$_s$	Fu's *F*$_s$ *p*-value
Total	65	6.65	-1.294	0.062	6.65	22.04	-24.814	0.000
Gulf of Thailand	56	4.22	-1.804	0.004	4.22	15.46	-25.670	0.000
Chonburi	21	3.67	-0.953	0.171	3.67	9.12	-16.562	0.000
Chumporn	18	5.10	0.250	0.661	5.10	9.49	-12.424	0.000
Chanthaburi	28	4.40	-1.549	0.043	4.40	8.65	-12.607	0.000
Rayong	27	5.32	-0.952	0.180	5.32	9.68	-16.697	0.000
Songkhla	9	1.16	-1.817	0.017	1.16	3.97	-5.717	0.000
Phetchaburi	3	2.33	0.000	0.854	2.33	2.24	-0.503	0.145
Prachuap Khiri Khan	12	3.19	-1.310	0.103	3.19	4.67	-4.123	0.004
Ranong	21	2.26	-2.314	0.002	2.26	5.75	-0.736	0.359

4.4.4 Genetic structure analyses

4.4.4.1 Analysis of molecular variance (AMOVA)

The AMOVA tests were performed on separately two data sets. Firstly, the result of first AMOVA test which was performed to test the genetic structure between two geographical groups which are the group of the overall population of the Gulf of Thailand (Chonburi, Chanthaburi, Rayong, Phetchaburi, and Prachuap Khiri Khan, Chumporn, and Songkhla provinces), and that of Andaman Sea (Ranong province) including the test between populations of *T. indicus* within each geographical group are shown in Table 4.6. The AMOVA result reveals that there was no significantly difference between the three population groups. However, the genetic differences among population within each geographical group were found to be significant (p-value = 0.000). From this AMOVA test, the genetic variation was found to be highest between the two groups (74.10%), and lowest for the variation among every population within each group (2.32%).

Secondly, the result from AMOVA on the second data set to test spatial genetic structure and genetic variation among the group of the populations of the upper Gulf of Thailand and the group of the lower Gulf of Thailand population (Table 4.7) shown that there was no significantly genetic difference between these two groups (p-value = 0.284). Nevertheless, genetic differences among populations within each group were found to be significant (p-value = 0.000). The highest variation found in the camparison between individuals within population (87.38%), followed by between groups (6.81%), and between populations within groups (5.81%).

Table 4.6 Analysis of molecular variance (AMOVA) results of a comparison between
T. indicus samples from the Gulf of Thailand and those of Andaman Sea.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	*p*-value
Among groups	1	219.01	5.83	74.10	0.125 ± 0.004
Among populations within groups	6	32.47	0.18	2.32	0.000 ± 0.000
Within populations	155	287.36	1.85	23.57	0.000 ± 0.000
Total	162	538.83	4.34		

Table 4.7 Analysis of molecular variance (AMOVA) results of a comparison between
T. indicus samples from the upper and the lower Gulf of Thailand.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	*p*-value
Among groups	1	10.26	0.15	6.80	0.284 ± 0.005
Among populations within groups	5	22.21	0.13	5.81	0.004 ± 0.001
Within populations	135	264.78	1.96	87.38	0.000 ± 0.000
Total	141	297.25	2.24		

4.4.4.2 Population comparisons using pairwise F_{st} values

The results of the population pairwise F_{st} analysis is shown in Table 4.8. The population pairwise F_{st} values ranged from -0.075 to 0.893. Interestingly, the pairwise F_{st} values between Ranong (Andaman Sea) samples and other samples of the Gulf of Thailand are high (0.731 to 0.893) revealing that there is high genetic differentiation between the populations in these two areas.

Ranong population was significantly different from every population from the Gulf of Thailand (p-value < 0.001). This finding consisted with the high F_{st} values (Table 4.8). Likewise, if the pairwise F_{st} values were low (i.e. the values between Chanthaburi and Chonburi samples, between Rayong and Chumporn, and between Phetchaburi against other populations in the Gulf of Thailand), there was no significantly genetic difference between such populations (p-value > 0.05).

Table 4.8 Population pairwise F_{st} of 163 *Thenus indicus* specimens. All significant levels were Bonferroni"s corrected.

Samples	Chonburi	Rayong	Chanthaburi	Phetchaburi	Prachuap Khiri Khan	Chumporn	Songkhla
Chonburi							
Rayong	0.044^{ns}						
Chanthaburi	-0.005^{ns}	0.061^{ns}					
Phetchaburi	-0.037^{ns}	0.045^{ns}	-0.075^{ns}				
Prachuap Khiri Khan	0.002^{ns}	0.093^{ns}	-0.028^{ns}	-0.059^{ns}			
Chumporn	0.08^{ns}	0.011^{ns}	0.113^{ns}	0.098^{ns}	0.153^{ns}		
Songkhla	0.092^{ns}	0.231^{***}	0.05^{ns}	0.08^{ns}	0.098^{ns}	0.295^{***}	
Ranong	0.795^{***}	0.742^{***}	0.79^{***}	0.851^{***}	0.843^{***}	0.731^{***}	0.893^{***}

^{ns} not significant, *** significant at 0.001, ** significant at 0.01, and * significant at 0.05.

4.4.5 Historical demographic parameters

The time since last expansion of the population in mutational time unit or Tau (τ) of each *T. indicus* population sample and those of the Gulf of Thailand were estimated to be 0.799 and 1.234, repectively (see Table 4.9). The τ values ranged from 0.758 for the Ranong samples to 2.953 for the Rayong samples. The Theta0 (θ_0), or the population size before the expansion, and Theta1 (θ_1), or the population size after the expansion, values were calculated and are shown in Table 4.9. The θ_0 values of every population were very low (from 0.00 to 2.43) whereas the θ_1 values of almost all populations (except for Chanthaburi and Rayong) reached 99999.00. Only populations of Total, the Gulf of Thailand, Chonburi, Chumporn, and Songkhla were given the low τ values with low θ_0 (nearly zero) and high θ_1 (reached 99999.00). Further analyses would be needed to confirm the expansions of the other populations.

Table 4.9 Demographic history parameters of 163 *Thenus indicus* specimens.

Samples	Tau	Theta0	Theta1	Sum of squared deviation (SSD)	SSDs' p-value	Harpending's Raggedness index (Hri)	Hris' p-value
Total	0.799	0.00	99999.00	0.248	0.000	0.008	1.000
Gulf of Thailand	1.234	0.00	99999.00	0.128	0.000	0.011	1.000
- Chonburi	1.109	0.00	99999.00	0.146	0.005	0.017	0.999
- Chumporn	1.609	0.00	99999.00	0.129	0.007	0.015	0.993
- Chanthaburi	2.797	0.00	22.73	0.014	0.238	0.030	0.764
- Rayong	2.953	2.43	99999.00	0.001	0.959	0.010	0.877
- Songkhla	1.182	0.00	99999.00	0.008	0.425	0.105	0.317
- Phetchaburi	2.719	0.00	99999.00	0.228	0.321	0.667	0.608
- Prachuap Khiri Khan	2.723	0.70	99999.00	0.018	0.470	0.093	0.347
- Ranong	0.758	0.00	9.33	0.019	0.209	0.075	0.843

4.4.6 Mismatch distribution analysis

The results from mismatch distribution analyses are shown in Figure 4.10 to 4.19. The sum of square deviation value (SSD), which is referred to the degree of deviation between the observed mismatch derived from nucleotide sequences of samples and the expected mismatch derived from the recent population expansion model. The p-value of SSD indicates the significance difference between observed and expected mismatch distribution or the model of recent population expansion. If the p-value less than 0.05, the null hypothesis of recent population expansion is rejected. The mismatch distribution of total of 163 *T. indicus* samples is shown in Figure 4.10. The SSD for overall samples was high (0.248) with its significant p-value equals to 0.000.This finding revealed that total of 163 *T. indicus* population deviated from recent population expansion model. In addition, the Harpending"s raggedness index (Hri) which is referred to the degree of the ragged or multimodal pattern of observed mismatch curve was low in overall population (0.008) reflecting the ragged pattern of mismatch curve. However, the p-value of Hri which is used to test for whether the observed mismatch deviated from the simulated non-smooth (or multimodal) pattern of mismatch observed from overall samples was greater than 0.05 (at 95% confidence level), revealing that the observed mismatch distribution curve of overall population is not smooth or has a multimodal pattern as shown in Figure 4.10.

For 142 *T. indicus* samples from the Gulf of Thailand, the mismatch distribution is shown in Figure 4.11. The SSD value is considerably high (= 0.128) with its p-value of SSD equal to 0.000, reflecting the significant difference of observed mismatch distribution from mismatch distribution simulating from the recent population expansion model. The Harpending"s raggedness index (Hri) is also low (0.011) reflecting the unimodal pattern but with p-value of Hri was greater than 0.05 and can be interpreted as the non-smooth or multimodal pattern of mismatch curve.
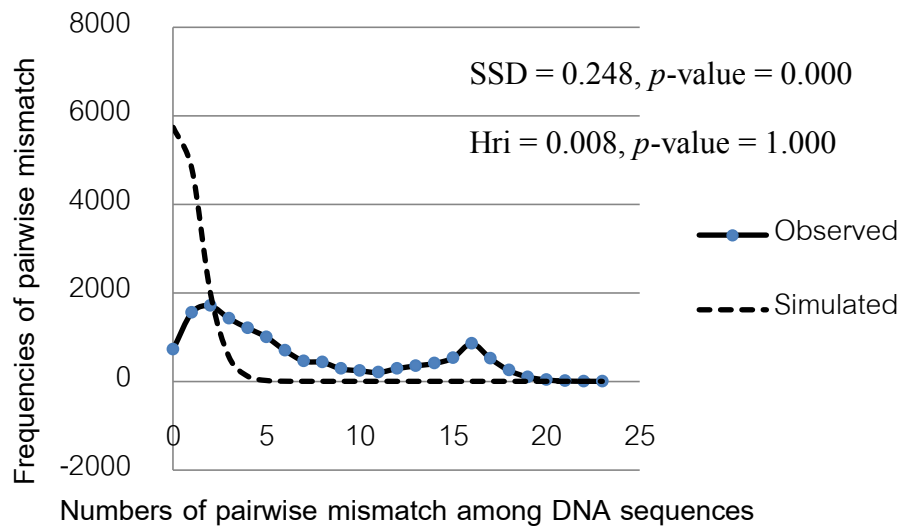
Figure 4.10 Mismatch distribution of total of 163 *T. indicus* samples. A dash line indicates the simulated mismatches and a flat line indicates the observed mismatches.
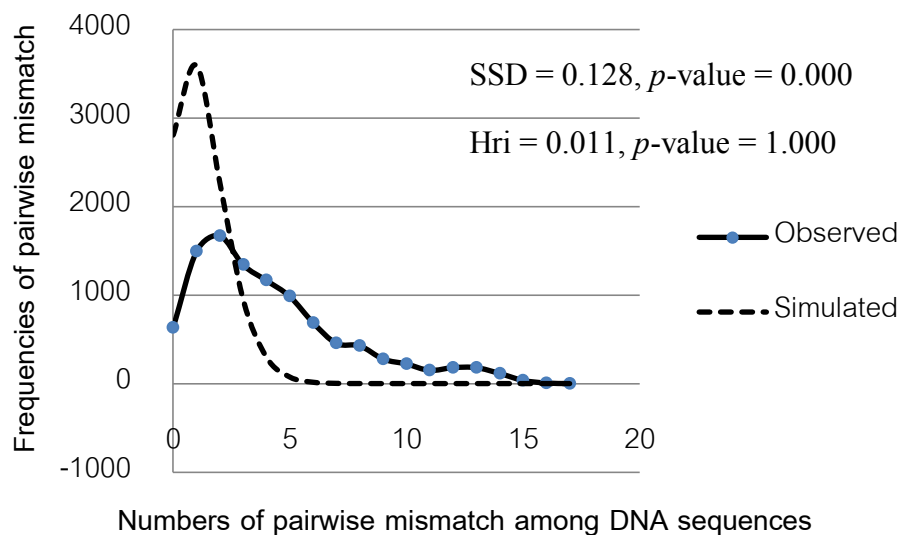
Figure 4.11 Mismatch distribution of 142 samples from the Gulf of Thailand samples. A dash line indicates the simulated mismatches and a flat line indicates the observed mismatches.

