ความหลากหลายทางพันธุกรรมของปลาจุมพรวด *Boleophthalmus boddarti*
ในอ่าวไทยโดยใช้คอนโทรลรีเจียน

นายเผชิญสุข ธีระนุกูล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาวิทยาศาสตร์ทางทะเล ภาควิชาวิทยาศาสตร์ทางทะเล
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2558

GENETIC DIVERSITY OF BLUE SPOTTED MUDSKIPPER *Boleophthalmus boddarti* POPULATIONS IN THE GULF OF THAILAND USING CONTROL REGION

Mr. Pachoensuk Theeranukul

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Marine Science
Department of Marine Science
Faculty of Science
Chulalongkorn University
Academic Year 2015

Thesis Title	GENETIC DIVERSITY OF BLUE SPOTTED MUDSKIPPER *Boleophthalmus boddarti* POPULATIONS IN THE GULF OF THAILAND USING CONTROL REGION
By	Mr. Pachoensuk Theeranukul
Field of Study	Marine Science
Thesis Advisor	Assistant Professor Sanit Piyapattanakorn, Ph.D.
Thesis Co-Advisor	Jes Kettratad, Ph.D.

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

.....Dean of the Faculty of Science
(Associate Professor Polkit Sangvanich, Ph.D.)

THESIS COMMITTEE

.....Chairman
(Associate Professor Voranop Viyakarn, Ph.D.)

.....Thesis Advisor
(Assistant Professor Sanit Piyapattanakorn, Ph.D.)

.....Thesis Co-Advisor
(Jes Kettratad, Ph.D.)

.....Examiner
(Associate Professor Charoen Nitithamyong, Ph.D.)

.....External Examiner
(Assistant Professor Jenjit Khudamrongsawut, Ph.D.)

เผชิญสุข ธีระนุกูล : ความหลากหลายทางพันธุกรรมของปลาจุมพรวด *Boleophthalmus boddarti* ในอ่าวไทยโดยใช้คอนโทรลรีเจียน (GENETIC DIVERSITY OF BLUE SPOTTED MUDSKIPPER *Boleophthalmus boddarti* POPULATIONS IN THE GULF OF THAILAND USING CONTROL REGION) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร. ศานิต ปิยพัฒนากร, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ดร. เจษฎ์ เกษตระทัต, 71 หน้า.