The mismatch distribution of Chonburi samples is shown in Figure 4.12. With the high SSD value (= 0.146) and the statistical test between SSD of Chonburi and simulated SSD of recent population expansion model is significantly diffrent (*p*-value = 0.005) revealed that the mismatch distribution constructed from Chonburi samples deviated from recent population expansion model. Its Hri is low (0.017) reflecting the unimodal pattern but the statistical test of Hri was not significant (*p*-value = 0.999). This reveals the mismatch distribution of Chonburi is ragged or has a multimodal pattern.

The mismatch distribution of Chumporn samples is shown in Figure 4.13. The SSD value is high (0.129) along with significant difference between the observed and expected mismatch (*p*-value = 0.007). This reveals that the Chumporn mismatch distribution is deviated from the model of recent population expansion. The Hri is low (0.015) and not significant (*p*-value = 0.993), which can be interpreted as the ragged or multimodal model.
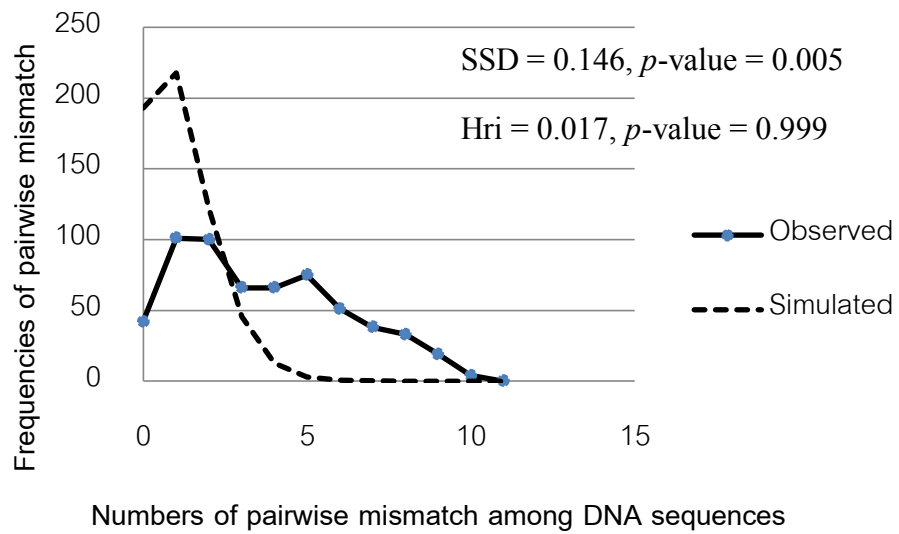
Figure 4.12 Mismatch distribution of Chonburi samples. A dash line indicates the simulated mismatches and a flat line indicates the observed mismatches.
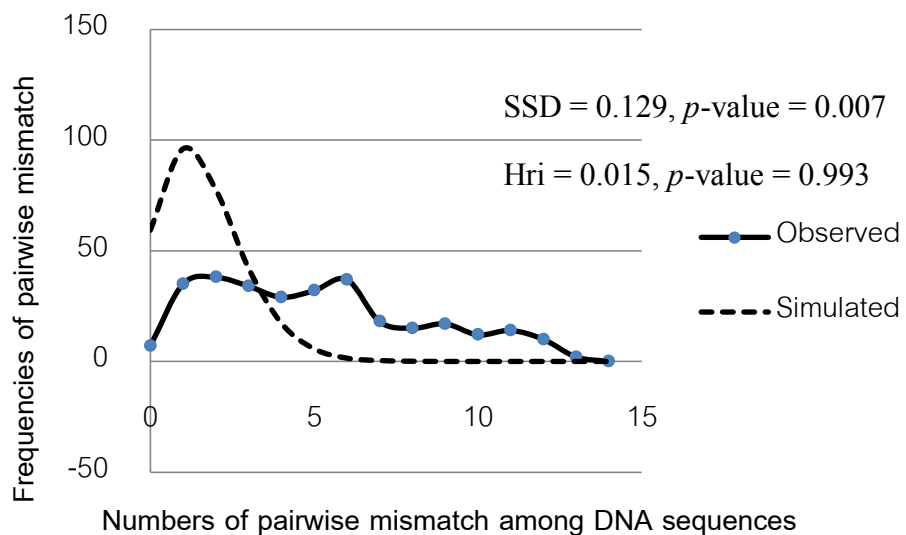
Figure 4.13 Mismatch distribution of Chumporn samples. A dash line indicates the simulated mismatches and a flat line indicates the observed mismatches.

For Chanthaburi population, the mismatch distribution is shown in Figure 4.14. The calculated SSD value was low (0.014) and not significant (p-value = 0.238), revealing that the observed mismatches do not significantly differ from the simulated mismatch from a recent demographic expansion model. The Hri of Chanthaburi population was low (0.030) and can be inferred that the mismatch curve was smooth or unimodal but the p-value of Hri was equal to 0.764 reflected non-significant difference between the observed mismatch and the model of multimodal pattern. This revealed that the Chanthaburi curve is ragged (or not smooth).

For Rayong population, the mismatch distribution is shown in Figure 4.15. The low SSD value and the statistical test for SSD value was not significant (p-value = 0.959). These revealed that the observed mismatch of Rayong was well-fit with simulated mismatch of recent demographic expansion model. The Hri value was low (0.010) revealing that the curve was smooth but the p-value of Hri value was equal to 0.877, reflecting the non-significance between the observed mismatch and the model of mutimodal pattern. This finding clarified that Rayong mismatch curve was not smooth. This is not consistent with the mismatch distribution shown in Figure 4.15 which is smooth or unimodal.
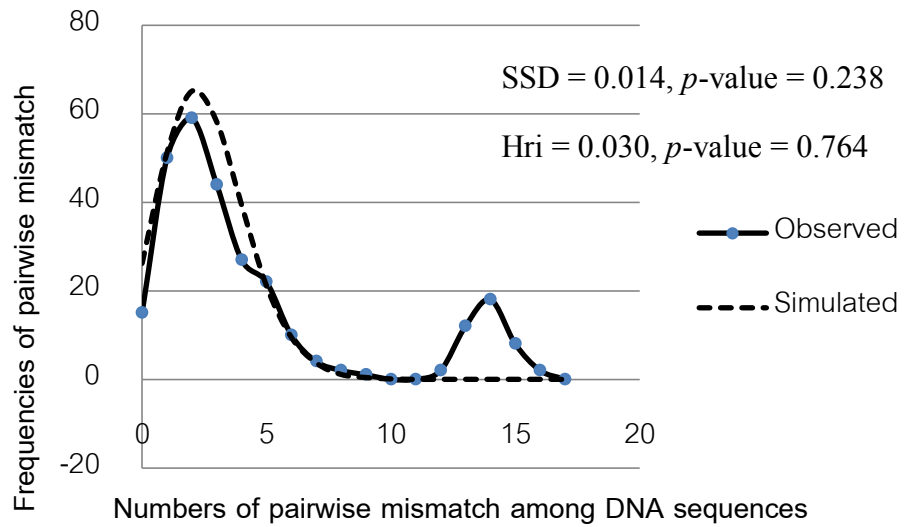
Figure 4.14 Mismatch distribution of Chanthaburi samples. A dash line indicates the simulated mismatches and a flat line indicates the observed mismatches.
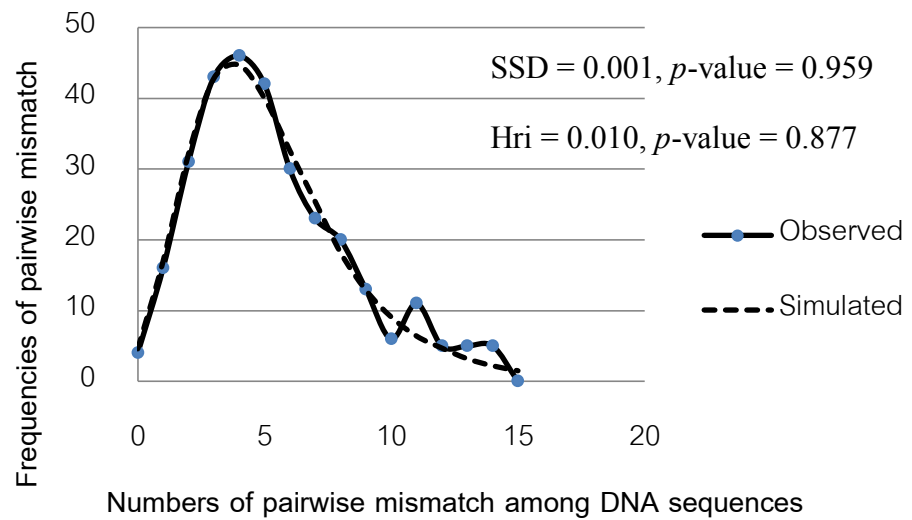
Figure 4.15 Mismatch distribution of Rayong samples. A dash line indicates the simulated mismatches and a flat line indicates the observed mismatches.

For Songkhla population, the mismatch distribution is shown in Figure 4.16. The low SSD value is low (0.008), and not significant (p-value = 0.425), revealing that the observed mismatch is also well-fit with the simulated model of recent demographic expansion. The low Hri value (0.105) clarifies that the mismatch curve of Songkhla is unimodal consistent with what shown in Figure 4.16. However, this finding was not consistent with the result from the statistical test between the observed Hri value, which is not significant (p-value = 0.317), reflecting the multimodal mismatch distribution of Songkhla population.

For Phetchaburi population, the mismatch distribution is shown in Figure 4.17. Even if the calculated SSD value was high (0.228) reflecting the unfit pattern between the observed mismatch and the mismatch simulating from recent population expansion model, but the p-value was equal to 0.321, revealing that the observed mismatch was well-fit with the simulated mismatch of demographic expansion model. The computed Hri value was considerably high (0.667) and not significant (p-value = 0.608) clarifying the non-smooth pattern of the mismatch curve of Phetchaburi population.
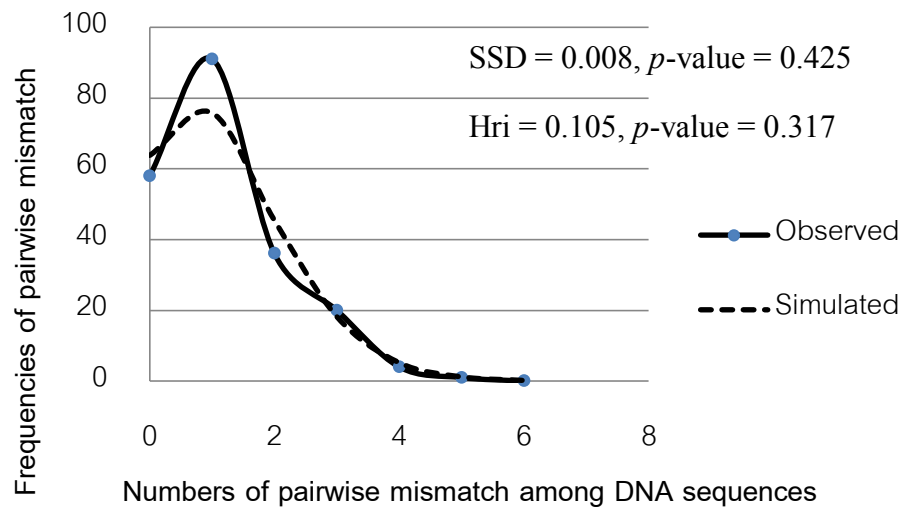
Figure 4.16 Mismatch distribution of Songkhla samples. A dash line indicates the simulated mismatches and a flat line indicates the observed mismatches.
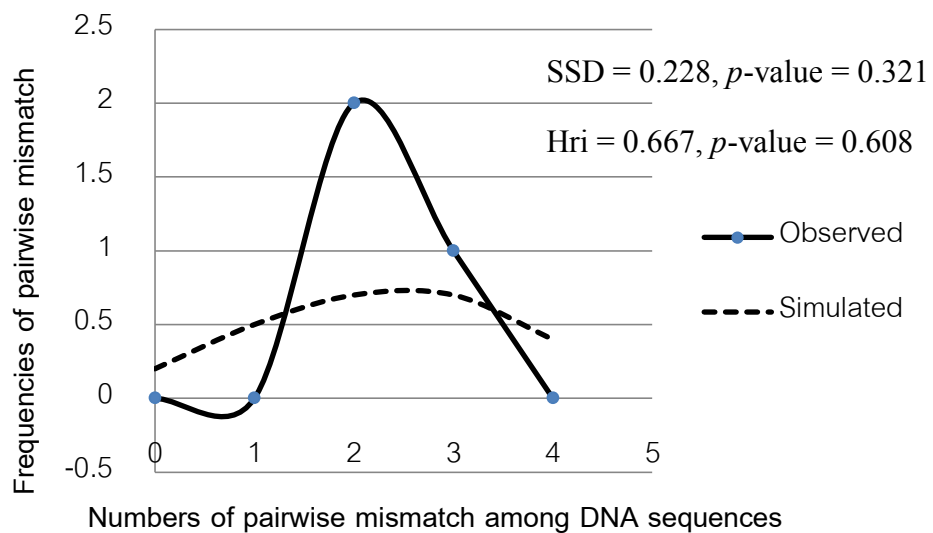
Figure 4.17 Mismatch distribution of Phetchaburi samples. A dash line indicates the simulated mismatches and a flat line indicates the observed mismatches.