ศึกษาโครงสร้างทางพันธุกรรมของปลาจุมพรวด *Boleophthalmus boddarti* ในอ่าวไทย โดยวิเคราะห์จากลำดับนิวคลีโอไทด์จำนวน 320 ตำแหน่ง บริเวณคอนโทรลรีเจียน ในไมโทคอนเดรีย ของตัวอย่างปลาทั้งหมด 179 ตัวอย่าง จาก 6 สถานี คือ ระยอง ฉะเชิงเทรา สมุทรสงคราม เพชรบุรี นครศรีธรรมราช และปัตตานี พบว่ามีรูปแบบทางพันธุกรรมทั้งหมด 56 รูปแบบ และมีตำแหน่งเบสที่มีความแปรปรวนอยู่ทั้งหมด 60 ตำแหน่ง รูปแบบพันธุกรรม H01 เป็นรูปแบบร่วมที่พบมากที่สุด โดยพบทั้งหมด 88 ตัวอย่าง จากตัวอย่างปลาทั้งหมด 179 ตัวอย่าง โดยความหลากหลายเฉลี่ยของแฮปโพลไทป (*h*) มีค่า 0.7879 ± 0.0676 และความหลากหลายเฉลี่ยของนิวคลีโอไทด์ (**TT**) มีค่า 0.004899 ± 0.003389 เมื่อวิเคราะห์โครงสร้างพันธุกรรมของประชากรด้วย AMOVA พบว่าไม่มีโครงสร้างพันธุกรรมระหว่างประชากรจากอ่าวไทยตอนบน ได้แก่ จังหวัดระยอง ฉะเชิงเทรา สมุทรสงคราม เพชรบุรี และ อ่าวไทยตอนล่าง ได้แก่ จังหวัดนครศรีธรรมราช ปัตตานี (F_{CT} = 0.02461, p > 0.05) แต่พบความแตกต่างทางพันธุกรรมระหว่างประชากรในแต่ละกลุ่ม (F_{SC} = 0.05474, p <0.001) และภายในประชากร (F_{ST} = 0.07800 p <0.001) เมื่อนำค่าระยะห่างทางพันธุกรรมของแต่ละประชากรมาสร้าง แผนผังแสดงความสันพันธ์ทางพันธุกรรม พบว่าประชากรปลาจุมพรวดในอ่าวไทยรูปตัว ก รวมอยู่ในกลุ่มเดียวกัน คือ ฉะเชิงเทรา สมุทรสงคราม เพชรบุรี แต่ภาพรวมของโครงสร้างทางพันธุกรรมทั้งหมดยังไม่ชัดเจน จากการสร้างแผนภูมิการกระจายตัวของแฮปโพลไทป์จะเห็นว่าแฮปโพลไทป์ของปลาตีนในอ่าวไทยแบ่งออกเป็นนัยได้ 2 กลุ่ม คือกลุ่มแรกที่เป็นกลุ่มหลัก พบปลาจุมพรวดจากทุกประชากร ซึ่งส่วนใหญ่มีรูปแบบพันธุกรรม H01 กลุ่มที่สองคือ กลุ่มที่รูปแบบพันธุกรรมส่วนใหญ่พบในประชากรจากอ่าวไทยตอนล่างและระยอง เมื่อทดสอบความสัมพันธ์ระหว่างระยะห่างทางพันธุกรรมและระยะห่างระหว่างพื้นที่ด้วย Mantel's test พบว่าไม่มีความสัมพันธ์ระหว่างระยะห่างทั้งสองอย่างมีนัยสำคัญ (p>0.05) จากผลการศึกษาข้างต้นไม่พบโครงสร้างพันธุกรรมที่ชัดเจนของประชากรปลาจุมพรวดในอ่าวไทย หากมีการวางแผนจัดการประชากรปลาจุมพรวดในพื้นที่นี้ ควรทำจัดการเป็นแบบประชากรเดียวกันทั้งอ่าวไทย

ภาควิชา วิทยาศาสตร์ทางทะเล ลายมือชื่อนิสิต .....

สาขาวิชา วิทยาศาสตร์ทางทะเล ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2558 ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

5572050023 : MAJOR MARINE SCIENCE
KEYWORDS: BLUE-SPOTTED MUDSKIPPER, GENETIC DIVERSITY, GENETIC STRUCTURE, CONTROL REGION, THE GULF OF THAILAND

PACHOENSUK THEERANUKUL: GENETIC DIVERSITY OF BLUE SPOTTED MUDSKIPPER *Boleophthalmus boddarti* POPULATIONS IN THE GULF OF THAILAND USING CONTROL REGION. ADVISOR: ASST. PROF. SANIT PIYAPATTANAKORN, Ph.D., CO-ADVISOR: JES KETTRATAD, Ph.D., 71 pp.

Genetic structure of blue-spotted mudskipper (*Boleophthalmus boddarti*) in the Gulf of Thailand was investigated, based on 320 base pairs of partial control region in mitochondrial DNA. A total of 179 samples were collected from 6 locations in the Gulf of Thailand, namely Rayong (RY), Chachoengsao (CH), Samut Songkram (SS), Petchburi (PB), Nakorn Sri Thammarat (NK), and Pattani (PT). There were 56 haplotypes, of which haplotype01 (H01) was the common haplotype, found in 88 samples from all locations. The numbers of variable sites were 60. Average haplotype diversity (*h*) and nucleotide diversity (**TT**) were 0.7879 ± 0.0676 and 0.004899 ± 0.003389, respectively. AMOVA results showed that there was no significantly genetic differentation between the populations in the upper (Rayong, Chachoengsao, Samut Songkram, Petchburi) and lower (Nakorn Sri Thammarat, and Pattani) Gulf of Thailand (F_{CT} = 0.02461, p > 0.05). However, there were significant difference between the populations within group (F_{SC} = 0.05474, p <0.05.) and individuals within population (F_{ST} = 0.07800 p <0.01). Dendogram, generated from pairwise genetic distances, indicated the group of the populations from the inner Gulf of Thailand, consisting of Chachoengsao, Samut Songkram, and Petchburi, but the genetic structure of all populations was unclear. Network diagram of Median joining haplotype can classified into two groups. Group I contained haplotypes that found in all populations, including the common haplotype H01 that can be detected in the samples from all sampling sites, while most of member in group II were from the lower Gulf of Thailand and Rayong. For Mantel test, there was no correlation between genetic and geographical distances (p>0.05). The result showed no genetic structure of the Blue-spotted mudskipper in the Gulf of Thailand which should be the result of larval dispersal. Therefore, as far as the management plan of this fish species in the Gulf of Thailand is concerned, the species should be treated as single stock.