For Prachuap Khiri Khan population, the mismatch distribution is shown in Figure 4.18. The SSD value was low (0.018) and not significant (p-value = 0.470), revealing that the observed mismatches was fit with the recent demographic expansion model. The Hri value of Prachuap Khiri Khan population was low (0.093) reflecting the smooth pattern of the mismatch curve but the statistical test of Hri is not significant (p-value = 0.347), reflecting the non-smooth pattern of the mismatch distribution.

For Ranong population, the mismatch distribution is shown in Figure 4.19. Even if the SSD value was low (0.019), but not significant (p-value = 0.209), revealing that the observed mismatches was fit with the mismatch simulating from recent demographic expansion model. The Hri value was low (0.075), reflecting the unimodal pattern of the observed mismatch curve, but the statistical test of Hri value is not significant (p-value = 0.843), revealing the multimodal mismatch distribution. In summary, Chanthaburi, Rayong, Songkhla, and Ranong populations underwent recent population expansion according to the mismatch distribution analyses.
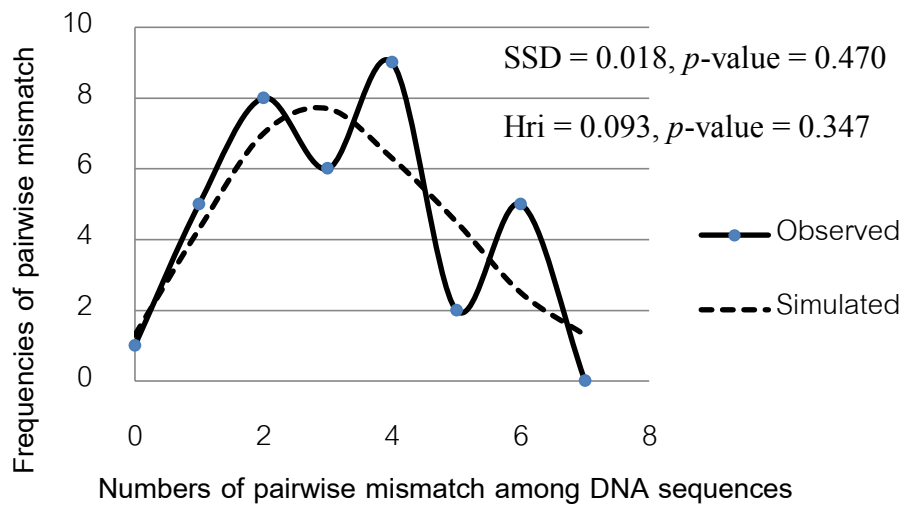
Figure 4.18 Mismatch distribution of Prachuap Khiri Khan samples. A dash line indicates the simulated mismatches and a flat line indicates the observed mismatches.
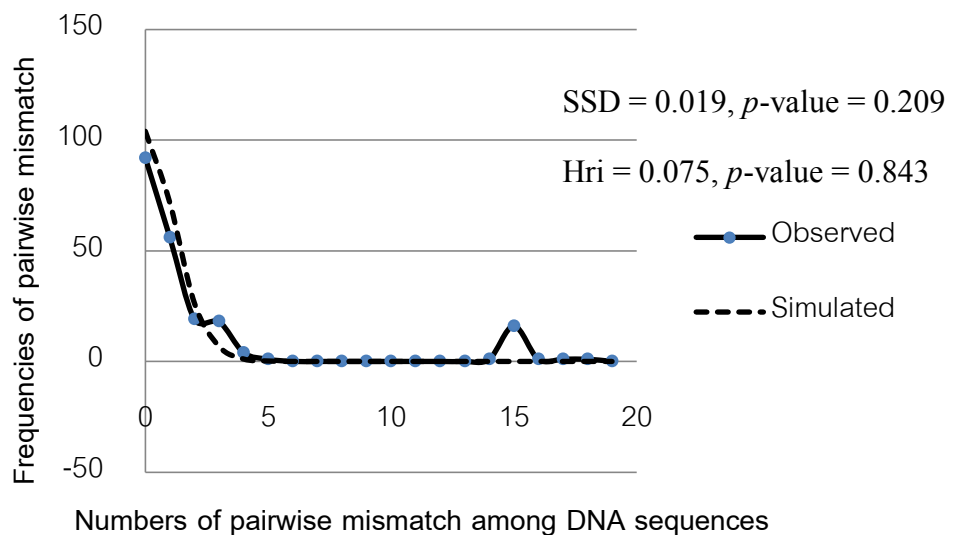
Figure 4.19 Mismatch distribution of Ranong samples. A dash line indicates the simulated mismatches and a flat line indicates the observed mismatches.

4.4.7 Coalescent-based Bayesian skyline plot

The results from Bayesian skyline plot analysis are shown in Figure 4.20 to 4.29. The median value of $N_e\tau$ values (N_e is the effective population size multiplied by τ which is time since expansion in mutational unit) (flat line) of total 163 *T. indicus* samples (Figure 4.20) infers that population size inclined in the past and declined recently. However, the Gulf of Thailand population has been constant (Figure 4.21). These indicated that the every *T. indicus* sample underwent expansion long time ago and has been through contraction recently. The median values for $N_e\tau$ of Rayong, Chumporn, Chonburi, Chanthaburi, Phetchaburi, and Prachuap Khiri Khan populations (Figure 4.22 to Figure 4.27, respectively) were found to be inclined in the past and have been declined recently. Therefore, these populations underwent expansion long time ago and have been through contraction recently.

Figure 4.28 to 4.29 showed that the median value $N_e\tau$ of of Songkhla and Ranong populations inclined in the past. Therefore, these populations underwent expansion long time ago.
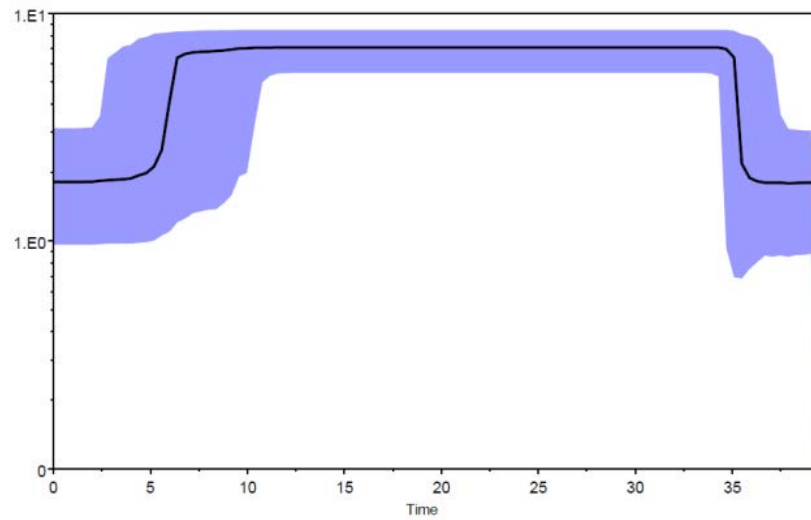
Figure 4.20 Bayesian skyline plot of 163 *T. indicus* samples. The flat line represents the median estimate for $N_e\tau$ against time (x1,000 years ago or KYA). The blue area indicated the upper and lower bounds of 95% HPD (highest posterior density).
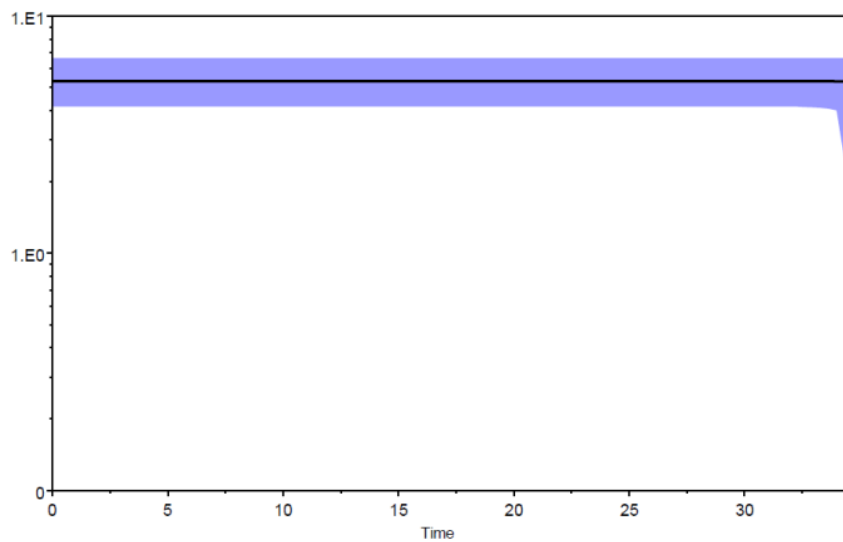
Figure 4.21 Bayesian skyline plot of Gulf of Thailand population. The flat line represents the median estimate for $N_e\tau$ against time (x1,000 years ago or KYA). The blue area indicated the upper and lower bounds of 95% HPD (highest posterior density).
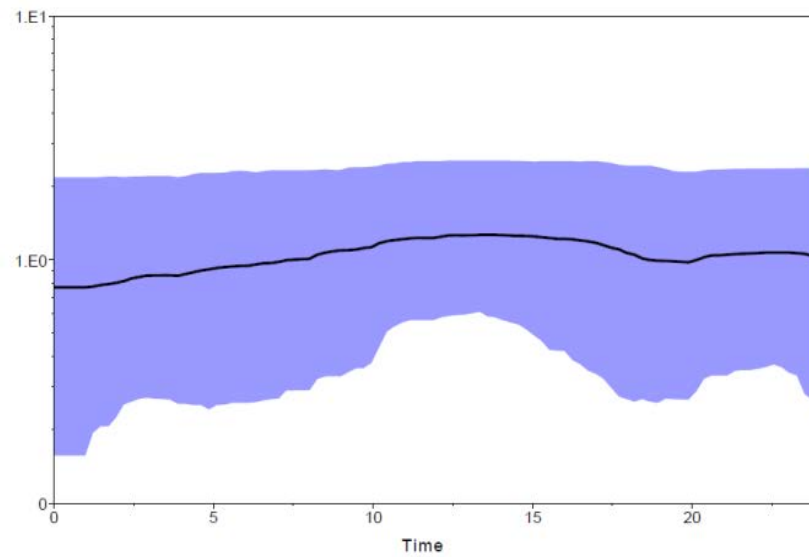
Figure 4.22 Bayesian skyline plot of Rayong population. The flat line represents the median estimate for $N_e\tau$ against time (x1,000 years ago or KYA). The blue area indicated the upper and lower bounds of 95% HPD (highest posterior density).
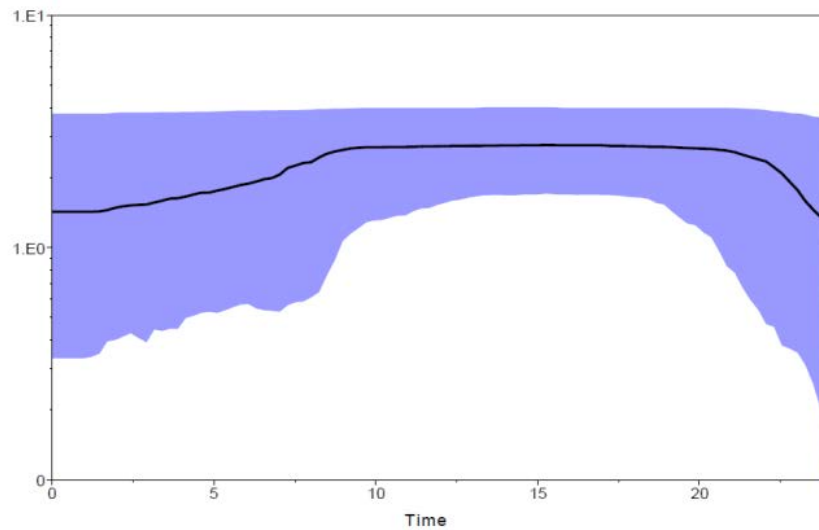
Figure 4.23 Bayesian skyline plot of Chumporn population. The flat line represents the median estimate for $N_e\tau$ against time (x1,000 years ago or KYA). The blue area indicated the upper and lower bounds of 95% HPD (highest posterior density).
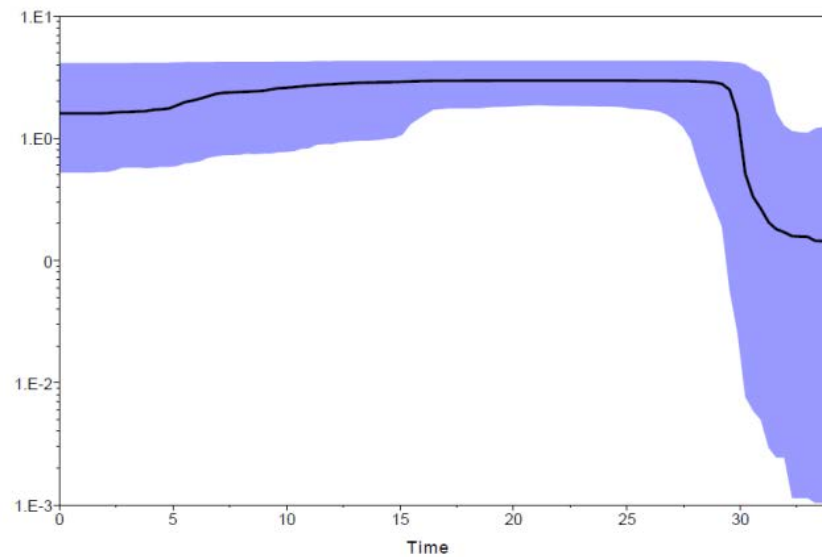
Figure 4.24 Bayesian skyline plot of Chonburi population. The flat line represents the median estimate for $N_e\tau$ against time (x1,000 years ago or KYA). The blue area indicated the upper and lower bounds of 95% HPD (highest posterior density).
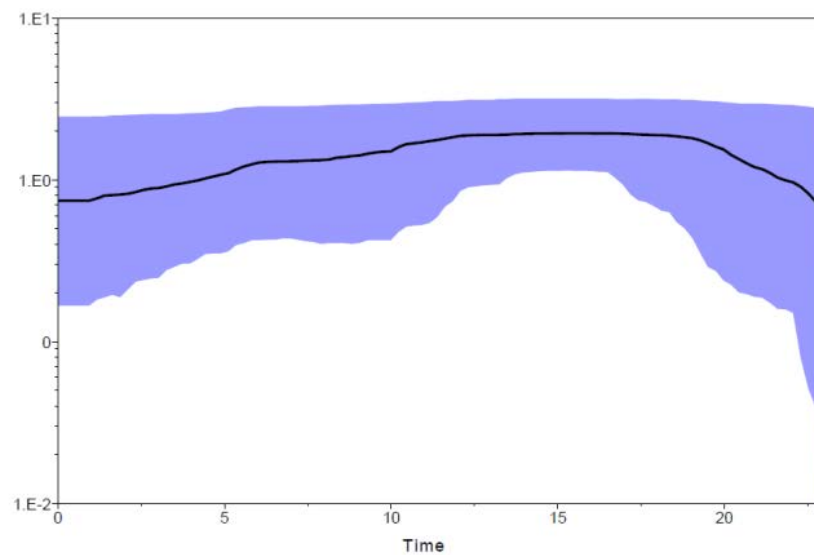
Figure 4.25 Bayesian skyline plot of Chanthaburi population. The flat line represents the median estimate for $N_e\tau$ against time (x1,000 years ago or KYA). The blue area indicated the upper and lower bounds of 95% HPD (highest posterior density).
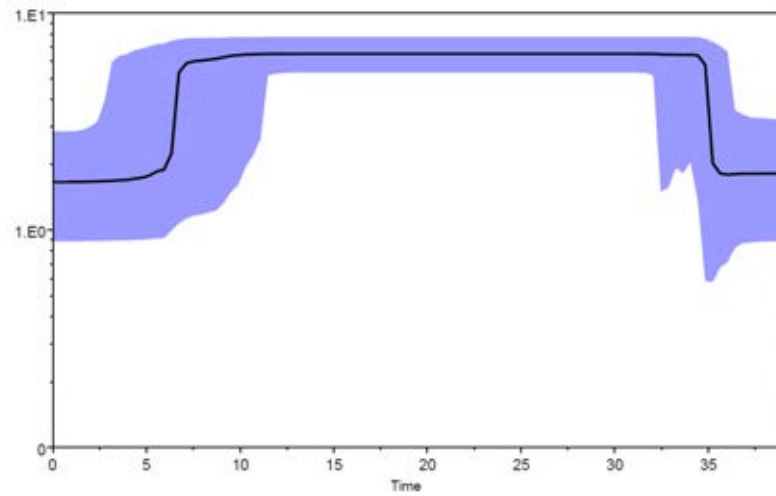
Figure 4.26 Bayesian skyline plot of Phetchaburi population. The flat line represents the median estimate for $N_e\tau$ against time (x1,000 years ago or KYA). The blue area indicated the upper and lower bounds of 95% HPD (highest posterior density).
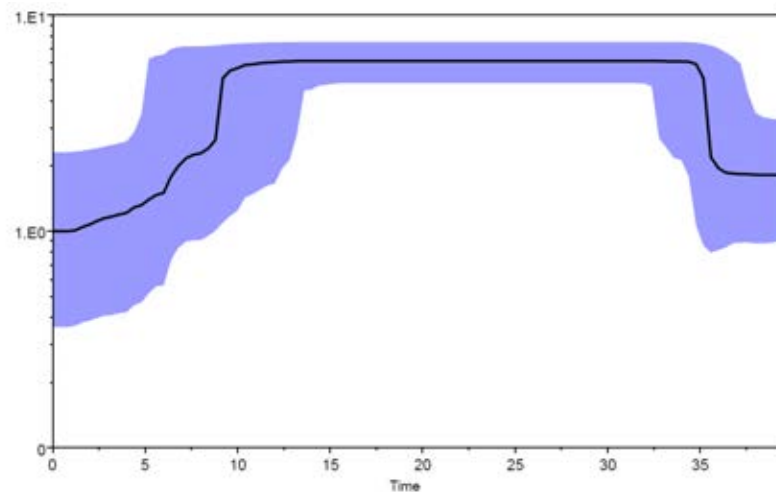
Figure 4.27 Bayesian skyline plot of Prachuap Khiri Khan population. The flat line represents the median estimate for $N_e\tau$ against time (x1,000 years ago or KYA). The blue area indicated the upper and lower bounds of 95% HPD (highest posterior density).
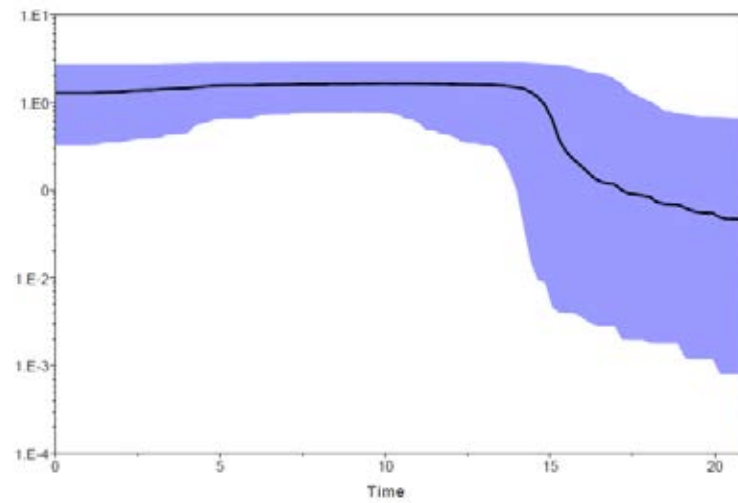
Figure 4.28 Bayesian skyline plot of Ranong population. The flat line represents the median estimate for $N_e\tau$ against time (x1,000 years ago or KYA). The blue area indicated the upper and lower bounds of 95% HPD (highest posterior density).
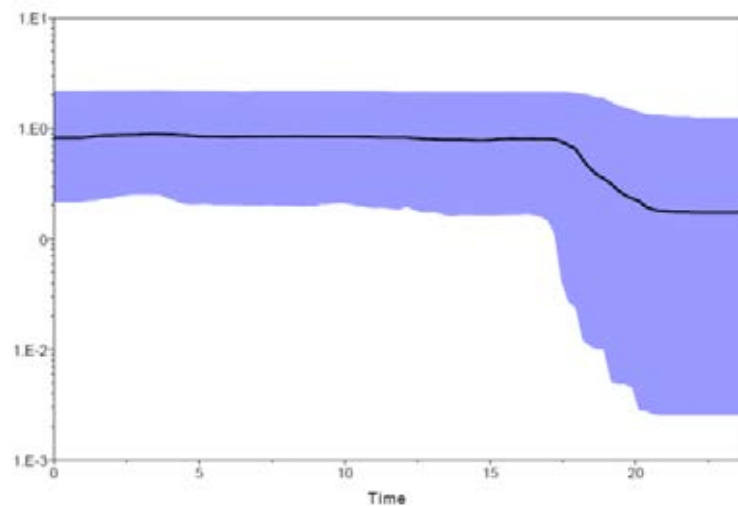
Figure 4.29 Bayesian skyline plot of Songkhla population. The flat line represents the median estimate for $N_e\tau$ against time (x1,000 years ago or KYA). The blue area indicated the upper and lower bounds of 95% HPD (highest posterior density).

4.4.8 Time to the most recent common ancestor (t_{mrca})

Time to the most recent common ancestor (t_{mrca}) between the population of the Gulf of Thailand and the Andaman Sea was estimated with strict molecular clock at rate 1.15%. The t_{mrca} between the population of Ranong (Andaman Sea) and the Gulf of Thailand was about 20,000 years ago (1.965 x 10^4 years ago with the 95% HPD (highest posterior density) = 1.1496 x 10^4 to 2.814 x 10^4 years ago; ESS (effective sample size) = 1040.697). Genetic divergence of the populations within the Gulf of Thailand was found between the population of Songkhla against the other populations and this divergence had been occurred around 14,000 years ago (1.417 x 10^4 years ago, with 95% HPD = 8.3471 x 10^3 to 2.1154 x 10^4 years ago; ESS = 270.549).

CHAPTER V

DISCUSSION

5.1 External morphological characteristics

From the results of this research, the external morphological characteristics proposed by Burton and Davie (2007) were confirmed to be useful for species identification of *Thenus* specimens from Thailand. This finding also agreed with Burton and Davie,,s identification key using propodus characteristics in that it can be properly applied to any sample of the three species of *Thenus* in Thailand. However, this identification technique must be used with the care in the case of *T. unimaculatus* as it could be wrongly recognised as *T. indicus*. This is because if the purple propodus on periopods of *T. unimaculatus* is so pale that its propodus would look similar to the non-spotted periopods of *T. indicus*. Moreover, these two species was also found to be in a sympatric habitat (Burton and Davie, 2007). This identification criterion also has another important limitation, which is that the propodus character could not be applied with any preserved specimen which probably losses pigments on the periopod. Thus, other identification methods such as molecular approach should be used to identify the preserved specimen.