Department:	Marine Science	Student's Signature	.....
Field of Study:	Marine Science	Advisor's Signature	.....
Academic Year:	2015	Co-Advisor's Signature	.....

ACKNOWLEDGEMENTS

I would like to thank my advisor, Assistant Professor Sanit Piyapattanakorn (Ph.D), and co-advisor, Jes Kettratad (Ph.D), for their support, encouragement and help throughout the duration of this project.

In addition, I would also thank my thesis committee: Associate Professor Voranop Viyakarn (Ph.D), Associate Professor Charoen Nitithamyong (Ph.D), and Assistant Professor Jenjit Khudamrongsawat (Ph.D) for their insightful comments, and useful questions.

I would like to thanks the Development and Promotion of Science and Technology Talents Project (DPST scholarship) for financial support for my thesis and opportunity for short time collaboration in School of Marine Bioscience, Kitasato University, Kanagawa, Japan for 6 months between February to August 2015.

My sincere thanks also go to Professor Shugo Watabe (Ph.D), Junior Associate Professor Daisuke Ikeda (Ph.D), and Associate Professor Shaheed Reza (Ph.D), in school of Marine Bioscience, Kitasato University, Kanagawa, Japan, for their suggestion useful guidance and support of the chemical reagents and automated sequencer.

Thanks to my colleagues and local fishermen for their help to collect the mudskippers in this study.

Finally, I gave special thanks to my family who really deserve my appreciation for their full support, encouragement and inspiration throughout these years.

CONTENTS

Page

Page

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A	Adenine
AMOVA	Analysis of molecular variance
bp	Base pair
C	Cytosine
D1	First dorsal fin
D2	Second dorsal fin
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
EDTA	Ethylenediaminetetraacetic acid
g	Gram
G	Guanine
mg	Milligram
MgCl2	Magnesium Chloride
ml	Milliliter
mm	Millimeter
mM	Millimolar
mtDNA	Mitochondrial DNA
NaCl	Sodium Chloride
PCR	Polymerase chain reaction
PEG	Polyethelene glycol
RNA	Ribonucleic acid
rpm	Revolution per minute
SDS	Sodium dodecyl sulfate

T	Thymine
TBE buffer	Tris/Borate/EDTA buffer
TE buffer	Tris/EDTA buffer
TNES buffer	Tris/NaCl /EDTA/SDS buffe
Tris	Tris (hydroxyl methyl) aminomathane
U	Unit
µl	Microliter
UV	Ultraviolet
v/v	Volume by volume
w/v	Weight by volume

CHAPTER 1
INTRODUCTION

1.1 Introduction

The blue-spotted mudskipper (*Boleophthalmus boddarti*) has many physiological, morphological, and behavioral adaptations to live in mangrove areas (Swanson & Gibb, 2004). Its muscles and pectoral fins are adapted to move effectively on the mud flat. The gill chambers can tightly be closed when the mudskipper is above water to keep the gill moist. With this method, they can use oxygen while they are on mud flats during low tide period (Graham, 1997). The fish can also be used as a bio-indicator for water quality and toxin in the mangrove (Ansari et al, 2014). *B. boddarti* can generally be found along the coasts of the Gulf of Thailand and Andaman Sea (Darumas, 1997). It spends most of its life in the mud flat and river mount, especially mangrove areas. Therefore, it has been defined as the true-resident in the mangrove (Clayton, 1993). As far as the life cycle of mudskipper is concerned, the breeding season of *B. boddarti* has been reported. It started from August to October (Quang et al, 2016). The eggs are laid in their burrows (Sasekumar et al, 1994) and larvae spawn during monsoon periods when the pH level and salinity are reduced, and the primary product is increased (Clayton, 1993). The planktonic larvae disperse and develop to juvenile in the water column. After that, they will settle on the mud flats and mangrove areas and become the recruits of the populations in the areas. Although the water current can facilitate the dispersal of the larvae, it can also play an important role as physical barrier on larval migration. Therefore, the water currents can have an effect on the distribution of the species since the larvae spend approximately 35 days in the water column (Koga et

al, 1989a). The other effect threatening on mudskipper populations can be the loss of their habitats. Almost fifty percent of mangrove areas in Thailand were invaded by anthropogenic activities, especially in Gulf of Thailand. The reduction of the mangrove areas may cause the distribution of the mangrove. As the result, the fragmentation of the mangrove may affect the genetic variation and structure of the species.

Population connectivity of marine organisms is mostly dependent on dispersal and migration ability of the organisms and their planktonic larvae. It can play a significant role in the intraspecific genetic diversity, genetic structure, and the evolutionary and ecological processes of the populations (Bowen et al, 2006; Shulman, 1998; Weersing & Toonen, 2009). An accurate population structure is important for the management of marine fish populations (Gjedrem et al, 1988; Hindar et al, 1991; Ryman & Utter, 1987). The miss identification on population units can cause local overfishing and ultimately severe declining of the populations (Waples, 1998). For the mudskippers, many morphological, physiological, and ecological studies on the species have been carried out (Clayton, 1993; Clayton & Vaughan, 1988; Murdy, 1989; Ravi, 2013; Ravi & Rajagopal, 2007). There were few reports on genetic diversity and structure of their populations which suggested that the dispersal patterns of larvae may be limited by water current, and habitat distances (Chen et al, 2015; Ramanadevi et al, 2013). In the mudskippers, juveniles and adults are likely be a permanent residence in their habitats after the settling (Hong et al, 2007; Murdy, 1989), while the adults of other fishes have higher migratory ability. Therefore, gene flow between populations of mudskipper should be mostly dependent on the dispersal ability of their pelagic larvae (Chen et al, 2015).

In this study, the genetic diversity and structure of the mudskipper, *B. boddarti,* in the Gulf of Thailand were examined using D-loop in mitochondrial genome. The results from this study provided basic knowledge in population dynamics and structure of the species since the life history of the mudskipper is different from other fishes.

1.2 Objective

1. To investigate the genetic diversity of Blue-spotted mudskipper (*B. boddarti*) populations in the Gulf of Thailand using mitochondrial DNA
2. To determine genetic structure of blue-spotted mudskipper (*B. boddarti*) populations in the Gulf of Thailand using mitochondrial DNA

CHAPTER 2
LITERATURE REVIEW

2.1 Blue-Spotted Mudskipper

2.1.1 Taxonomy of the Blue-Spotted Mudskipper

Blue-Spotted mudskipper (Figure 2.1) has been classified in Kingdom Animalia, Phylum Chordata, Class Actinopterygii, Order Perciformes, and Family Gobiidae. All of mudskippers were grouped in sub-family Oxudercinae. The blue-spotted mudskipper was specified in the genus Boleophthalmus, and its scientific name is *B. boddarti* (Pallas, 1770)

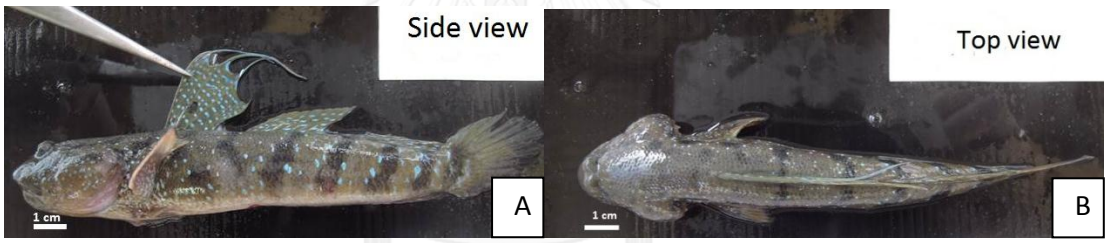


Figure 2.1 The Blue-spotted mudskipper, *Boleophthalmus boddarti* (Pallas, 1770) (A) Side view (B) Top view