According to the interviews with the fishermen and local seafood sellers in the field trips, shovel-nosed lobsters in Thailand can be found and caught throughout the year and, they are most abundant in a rainy season and decrease in number in November to March. This finding is consistent with the previous report by Naiyanetr (1963) also closely consistent with Jones" report (2007) that there were two periods of increasing abundance of Australian shovel-nosed lobster, first in April to March and second in October. However, the period of abundance purposed in this thesis is not consistent with the suggestion of Uraiwan (1977) who reported that Thai shovel-nosed lobsters were most abundant in January and February. Notably that the availability of lobsters in the market is not only up to the recruitment pulses of postlarval individuals (Jones, 2007), but also indirectly to the weather. If there is rainfall, the trawling boats cannot go out and capture the lobsters. Since the peak of

rainfalls would be in November to January for the Gulf of Thailand and September for Andaman Sea (http://www.tmd.go.th/ en/archive /rainfall.php), the numbers of shovel-nosed lobsters in the market are usually very low in monsoon season (from November to January), especially in the South of Thailand.

From this study, the distributions of *Thenus* species in Thailand are as follow: *Thenus indicus* can be found in both the Gulf of Thailand and the Andaman Sea; *T. orientalis* can be found only in the Gulf of Thailand; and, *T. unimaculatus* has the limited distribution area, which is only found in the Andaman Sea. These data are consistent with the previous study of Burton and Davie (2007). Furthermore, the difference in distribution ranges between *Thenus* species might have been resulted from their ecological distinction. According to the information from the fishermen, each *Thenus* species preferred to live in a specific water depth. *Thenus indicus* is usually found at 10 to 30 metres depth under the sea surface and captured by small local fishing boats. But, *T. orientalis*, which can be captured by commercial large boats only, can be found at more than 30 metres depth, and *T. unimaculatus* can be captured from a broader range of 10 to over 100 metres depth. This data is consistent with Jones" suggestions (1993; 2007) in that there should be different preferences of water depth between *Thenus* species. For example, *T. parindicus* prefers to live in shallow water (10 to 30 metres), and in deeper water for *T. australiensis* (30 to 50 metres). In addition, there may be a difference in the sediment preferences between *T. indicus*, *T. orientalis*, and *T. unimaculatus* because the Australian species *T. parindicus* (also known as a mud bug) is found preferring to live in fine sand whereas *T. australiensis* (also known as a reef bug) prefers to live in medium-coarse sand (Jones 1993; 2007). Additional ecological studies of *Thenus* spp. in Thailand are needed to clarify this hypothesis.

5.2 Morphometric analyses

The sex ratios of three *Thenus* species in Table 4.1 suggested that there were more female shovel-nosed lobsters in Thailand than males (*T. indicus* = 1:1.44, *T. orientalis* = 1:4, and *T. unimaculatus* = 1:1.81). This data is similar with the sex

ratios of *T. parindicus* (1:1), but not consistent with that of *T. australiensis* (1:0.57) suggested by Jones (2007). Naiyanetr (1963) and Uraiwan (1977) also previously reported that there were more male shovel-nosed lobsters than females. The average total length of *Thenus* spp. ranged between around 16 cm to 22 cm and this length range is consistent with the previous reports by the Department of Fisheries (1993, as 15 to 23 cm), by Uraiwan (1977, as 16 cm to 20 cm), and by Radhakrishnan et al. (2007, as 11 cm to 21 cm). When comparing between the three species of *Thenus*, *T. indicus* has the smallest size (16.67 ± 3.05 cm) following by *T. unimaculatus* (19.29 ± 2.23 cm), and the largest species was *T. orientalis* (21.72 ± 2.98 cm). This data indicated that there might be difference in size among each species of the genus *Thenus*, similar to the previous finding that *T. parindicus* is smaller than *T. australiensis* (Jones, 1993; 2007).

The identification method based on the morphometric ratios shown in Figure 4.3 cannot be applied to separate the specimens from different geographical locations even if there is a genetic difference found between *T. indicus* specimens from the Gulf of Thailand and from the Andaman Sea. The morphometric ratios using the morphometric criterions described by Burton and Davie (2007) cannot be applied for *Thenus* samples in Thailand since only 11.3% was correctly identified. Thus, the morphometric ratios are needed to be adjusted. For example, the ML3/CL ratio should be adjusted to be more than 0.48 (up from 0.45) for *T. indicus*, and the PW1/PL1 ratio should be changed to be more than 0.33 (from 0.35) to distinguish *T. unimaculatus* from the other species more accurately. Moreover, in order to study the population structure of any specific *Thenus* species, the DNA data is more robust than the morphometric ratio data.

5.3 Molecular phylogenetic analyses

From this thesis study, CO1 pairwise divergence found between *Thenus* species (4% to 14%) is similar to the finding of Burton and Davie (2007) and also consistent with the >2% genetic distance criteria for delimiting species in invertebrate phyla of Hebert et al. (2003). The CO1 genetic distance between invertebrate species

is typically different from ten to 25% (Hebert et al., 2003). The genetic differences of other decapods species using CO1 sequences were previously reported to be 6% to 20% for the snapping shrimp *Alpheus* spp. (Anker et al., 2008), 3% for red-snow-crab *Chionoecetes* spp. (Azuma et al., 2011), 15% to 16% for squat lobster *Babamunida* spp., 17% to 23% for *Munida* spp. (Cabezas et al., 2008), 8% to 18% for *Agononida* spp., 3% to 17% for *Paramunida* spp., 11% of *Plesionida* spp. (Cabezas et al., 2009), 11% to 16% for *Allogalathea* spp. (Cabezas et al., 2011), 9% for *Munidopsis* spp. (Jones and Macpherson, 2007), 8% to 16% for *Munida* and related genera (Machordom and Macpherson, 2004), 28% to 33% for *Uroptychus* spp. (Poore and Andreakis, 2011), 10% for spiny lobster *Panulirus* spp. according to Cannas et al. (2006) or 13% to 21% according to Ptacek et al. (2001), 8% for *Palinurus* spp. (Groeneveld et al., 2007), 2% to 16% for the clawed lobster *Metanephrops* spp. (Chan et al., 2009) 14% to 16% for marine porcelain crab *Petrolisthes* spp. (Hiller and Werding, 2007), 11% to 20% for palaemonid shrimp *Periclimenes* spp. (Komai et al., 2010), 4% to 25% for hermit crab *Calcinus* spp. (Malay and Paulay, 2009), 11% to 25% for crab *Cancer* spp. (Pardo et al., 2009), 7% to 15% for fiddler crab *Uca* spp. (Shih et al., 2009), the 8% to 54% for spear lobster *Linuparus* spp. (Tsoi et al., 2011), 14% to 18% for slipper lobster *Galearctus* spp. (Yang et al., 2011), and 3% to 59% for freshwater crab *Johora* spp. (Yeo et al., 2007).

The NJ tree in Figure 4.4 clarified the phylogenetic relationships between *Thenus* spp. samples from Thailand. Each of the three *Thenus* species is a monophyletically natural group. This is because all 163 samples of *T. indicus* and HM015445 *T. indicus* were monophyletically grouped together in Clade A while *T. orientalis* and *T. unimaculatus* specimens are independently grouped within Clade B and Clade C, respectively. *Thenus* specimens are not only separated into three monophyletic clades as described, but also given the scientific names congruent with the external morphological criteria. Therefore, this thesis strongly confirms the usage of CO1 nucleotide sequences as a suitable barcoding region for species boundaries delimitation between *Thenus* spp. as purposed in the previous phylogenetic study of *Thenus* by Burton and Davie (2007).

The strict consensus tree from maximum parsimony (MP) analysis shown in Figure 4.6 supported the groupings of each *Thenus* species on the maximum likelihood (ML) tree (Figure 4.7) with a very similar topology. One of 11 MP trees (as an example in Figure 4.5) suggested that the evolutionary step changes in each *Thenus* lineage reflecting the species boundary are not equal. The evolutionary step changes between *T. indicus* and the sister group of *T. orientalis* / *T. unimaculatus* (with 68 bp branch length support) is much larger than the changes between *T. orientalis* and *T. unimaculatus* (26 bp). In addition, the intraspecific variation of *T. indicus* between the samples from the Gulf of Thailand and those from Andaman Sea is rather high with the nucleotide pairwise difference from 13 to 26 bp.

5.4 Genetic diversity

High haplotype diversity (as high as 0.7 and more) and close genetic similarity between the haplotypes are found in six of eight provincial populations Chonburi, Chumporn, Chanthaburi, Rayong, Phetchaburi, and Prachuap Khiri Khan and also the overall populations of the Gulf of Thailand, and the total 163 samples of *T. indicus* in Thailand. This indicates the evidence of the recent population expansion (see the explanation in Rogers (1995) and Grave (1998)). However, this finding is not consistent with the haplotype network (Figure 4.9) which does not represent any star-like pattern referring to a rapid expansion. Therefore, the populations of *T. indicus* in the Gulf of Thailand might have actually expanded but long time ago. Moreover, that narrow genetic similarity found might be resulted from a recent diversification process of those populations but their evolutionary times may not be sufficient enough to acquire any significant genetic difference (Cassone and Boulding, 2006).

From Table 4.4, the nucleotide diversity of every *T. indicus* population in Thailand is low (not exceed 0.01). This may be a result of some newly existed haplotypes which obtain only few basepair differences and have gone extinct (Cassone and Boulding, 2006). The levels of nucleotide diversity of all eight populations of *T. indicus* in Thailand are considerably lower than that of several marine organisms (Ovenden, 1990; Palumbi and Wilson, 1990; Boulding et al., 1993). When considering within decapods, the overall nucleotide diversity of Thai *T. indicus*

is lower than that of *Panaeus monodon* (0.033) (Klinbunga et al., 2001) but greater than that of the lined shore crab, *Pachygrapsus crassipes* (less than 0.005) (Cassone and Boulding, 2006).

5.5 Population structure

After compared these MP and ML phylogenetic trees with Bayesian Inference (BI) tree (Figure 4.8), none of geographically grouping of *T. indicus* within the Gulf of Thailand could be significantly recognised. This unexpected finding could result from the fact that shovel-nosed lobster cannot migrate over long distances and the circulation of sea currents in the Gulf of Thailand must have played an important role on a homogenisation between their planktonic larvae from different localities in the Gulf. Some might link this hypothesis with the changing of the direction of the Gulf of Thailand current as the current moves in a counterclockwise direction under the influence of northeastern monsoon on November to January and in a clockwise or counterclockwise direction under the influence of southwestern monsoon on May to August (Buranapratheprat, 2008). Nevertheless, the BI tree also suggested that the speciation event would occur in the future in the case of *T. indicus* populations because of the disruption of the gene flow between the populations of the Gulf of Thailand and Andaman Sea. Interestingly, one sample from Ranong (Rano17) was grouped with other samples of the Gulf of Thailand instead of those from Andaman Sea and possibly indicated the genetic hitchhiking or translocation by human of *T. indicus* between the Gulf of Thailand and Andaman Sea.

According to the AMOVA analyses for the three geographical groups assigned (the upper Gulf of Thailand, the lower Gulf of Thailand, and Andaman Sea), the result suggested that there could be spatial genetic structure of *T. indicus* in Thailand, meaning that the population of the Gulf of Thailand had different genetic background from that of Andaman Sea. This suggestion is also confirmed by the F_{st} pairwise population comparison which Ranong samples significantly differentiated from the samples of the Gulf of Thailand. Likewise, the phylogenetic result of BI tree of *T. indicus* also confirmed that Andaman Sea population was separated from that of the

Gulf of Thailand. This pattern of genetic heterogeneity in *T. indicus* between the Gulf of Thailand and Andaman Sea was also consistent with other marine organisms habiting in Thai waters, such as the giant tiger shrimp *Panaeus monodon* (Khamnamtong et al, 2009; Klinbunga et al., 2001), banana shrimp *P. merguiensis* (Hualkasin et al, 2003), abalone *H. asinina* (Praipue et al., 2010), *H. varia* (Klinbunga et al., 2003), and swimming crab *Portunus pelagicus* (Klinbunga et al., 2007). Nevertheless, the other result of another analysis testing, only two assigned groups (the upper Gulf of Thailand and the lower Gulf of Thailand) did not indicate a possible spatial genetic structure of *T. indicus* within the Gulf. The reason might lay on the fact that *T. indicus*, like other *Thenus* species, possesses planktonic larvae which normally are dispersed by ocean currents (McEdward, 1995). Therefore, it has been characterised by low genetic heterogeneity among populations over spatial scale (Lui et al., 2010).