Boleophthalmus depict their eyes that are ejected out their orbit. Their eyes above the level of their orbital cavities: from bole (ejected) and ophthalmon (eye) (Cuvier & Valenciennes, 1829). Moreover, the specific name is the name of Pierre Boddaer, who collected the specimen for the original description (Pallas, 1770). The genus can be characterized by the thick epidermis, especially in their heads and dorsal parts covered by the small dermal papillae. Their pelvic fins were fused with each other (Swennen et al, 1995). In addition, the pelvic fins have the intercleithral cartilage spaning along the width of the fins. The marginal pectoral fins are black (Murdy, 1989; Polgar & Crosa, 2009). There is no barbel on underside of their head.

Fine compressed teeth are found on the lower jaw and a single row of teeth is found on the upper jaw. There is a recurved symphyseal canine tooth on each side on lower jaw. Their first dorsal fin or D1 have 5 spines (schematic of mudskipper show in (Figure 2.2). The middle of D1 spine is the longest. There are 21-27 elements in D1 and 19-27 elements in the anal fin. The pectoral fin rays are about 17-21. The first of second dorsal fin (D2) element is soft spine. The other elements are usually segmented and branched (Darumas, 1997). Swennen et al (1995) reported that the sexual dimorphism was found on this fish species. The third of D1 spine in the female is longer than the male. On the other hand, Ansari et al (2014) reported that the mudskippers are sexually monomorphic. The longitudinal scales on their body are around 67-106; transverse scales are 16-33; and predorsal scales are 25-44 (Darumas, 1997). Their colors are greenish-brown in the dorsal and lateral of the bodies. The ventral color is bright grey, white, or pale yellow, but darker in the head and tail areas. The distinct characteristic of this fish is the iridescent blue spots on their brown body skin, dorsal fins, especially on the latero-ventral origin of pectoral fins. The pectoral fins are yellow to orange and its border is black. The muscle of pectoral fin base is brown and scattered of blue spots. There is the black stripe which present from the anterior nostril to the operculum (Murdy, 1989). Moreover, there are 7 diagonal black stripes in each lateral site; the first at D1 base, the second between D1 and D2, the third to the fifth under the D2 base, the sixth at posterior end of D2 base, and the seventh near the caudal fin (Darumas, 1997).

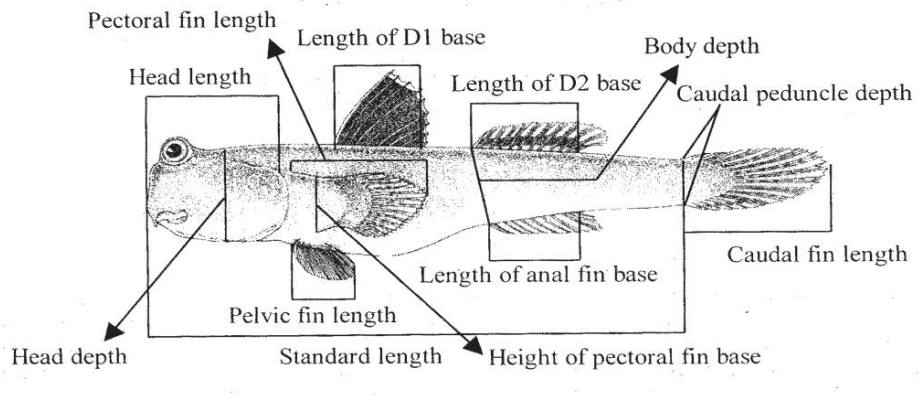


Figure 2.2 The schematic drawing of a mudskipper explains structure and measurements (Darumas & Tantichodok, 2002)

2.1.2 Adaptation

The blue-spotted mudskipper (*B. boddarti*) is a member of family Gobiidae, sub-family Oxudercinae. It is an amphibious fish, which can live in the intertidal mud flat, river mount, especially in the mangrove. While the other fish can survive the retreat of the water by live in tide pools, mudskipper can emerge from water to feed, defend territories and show the courtship behavior during the low tide (Clayton, 1993). Therefore, there are many physiological, morphological, and behavioral adaptations for the mudskipper.