Interestingly, according to the F_{st} values from AMOVA test, Songkhla population was found being genetically different from every population in the Gulf of Thailand except Phetchaburi province. This result suggests that some genetic differentiation within the Gulf of Thailand might still exist even if the larvae could be widely distributed (Palumbi, 2003). Such genetic differentiation might come from other environmental factors such as the unusual pattern of oceanic circulation within the Gulf of Thailand causing some physical barriers and eventually disrupting the gene flow (Perrin et al, 2004; Palumbi, 1994). The restricted level of dispersal ability of larvae of *T. indicus* may occasionally bring about the genetic heterogeneity of shovel-nosed lobster in the Gulf of Thailand as found in the Kuruma prawn (Tzeng et al., 2004). Further investigation on larval biology of *T. indicus* is needed to confirm this hypothesis.

5.6 Neutrality tests and demographic history

From the neutrality tests shown in Table 4.5, the populations with negative Tajima"s D values (the overall population, the populations of the Gulf of Thailand, and those of Chonburi, Chanthaburi, Rayong, Songkhla, Prachuap Khiri Khan, and Ranong provinces) did not undergo neutral evolution and might experience population expansion or population bottleneck. The population from Chumporn province with positive Tajima"s D value deviated from neutral evolution and might undergo some forms of balancing selection. On the other way round, Tajima"s D value of Phetchaburi province was equal to zero and indicated that this population might undergo neutral evolution and in mutation-drift equilibrium (Tajima, 1989). Similarly for Fu"s F_s values, every population in this study had negative values and this phenomenon suggested that these populations deviated from neutral evolution and might experience population expansion (Fu, 1997). Notably, only *T. indicus* samples from Chanthaburi, Songkhla, and Ranong provinces gave Tajima"s D test results which were consistent with the negative Fu"s F_s values and significant.

From Figure 4.10, 4.12, 4.13, and 4.17, the mismatch distribution results of overall 163 *T. indicus* samples in Thailand, the overall populations of the Gulf of Thailand, Chonburi, Phetchaburi, and Chumporn populations showed that they were under a multimodal mismatch distribution. Moreover, an unfit between the observed mismatch and the population expansion model (from high SSD values and/or significant *p*-values of SSD) suggested that there would not be a population expansion occurred within those populations (Rogers and Harpending, 1992). On the contrary, an expansion of populations in Chanthaburi, Rayong, Songkhla, Prachuap Khiri Khan, and Ranong provinces was suggested by the unimodal mismatch distribution (from low SSD values and/or non-significant *p*-values of SSD) (Rogers and Harpending, 1992). However, the coalescent-based Bayesian skyline plot results (Figure 4.20 to 4.29) differently suggest that only Ranong and Songkhla populations have been continuously expanded. Moreover, it indicated that the populations of all samples and the specific populations of Rayong, Chumporn, Chonburi, Chanthaburi, Phetchaburi, and Prachuap Khiri Khan provinces probably expanded in the past and have

contracted recently. The Gulf of Thailand population was the only group which was suggested that it did not expand.

The divergent time between *T. indicus* populations of the Gulf of Thailand and Andaman Sea was estimated to be around 20,000 years ago. This is consistent with the last glaciation event in Pleistocene. The glaciation had an impact on decreasing the sea level and brought up some land bridges. On that time, the sea level of the Gulf of Thailand was low and the land bridge called Sundaland connecting Malay Peninsula and Sumatra and Java islands was occurred (Sathiamurthy and Vorris, 2006). Therefore, some ancestral shovel-nosed lobster populations in Andaman Sea were probably separated from those of the Gulf of Thailand on that time. After the end of the ice age, the sea level rose again the geographic barrier between the Gulf of Thailand and Andaman Sea was gone (Sathiamurthy and Vorris, 2006), making the populations of *T. indicus* shovel-nose lobster on both ocean basins could transgress between each other, though with very low level of chance. Additional samples from the lower part of the Andaman Sea are needed to clarify where the genetic break between both sides is.

CHAPTER VI

CONCLUSIONS

From this thesis, morphological examinations combined with molecular phylogenetic analysis can reveal species diversity and genetic diversity of shovel-nosed lobster of the genus *Thenus* in Thailand. New local Thai names were recorded in this study: Kang Kradan Thammada (common shovel-nosed lobster) for *T. indicus*, Kang Kradan Kha-lai (spotted-leg shovel-nosed lobster) for *T. orientalis*, and Kang Kradan Kha-muang (purple leg shovel-nosed lobster) for *T. unimaculatus*.

According to the genetic analyses of 163 samples of *T. indicus*, the results revealed that 87 haplotypes were found and the overall nucletide diversity is 0.0104. The haplotype network showed 2 major clades and suggested that the expansion of *T. indicus* populations might have happened since long time ago. From AMOVA test, there was the highest genetic variation found among two geographical groups (a group of seven populations from the upper Gulf of Thailand, and a group of one population from Andaman Sea). In the case of Gulf of Thailand, the highest genetic variation was found within each of all seven populations. The population pairwise comparison indicated that the genetics of the Ranong population was significantly different from that of other *T. indicus* populations. The neutrality tests also suggested that every *T. indicus* population did not undergo neutral evolution and might have experienced population expansion. The mismatch distribution analysis further indicated that Rayong, Songkhla, and Ranong populations were rapidly expanded. However, the coalescent-based Bayesian skyline plots clarified that every population except Songkhla and Ranong underwent population expansion long time ago followed by recent contraction, and only Ranong and Songkhla populations have been continuously expanding. Interestingly, the *T. indicus* populations between the Gulf of Thailand and Andaman Sea were estimated to be separated from each other about 20,000 years ago. In summary, the populations of *T. indicus* in Thailand have a high level of genetic diversity (except for Ranong population), and this data should fulfill

the necessary knowledge for establishing conservation and management strategies of the exploitation of shovel-nosed lobsters in the future.

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APPENDIX

Table A Raw data of morphological measurements of *T. indicus* samples of this study.

Column groups: Carapace (CL1, CW1, CW2); Antenna I (A1L, A1W); Antenna II (A2L, A2W); 1ˢᵗ abdom. seg. (AL1, AW1); Periopod 1 — Propodus (PL1, PW1), Merus (ML1, MW1); Periopod 2 — Propodus (PL2, PW2), Merus (ML2, MW2); Periopod 3 — Propodus (PL3, PW3), Merus (ML3, MW3); 6ᵗʰ abdom. seg. (AL2, AW2); Telson (TL, TW).

Samples	Species	Total length (cm)	Sex	CL1	CW1	CW2	A1L	A1W	A2L	A2W	AL1	AW1	PL1	PW1	ML1	MW1	PL2	PW2	ML2	MW2	PL3	PW3	ML3	MW3	AL2	AW2	TL	TW
Chon01	T. indicus	-	-	4.00	5.17	3.70	1.19	1.64	1.90	2.13	0.85	3.35	-	-	1.67	0.37	1.06	0.25	1.51	0.35	1.33	0.18	1.82	0.30	0.71	2.16	0.48	1.76
Chon02	T. indicus	-	-	4.28	5.54	3.90	1.39	1.86	1.97	2.29	-	-	1.21	0.23	-	-	-	-	2.20	0.33	-	-	2.20	-	0.57	2.45	0.50	1.99
Chon03	T. indicus	-	-	6.12	7.61	5.50	1.77	2.42	2.74	3.13	1.20	5.16	1.64	0.45	2.06	0.58	2.03	0.39	2.77	0.51	1.74	0.33	2.87	0.43	1.16	3.27	0.93	2.75
Chon04	T. indicus	-	-	5.88	7.55	5.25	1.83	2.52	2.66	3.08	1.17	4.98	1.57	0.47	1.95	0.58	2.08	0.43	2.65	0.49	1.71	0.33	2.67	0.46	1.05	3.25	0.70	2.75
Chon05	T. indicus	-	-	6.05	7.42	5.44	1.73	2.64	2.80	3.17	1.05	5.00	1.60	0.43	2.05	0.58	2.06	0.36	2.80	0.50	1.74	0.31	2.89	0.46	1.10	3.44	0.90	2.74
Chon06	T. indicus	-	-	5.82	7.38	5.50	2.00	2.55	2.76	3.25	1.15	5.07	1.47	0.42	2.05	0.55	2.00	0.40	2.67	0.46	1.74	0.35	2.82	0.44	1.10	3.24	0.78	2.68
Chon07	T. indicus	-	-	4.88	7.00	4.92	1.67	2.26	2.50	2.89	1.10	4.76	1.38	0.38	2.01	0.49	2.49	0.37	2.59	0.43	1.61	0.35	2.64	0.44	1.07	2.97	0.66	2.45
Chon08	T. indicus	-	-	5.66	6.80	5.09	1.55	2.28	2.60	2.90	1.12	4.82	1.42	0.41	1.98	0.51	2.06	0.34	2.61	0.49	1.60	0.31	2.66	0.45	1.03	3.10	0.61	2.45
Chon09	T. indicus	-	-	5.35	6.66	4.84	1.69	2.25	2.41	2.70	1.01	4.67	1.37	0.42	1.77	0.51	1.88	0.36	2.46	0.45	1.59	0.33	2.50	0.46	1.20	2.91	0.71	2.44
Chon10	T. indicus	-	-	5.91	7.08	5.05	1.66	2.42	2.15	3.06	1.15	5.01	1.60	0.43	2.27	0.57	2.16	0.37	2.94	0.48	1.61	0.32	2.88	0.44	1.00	3.27	0.80	2.64
Chon11	T. indicus	18.7	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chon12	T. indicus	16.6	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chon13	T. indicus	19.4	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chon14	T. indicus	18.2	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chon15	T. indicus	20.3	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chon16	T. indicus	18.2	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chon17	T. indicus	17.2	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chon18	T. indicus	17.9	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chon19	T. indicus	20.4	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chon20	T. indicus	21.2	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chon21	T. indicus	17.2	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chon22	T. indicus	18.2	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chon23	T. indicus	18.6	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chon24	T. indicus	17.9	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chon25	T. indicus	18.5	F	6.12	8.06	5.58	1.63	2.42	2.16	3.18	1.30	4.47	1.40	0.45	1.72	0.62	1.76	0.44	2.35	0.61	1.57	0.28	2.49	0.52	1.26	3.45	0.80	2.85
Chon26	T. indicus	15.1	M	5.41	6.60	4.77	1.35	2.00	1.95	2.94	1.03	3.77	1.18	0.38	1.55	0.54	1.40	0.35	2.16	0.49	1.22	0.26	2.02	0.48	1.00	2.85	0.65	2.45
Chon27	T. indicus	14	M	5.18	6.33	4.60	1.35	1.92	1.59	2.57	1.30	3.79	1.13	0.38	1.51	0.47	1.35	0.31	1.97	0.42	1.23	0.24	2.00	0.41	0.96	2.79	0.79	2.10
Chon28	T. indicus	14.84	M	5.39	6.51	4.66	1.26	1.81	1.74	2.72	1.20	3.82	1.17	0.38	1.53	0.50	1.52	0.37	2.13	0.47	1.25	0.28	2.41	0.43	1.00	2.83	0.74	2.22
Chon29	T. indicus	16.8	F	5.92	7.20	5.38	1.49	2.12	2.03	3.07	1.20	4.14	1.25	0.43	1.49	0.56	1.66	0.38	2.23	0.55	1.32	0.32	2.20	0.51	1.00	3.20	0.74	2.58
Chon30	T. indicus	13.75	F	4.91	6.22	4.42	1.16	1.68	1.62	2.61	1.00	3.47	1.03	0.35	1.39	0.45	1.34	0.30	1.88	0.40	1.18	0.24	1.94	0.43	0.96	2.65	0.60	2.24
Chon31	T. indicus	14.9	M	5.24	6.34	4.59	1.20	1.89	1.69	2.75	1.13	3.75	1.13	0.33	1.45	0.50	1.50	0.30	2.13	0.51	1.30	0.27	2.12	0.41	0.94	2.75	0.63	2.24
Chon32	T. indicus	14.05	M	4.90	6.00	4.40	1.18	1.87	1.70	2.57	1.15	3.80	1.20	0.31	1.43	0.48	1.38	0.29	1.87	0.42	1.09	0.22	1.90	0.37	0.82	2.59	0.60	2.09
Chon33	T. indicus	14.69	F	5.15	6.30	4.44	1.19	1.81	1.61	2.61	1.05	3.70	0.96	0.36	1.41	0.49	1.28	0.30	1.75	0.43	1.14	0.23	1.86	0.42	0.94	2.68	0.60	2.15
Chon34	T. indicus	17.5	F	5.84	7.19	5.50	1.46	2.16	1.92	2.93	1.20	4.27	1.30	0.41	1.64	0.55	1.79	0.39	2.20	0.49	1.41	0.36	2.25	0.53	1.00	3.30	1.13	2.70
Chon35	T. indicus	15.13	M	5.26	6.45	4.75	1.34	1.83	1.77	2.83	1.02	3.88	1.11	0.38	1.43	0.52	1.39	0.32	2.04	0.46	1.27	0.24	2.10	0.40	0.79	2.84	0.69	2.30