2.1.2.1 Movement Adaptation

There are the unique physiological and behavior specializations that make the species to move effectively on the mud flat. Their pelvic fins are synchronized with each other and served as a paddle to walk on the mud flat (Swanson and Gibb 2004). The movement on mud flat is facilitated by their appendicular skeleton and muscular adaptations. Their abductor

superficialis muscle's form is adapted for greater control and flexibility of the limb during terrestrial forays. Moreover, they can flip and jump themselves into the air (Piper, 2007). There are 4 modes of locomotion of this fish a part from swimming, namely crutching, skipping, skimming, and climbing. Crutching is the locomotion for taking their foods. Skipping and skimming are used to escape their predators. Climbing is the movement for attaining higher level in the mangrove forest (De & Nandi, 1984).

2.1.2.2 Anoxic Environment Adaptation

The mudskipper's breathing system has been adapted to stay in the intertidal zone. They can breathe through their skin, the buccal-branchial cavity, but their skin is needed to be wet. This mode of breathing is similar to the amphibian, called "cutaneous air breathing" (Graham, 1997). In some species of mudskippers, their buccal-branchial cavity volume is 2-6% larger than cavity volume of gobies that are not specified as amphibious fish (Gee & Gee, 1991). Another importance adaptive breathing mode is their special gills. The species can breathe, while they are out of water using these gills. These gill chambers are tightly closed when the mudskippers stay out of water. This can make the fishes keeping the gill moist and can use oxygen, while they are on lands (Graham, 1997).

2.1.2.3 Euryhaline Adaptation

The species can adapt themselves to tolerate the dramatically change of environmental conditions. The blue-spotted mudskipper was classified to be the euryhaline fish. Chew and Ip (1990) reported that the blue-spotted

mudskipper can live in the wide range of salinity changing the water content in their muscles to stabilize the ion concentration in their blood (Clayton, 1993).

2.1.2.4 Vary Temperature Adaptation

The temperature can be a major problem for this fish since they stay mostly out of water. However, the mudskippers use thermoregulatory behavior, evaporating process, and body color change to regulate their body's temperature (Tytler & Vaughan, 1983).

2.1.2.5 Predators

Predators are also a big problem for the blue-spotted mudskippers. They are eaten by several predators, such as sea snakes, cat fish, stone fish, marine bird, aquatic bird and reptiles (Clayton, 1993). Tytler and Vaughan (1983) reported that the mudskipper deep borrows in the soft sediments allow the mudskipper to thermoregulate. In addition, they can avoid theire predators during the high tide when their burrow are submerged (Sasekumar et al, 1994) and laying their eggs.

2.1.3 Reproduction and Life Cycle

The courtship behavior of this fish is very interesting. The male jumps to the female. After that the male waves its fin and tail for showing. The female goes to his egg-chamber room in the male hole and lays their eggs. Although, there is the complex courtship behavior (male-territory-visiting), but Takegaki (2008) reported that

the *Boleophthalmus pectinirostris*, the same genus of blue-spotted mudskipper, is polygamy. In Japan, the mudskipper (*Periophthalmus modestus*) produces J shaped burrows that have two or three openings on mudflat surface. The brooding chamber is located at the end of the burrow (Figure 2.3) (Dotsu & Matoba, 1977). After that the male guard its burrow for about 1 week or until the eggs hatch (Ishimatsu et al, 2007) . The male mudskipper deposits the air into the chamber by mouth during low tide. This method can protect the brood chamber from the hypoxia. When the eggs are completely developed, the male will remove the air from the chamber and release the pelagic larva to the water mass on the nocturnal rising tide. This flood induces eggs hatching (Ishimatsu et al, 2007) within 6-9 days (Koga et al, 1989b). The breeding seasons are August to October (Quang et al, 2016). This indicates that the fish spawn during monsoon periods when the pH level and salinity are reduced, and the primary product is increased (Clayton, 1993). The degree of hatching success depends on many factors, for example, the temperature and the salinity. The optimum temperature and salinity for hatching were 28 degree of Celsius and 15-25 ppt, respectively ((Zhang et al, 1989)quoted in(Clayton, 1993)). After the eggs hatched, the pelagic larva will stay in the water mass. The periods of pelagic larva are 14 days to more than 200 days dependent on the species (Clayton et al, 1994). The periods of being pelagic larva in *Boleophthalmus pectinirostri*, closely related species to blue-spotted mudskipper, are 35 days (Koga et al, 1989a).

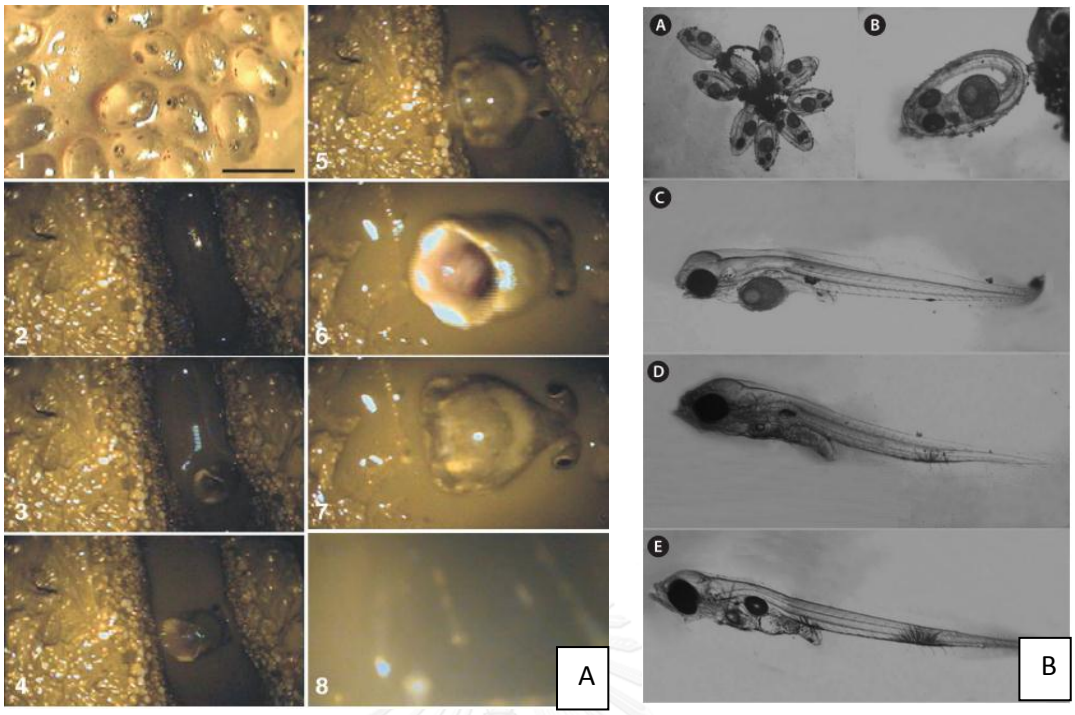


Figure 2.3 The brood chamber (A) (Ishimatsu et al, 2007) and the stages of pelagic larva of mudskipper (B) (Kim et al, 2011)

2.1.4 Feeding behavior

The blue-spotted mudskippers actively feed on the mudflats during the low tide. On the other hand, they remain in their burrows hiding themselves from their predators during the high tide (Milward, 1974). Previous investigations indicated that mudskippers are herbivores (Murdy, 1989) or carnivores (Colombini et al, 1996). The blue-spotted mudskipper feed on the variety of foods (Ravi, 2013). The main food item of the blue-spotted mudskipper is diatom. However, they also consume the other food item such as nematodes, polychaetes, algae, and fish eggs. Moreover, the detritus, mud, and sand particles are found in their stomach as well. This indicated that the blue-spotted mudskipper is the herbivore. The ratio of their foods varies by their age stage, and season. For example, the juvenile fish stomach contant contained more diatoms than adults (Ravi, 2013). It grazes on benthic flora on the

mud flat; takes mud into its mouth, keep the algal or diatom, and blow out the mud. Sometimes, they feed benthic green algae in the mangrove area especially in the pneumatophore zone. The bigger one occupies near the water and the smaller one occupies further up on the dry zone (pneumatophore zone). On the other hand, the mudskipper larva take the particulate organic detritus of the benthic diatom that was decomposed by nature (Qiyong et al, 1988).