Table A (continue)

Samples	Species	Total length (cm)	Sex	Carapace			Antenna I		Antenna II		1st abdom.		Periopod 1				Periopod 2				Periopod 3				6th abdom. seg.		Telson	
													Propodus		Merus		Propodus		Merus		Propodus		Merus					
				CL1	CW1	CW2	A1L	A1W	A2L	A2W	A1	AW1	PL1	PW1	ML1	MW1	PL2	PW2	ML2	MW2	PL3	PW3	ML3	MW3	AL2	AW2	TL	TW
Phet01	T. indicus	-	-	5.64	7.06	5.22	1.64	2.31	2.52	3.20	1.08	4.97	1.7	0.4	2	0.5	2.03	0.34	2.64	0.45	1.45	0.32	2.72	0.41	0.99	3.18	0.66	2.55
Phet02	T. orientalis	-	-	6.48	8.05	6.08	1.87	2.47	2.92	3.27	1.3	5.6	1.73	0.53	2.35	0.65	2.2	0.5	2.90	0.60	2	0.4	3.08	0.54	1.20	3.58	1.00	2.97
Phet03	T. orientalis	-	-	7.15	8.85	6.27	2.00	2.98	2.99	3.71	1.45	5.97	1.73	0.59	2.41	0.71	2.24	0.53	3.11	0.65	2.10	0.44	3.13	0.58	1.19	3.92	1.10	3.32
Phet04	T. orientalis	-	-	6.37	7.78	5.55	1.71	2.44	1.97	3.26	1.12	5.23	1.67	0.50	2.15	0.63	2.00	0.44	2.77	0.57	1.93	0.36	2.93	0.51	0.86	3.32	0.80	2.75
Phet05	T. orientalis	-	-	5.80	7.02	5.30	2.43	2.10	2.55	2.90	1.17	5.09	1.50	0.46	2.02	0.52	2.04	0.38	2.62	0.49	1.80	0.34	2.66	0.42	0.77	3.02	0.80	2.50
Phet06	T. orientalis	-	-	7.51	9.08	6.05	2.08	3.07	3.23	3.83	1.60	6.52	1.92	0.63	2.53	0.70	2.44	0.58	3.27	0.64	2.99	0.47	3.38	0.60	1.31	4.31	1.01	3.46
Phet07	T. indicus	-	-	5.60	7.17	5.05	2.03	2.32	2.96	2.62	1.10	4.47	1.52	0.40	2.07	0.50	1.77	0.34	2.87	0.49	1.55	0.30	2.94	0.43	0.67	2.92	0.71	2.44
Phet08	T. indicus	-	-	5.74	7.30	5.16	2.05	2.56	3.05	3.23	1.19	4.95	1.68	0.45	2.21	0.57	2.16	0.30	3.05	0.46	1.82	0.30	2.96	0.45	1.02	3.07	0.68	2.55
Phet09	T. orientalis	-	-	6.60	8.02	5.91	1.68	2.37	2.70	3.36	1.29	5.54	1.65	0.50	2.20	0.62	2.05	0.47	2.73	0.53	1.88	0.37	2.93	0.45	0.96	3.45	0.82	2.90
Phet10	T. orientalis	-	-	6.94	8.59	6.44	1.88	2.80	2.90	3.45	1.42	6.45	1.80	0.52	2.42	0.64	2.26	0.40	3.00	0.60	2.05	0.39	3.19	0.52	1.17	3.80	0.99	3.20
Prac01	T. indicus	5.38	-	2.1	2.7	2	0.5	0.7	0.7	0.9	0.5	1.5	0.4	0.1	0.6	0.2	0.6	0.1	0.8	0.1	0.4	0.1	0.8	0.1	0.4	1.2	0.3	0.9
Prac02	T. indicus	10.05	F	3	4.4	3.2	0.9	1.5	1.3	1.9	0.6	3.1	0.7	0.2	1.2	0.4	0.7	0.3	1.6	0.3	0.9	0.3	1.6	0.3	0.4	1.8	0.4	1.5
Prac03	T. indicus	11.27	M	4	5	3.7	1.1	1.5	1.4	2	0.8	2.8	0.6	0.2	1	0.4	1	0.2	1.5	0.3	0.8	0.2	1.5	0.3	0.7	2	0.7	1.7
Prac04	T. indicus	13.75	M	4.7	5.9	4.4	1.3	2	1.6	2.6	0.9	3.1	1	0.3	1.3	0.5	1.3	0.3	1.9	0.4	1	0.2	1.7	0.3	0.9	2.6	0.5	2
Prac05	T. indicus	9.5	M	3.7	4.6	3.2	0.9	1.4	1.2	1.9	0.7	2.7	0.8	0.2	1.2	0.3	1	0.2	1.4	0.3	0.9	0.2	1.4	0.3	0.7	1.9	0.5	1.5
Prac06	T. indicus	13	F	4	5.8	4.4	1.1	1.9	1.5	2.3	0.9	3.3	0.8	0.3	1.2	0.4	1.1	0.3	1.7	0.4	0.9	0.2	1.7	0.3	0.8	2.6	0.5	2
Prac07	T. indicus	9.79	M	3.3	4.2	3.1	0.9	1.3	1.1	1.8	0.6	2.9	0.8	0.2	1.1	0.3	1.1	0.3	1.5	0.3	1.2	0.2	1.6	0.3	0.4	1.9	0.5	1.5
Prac08	T. indicus	14.68	F	5.3	6.8	5.1	1.5	2.1	1.8	2.8	1.2	4.1	1.1	0.4	1.6	0.5	1.5	0.4	2	0.5	1.3	0.3	2.1	0.5	1	2.9	0.8	2.4
Prac09	T. indicus	9.74	F	3.3	4.2	3.1	0.9	1.3	1.2	1.8	0.4	2.5	0.7	0.2	0.8	0.3	0.8	0.2	1.3	0.2	0.7	0.1	1.3	0.2	0.6	1.7	0.4	1.4

Table A (continue)

Samples	Species	Total length (cm)	Sex	Carapace			Antenna I		Antenna II		1st abdom.		Periopod 1				Periopod 2				Periopod 3				6th abdom. seg.		Telson	
													Propodus		Merus		Propodus		Merus		Propodus		Merus					
				CL1	CW1	CW2	A1L	A1W	A2L	A2W	AL1	AW1	PL1	PW1	ML1	MW1	PL2	PW2	ML2	MW2	PL3	PW3	ML3	MW3	AL2	AW2	TL	TW
Chum01	T. indicus	15.7	F	5.21	6.55	4.80	1.37	2.00	1.83	2.20	1.10	4.45	1.3	0.5	1.5	0.6	1.70	0.44	1.99	0.52	1.40	0.40	2.10	0.51	0.95	2.85	0.83	1.76
Chum02	T. indicus	15.3	M	5.25	6.45	4.70	1.35	2.04	1.74	2.30	1.2	4.4	1.16	0.41	1.51	0.52	1.6	0.4	1.98	0.50	1.4	0.4	2.04	0.43	0.85	2.70	0.79	1.99
Chum03	T. indicus	16	F	5.46	7.03	5.00	1.37	2.13	1.80	2.54	1.21	4.53	1.32	0.48	1.46	0.63	1.57	0.47	2.06	0.52	1.52	0.43	2.09	0.55	0.91	2.95	0.89	2.75
Chum04	T. indicus	14.6	M	5.04	6.35	4.14	1.26	2.00	1.71	2.03	0.95	4.25	1.31	0.44	1.43	0.58	1.63	0.47	1.91	0.47	1.40	0.38	1.94	0.51	0.85	2.57	0.73	2.75
Chum05	T. indicus	11.8	M	4.23	5.20	3.18	1.08	1.59	1.41	1.68	0.95	3.45	0.98	0.38	1.12	0.47	1.32	0.35	1.55	0.44	1.05	0.29	1.63	0.39	0.65	2.18	0.74	2.74
Chum06	T. indicus	16.7	F	5.30	6.25	4.77	1.35	2.04	1.79	2.28	1.17	4.47	1.32	0.47	1.55	0.60	1.71	0.48	1.98	0.55	1.45	0.41	2.08	0.52	0.92	2.90	0.96	2.68
Chum07	T. indicus	17.4	M	6.11	7.90	5.55	1.53	2.37	2.06	2.74	1.30	5.03	1.52	0.52	1.64	0.68	1.83	0.49	2.21	0.66	1.68	0.50	2.25	1.58	1.03	3.15	0.95	2.45
Chum08	T. indicus	14.7	F	5.10	6.43	4.50	1.28	1.97	1.72	2.29	1.20	4.26	1.28	0.46	1.33	0.62	1.45	0.43	1.82	0.48	1.25	0.36	1.94	0.53	0.76	2.14	0.78	2.45
Chum09	T. indicus	15.6	M	5.13	6.55	4.65	1.31	2.00	1.79	2.18	1.05	4.30	1.17	0.43	1.38	0.58	1.58	0.39	2.03	0.56	1.44	0.37	2.02	0.55	0.74	2.60	0.80	2.44
Chum10	T. indicus	14.4	M	5.17	6.53	4.65	1.20	1.70	1.84	2.13	1.05	4.29	1.20	0.46	1.45	0.56	1.58	0.40	1.92	0.54	1.47	0.36	2.03	0.46	0.90	2.52	0.73	2.64
Chum11	T. indicus	17.6	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chum12	T. indicus	18.6	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chum13	T. indicus	12.8	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chum14	T. indicus	16	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chum15	T. indicus	15.3	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chum16	T. indicus	16.7	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chum17	T. indicus	14.9	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chum18	T. indicus	14.3	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chum19	T. indicus	17.6	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chum20	T. indicus	15.7	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chum21	T. indicus	15	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chum22	T. indicus	13.8	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chum23	T. indicus	14.1	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chum24	T. indicus	14.3	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chum25	T. indicus	12.9	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table A (continue)

Samples	Species	Total length (cm)	Sex	Carapace			Antenna I		Antenna II		1st abdom.		Periopod 1				Periopod 2				Periopod 3				6th abdom. seg.		Telson	
													Propodus		Merus		Propodus		Merus		Propodus		Merus					
				CL1	CW1	CW2	A1L	A1W	A2L	A2W	AL1	AW1	PL1	PW1	ML1	MW1	PL2	PW2	ML2	MW2	PL3	PW3	ML3	MW3	AL2	AW2	TL	TW
Song01	T. orientalis	19.2	F	6.35	7.10	5.75	1.48	2.44	1.91	2.58	1.40	5.46	1.5	0.6	1.7	0.7	2.00	0.59	2.24	0.70	1.74	0.51	2.50	0.65	0.96	3.39	0.99	2.87
Song02	T. indicus	21.3	M	7.00	8.30	6.30	1.74	2.95	2.41	3.08	1.5	5.7	1.92	0.70	2.18	0.80	2.5	0.6	2.81	0.72	2.2	0.6	2.85	0.70	1.33	3.86	1.03	3.20
Song03	T. orientalis	23	F	8.22	9.39	7.07	1.86	3.19	2.46	3.15	1.34	6.82	2.03	0.83	2.29	0.94	2.69	0.81	3.03	0.84	2.50	0.68	3.21	0.77	1.30	4.46	1.11	3.72
Song04	T. orientalis	26	F	8.08	9.56	7.62	2.13	3.61	2.61	3.72	1.67	7.16	2.20	0.80	2.72	0.97	2.78	0.81	3.13	0.93	2.44	0.74	3.45	0.83	1.43	4.29	1.31	3.91
Song05	T. orientalis	18.7	M	6.40	7.18	5.64	1.41	2.46	1.95	2.76	1.08	5.36	1.66	0.56	1.78	0.66	1.92	0.58	2.27	0.64	1.91	0.51	2.53	0.61	1.06	3.30	0.96	2.74
Song06	T. indicus	18.3	M	6.07	7.24	5.48	1.51	2.52	2.15	2.63	1.16	5.19	1.64	0.61	2.02	0.69	2.16	0.58	2.62	0.63	1.84	0.49	2.67	0.61	0.89	3.34	0.85	2.84
Song07	T. indicus	16.2	M	5.37	6.38	4.89	1.38	2.25	1.96	2.35	1.12	4.61	1.46	0.49	1.58	0.59	2.00	0.47	2.28	0.56	1.60	0.48	2.40	0.54	1.01	2.93	0.89	2.49
Song08	T. indicus	16.5	M	5.32	6.80	5.09	1.39	2.31	1.99	2.50	1.20	4.89	1.46	0.53	1.67	0.62	2.06	0.46	2.36	0.57	1.72	0.43	2.36	0.55	0.95	3.14	0.81	2.48
Song09	T. indicus	14.6	M	4.92	5.84	4.51	1.20	2.06	1.73	2.12	1.02	4.01	1.16	0.38	1.40	0.51	1.68	0.42	1.91	0.47	1.38	0.35	2.06	0.43	0.85	2.66	0.66	2.15
Song10	T. indicus	13.2	M	4.50	5.36	4.00	1.12	1.85	1.49	1.94	0.95	3.23	1.20	0.41	1.33	0.48	1.59	0.42	1.72	0.46	1.36	0.34	1.91	0.45	0.64	2.40	0.61	1.95
Song11	T. indicus	20.2	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Song12	T. indicus	18.7	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Song13	T. indicus	23.3	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Song14	T. indicus	22.7	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Song15	T. orientalis	21.7	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Song16	T. indicus	22.1	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Song17	T. indicus	16	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Song18	T. indicus	20.8	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Song19	T. indicus	19.5	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Song20	T. indicus	18.6	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Song21	T. indicus	16.9	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Song22	T. indicus	18.7	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Song23	T. indicus	18.9	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Song24	T. indicus	20.1	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Song25	T. indicus	20.1	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Song26	T. indicus	18.1	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table A (continue)

Samples	Species	Total length (cm)	Sex	Carapace			Antenna I		Antenna II		1st abdom.		Periopod 1 Propodus		Merus		Periopod 2 Propodus		Merus		Periopod 3 Propodus		Merus		6th abdom. seg.		Telson	
				CL1	CW1	CW2	A1L	A1W	A2L	A2W	AL1	AW1	PL1	PW1	ML1	MW1	PL2	PW2	ML2	MW2	PL3	PW3	ML3	MW3	AL.2	AW2	TL	TW
Satu01	unimaculatu	23.6	F	7.20	9.34	7.09	1.97	2.91	2.45	2.97	1.47	6.57	1.8	0.8	2	0.9	2.48	0.73	2.91	0.74	2.16	0.67	2.91	0.75	1.30	4.14	1.30	3.52
Satu02	unimaculatu	19.2	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phuk01	unimaculatu	18.8	M	6.25	8.39	5.87	1.62	2.40	2.28	2.85	1.30	5.13	1.76	0.60	1.95	0.81	2.20	0.63	2.54	0.69	1.94	0.54	2.66	0.71	0.85	3.34	0.80	2.90
Phuk02	unimaculatu	22.6	F	7.86	9.95	7.15	2.08	3.04	2.62	3.10	1.63	6.65	1.90	0.73	2.19	0.98	2.59	0.75	2.92	0.90	2.30	0.67	2.99	0.81	1.35	4.35	1.25	3.65
Phuk03	unimaculatu	17.2	M	6.22	7.88	5.60	1.47	2.29	1.95	2.54	1.37	5.15	1.55	0.56	1.68	0.75	1.86	0.52	2.35	0.62	1.76	0.46	2.48	0.55	0.85	3.16	0.95	2.58
Phuk04	unimaculatu	17.5	M	6.25	7.65	5.41	1.59	2.30	2.06	2.69	1.44	5.13	1.53	0.54	1.67	0.69	1.93	0.57	2.30	0.63	1.72	0.46	2.37	0.59	0.81	3.13	0.95	2.68
Phuk05	unimaculatu	19.6	F	6.79	8.34	6.15	1.63	2.57	2.17	2.82	1.48	5.80	1.59	0.67	1.74	0.76	2.00	0.62	2.44	0.66	1.82	0.53	2.58	0.63	0.93	3.57	1.05	2.95
Phuk06	unimaculatu	14.5	M	5.05	6.11	4.54	1.04	1.65	1.61	2.12	1.06	4.26	1.17	0.45	1.26	0.52	1.54	0.44	1.67	0.50	1.34	0.40	1.83	0.50	0.73	2.52	0.69	2.13
Phuk07	unimaculatu	20.6	M	6.87	8.63	6.15	1.75	2.51	2.29	2.60	1.52	5.85	1.65	0.61	1.98	0.78	2.07	0.65	2.68	0.67	1.91	0.50	2.64	0.66	0.94	3.58	1.10	3.02
Phuk08	unimaculatu	17.6	M	6.15	7.57	5.50	1.55	2.27	2.03	2.61	1.40	5.07	1.66	0.55	1.65	0.73	2.00	0.58	2.43	0.62	1.83	0.49	2.44	0.61	0.77	3.13	0.89	2.58
Phuk09	unimaculatu	21.4	F	7.4	8.9	6.5	1.7	2.7	2.4	2.9	1.5	6.2	1.7	0.7	2.1	0.9	2.2	0.7	2.7	0.8	2	0.5	2.6	0.7	1.1	3.9	1.1	3.3
Phuk10	unimaculatu	16.7	M	6	7.4	5.1	1.5	2.1	1.9	2.5	1.3	4.9	1.5	0.6	1.6	0.6	1.8	0.5	2.1	0.6	1.6	0.5	2.2	0.6	0.9	3.1	0.9	2.5
Phuk11	unimaculatu	22.1	F	7.4	9.2	6.6	1.8	2.7	2.4	3.3	1.7	6.2	1.9	0.7	1.9	0.8	2.5	0.7	2.7	0.8	2.2	0.6	2.9	0.7	1.2	4	1.1	3.2
Phuk12	unimaculatu	18.6	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phuk13	unimaculatu	19.9	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phuk14	unimaculatu	16	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phuk15	unimaculatu	22.6	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phuk16	unimaculatu	17.6	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phuk17	unimaculatu	18.2	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phuk18	unimaculatu	21.3	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phuk19	unimaculatu	20.3	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phuk20	unimaculatu	20.4	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phuk21	unimaculatu	21.6	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phuk22	unimaculatu	16.6	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phuk23	unimaculatu	18.9	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phuk24	unimaculatu	20.5	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phuk25	unimaculatu	17.6	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phuk26	unimaculatu	19.6	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phuk27	unimaculatu	16.7	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phuk28	unimaculatu	21.9	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table A (continue)

Samples	Species	Total length (cm)	Sex	Carapace			Antenna I		Antenna II		1st abdom.		Periopod 1				Periopod 2				Periopod 3				6th abdom. seg.		Telson	
													Propodus		Merus		Propodus		Merus		Propodus		Merus					
				CL1	CW1	CW2	A1L	A1W	A2L	A2W	AL1	AW1	PL1	PW1	ML1	MW1	PL2	PW2	ML2	MW2	PL3	PW3	ML3	MW3	AL2	AW2	TL	TW
Rano01	T. indicus	17.2	M	5.71	6.72	5.01	1.52	2.50	2.07	2.60	1.11	4.79	1.4	0.5	1.6	0.6	1.92	0.48	2.24	0.58	1.61	0.38	2.39	0.57	1.03	2.89	0.81	2.49
Rano02	T. indicus	15.2	M	4.95	5.64	4.38	1.33	2.02	1.84	2.16	1	4.2	1.22	0.42	1.41	0.53	1.6	0.4	2.04	0.46	1.4	0.4	2.00	0.40	0.79	2.68	0.67	2.07
Rano03	T. indicus	16.8	M	5.90	6.23	5.06	1.46	2.40	2.01	2.55	1.26	4.67	1.43	0.54	1.60	0.65	1.91	0.51	2.27	0.58	1.59	0.48	2.41	0.60	0.89	3.00	0.79	2.51
Rano04	T. indicus	16.6	M	5.83	6.96	5.08	1.50	2.38	1.89	2.48	1.30	4.64	1.32	0.47	1.54	0.64	1.76	0.48	2.25	0.59	1.39	0.41	2.34	0.62	0.94	2.92	0.75	2.52
Rano05	T. indicus	17.6	M	5.92	7.00	5.25	1.53	2.30	2.08	2.50	1.35	4.95	1.56	0.53	1.66	0.65	1.99	0.50	2.27	0.60	1.58	0.45	2.28	0.56	0.94	3.11	0.85	2.61
Rano06	T. indicus	20.2	F	6.80	8.27	5.90	1.75	2.70	2.16	3.02	1.25	5.51	1.71	0.58	1.73	0.72	2.18	0.59	2.57	0.68	1.74	0.59	2.67	0.56	1.24	3.45	0.98	2.93
Rano07	T. indicus	17.3	M	5.70	6.10	4.97	1.53	2.39	2.02	2.52	1.08	4.86	1.46	0.47	1.64	0.63	1.95	0.49	2.36	0.55	1.55	0.40	2.39	0.55	1.00	3.03	0.83	2.52
Rano08	T. indicus	18	M	5.90	6.60	5.21	1.68	2.55	2.08	2.70	1.25	4.99	1.49	0.55	1.79	0.77	2.01	0.54	2.46	0.71	1.67	0.44	2.45	0.57	0.83	3.22	0.80	2.70
Rano09	T. indicus	23	F	6.71	8.13	5.78	1.78	2.76	2.56	2.85	1.49	5.46	1.68	0.55	1.96	0.73	2.28	0.59	2.61	0.70	1.79	0.56	2.80	0.68	1.05	3.72	0.85	2.99
Rano10	T. indicus	21.4	M	7.36	9.03	5.80	1.74	3.16	2.70	3.11	1.44	5.69	1.88	0.70	1.93	0.90	2.47	0.71	2.76	0.83	2.04	0.58	2.90	0.66	1.13	3.78	0.99	3.27
Rano11	unimaculatu	18.3	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rano12	T. indicus	18.1	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rano13	T. indicus	19.2	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rano14	T. indicus	16.7	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rano15	T. indicus	22.4	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rano16	T. indicus	22.8	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rano17	T. indicus	20.1	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rano18	T. indicus	19.6	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rano19	T. indicus	19.8	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rano20	T. indicus	18.5	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rano21	T. indicus	20.2	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rano22	T. indicus	20.9	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

BIOGRAPHY

Mr. Apinan Iamsuwansuk was born on January 8th, 1987 in Bangkok province. He completed his high school from Wat Suthiwararam School, Bangkok, in 2005. He received Bachelor of Science degree in Biotechnology with first class honour from Department of Biotechnology, Faculty of Science and Technology, Thammasat University, Pathum Thani, in 2009. He has taken Master of Science degree in the programme of Zoology from Department of Biology, Faculty of Science, Chulalongkorn University, since 2009.

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