2.1.5 Distribution

The mudskippers are widely distributed in tropical and sub-tropical areas (Murdy, 1989). The blue-spotted mudskipper is mostly found in Indo-Pacific Ocean from the West coast of India Ocean to the North of Borneu Island, Sabah, Indonesia and East Malaysia. It can also be found in the southern part of Vietnam (Murdy, 1989). In Thailand, 4 genus of mudskippers were found including *Scartelaos sp*., *Periophthalmodon sp.*, *Periophthalmus sp*., and *Boleophthalmus sp*. The blue-spotted mudskipper is a common species in Thailand, found along the coasts of the Gulf of Thailand and Andaman Sea, especially on the southern part of the Gulf, namely Chumphon, Surat Thani, Nakhon Si Thammarat, Songkhla, Pattani, Trang, Satun, Krabi, Phangnga, Phuket, and Ranong (Darumas, 1997).

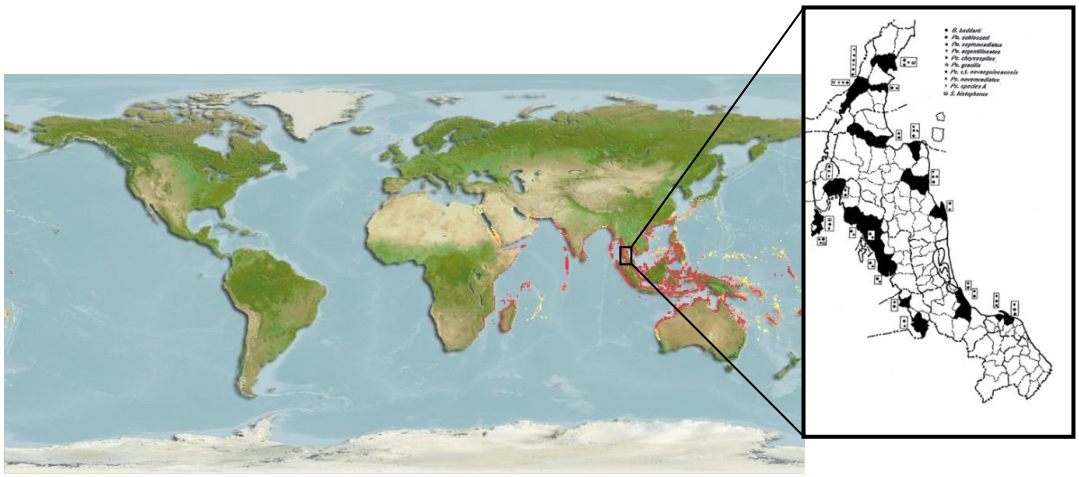


Figure 2.4 The distribution of the Blue-spotted mudskipper (*B. boddarti*) around the world and in the southern part of Thailand (Darumas, 1997) (http://www.fishbase.org/summary/Boleophthalmus-boddarti.html)

Murdy (1989) reviewed the mudskippers, including the distribution. Some species (Figure 2.4) continuously distribute along the coastal area, for instance, *Parapocrytes rictuosus* (Valenciennes), which can be only found in the east coast of India. However, some species were separated by geographical barrier and long distance, including *Parapocryptes seperaster* (Richardson) that are found on the East of China, Indonesia, Philippines (Herre, 1953) and Sri Lanka (Koumans, 1953). For the genus Boleophthalmus (Murdy, 1989), the distribution of *B. caeruleomaculatus* and *B. birdsong*, is continuous along the coast of Australia, while *B. Boddarti* and *B. pectinirostris* were grouped as patchy distribution from the mainland and some islands in the sub-tropical zone and tropical zone, respectively. In the South of Thailand, Darumas (1997) normally found the of *B. boddarti* both site of Thailand. In addition, Chen et al (2015) reported the new recorded area of *B. pectinirostris* in Malaysia (Figure 2.5).

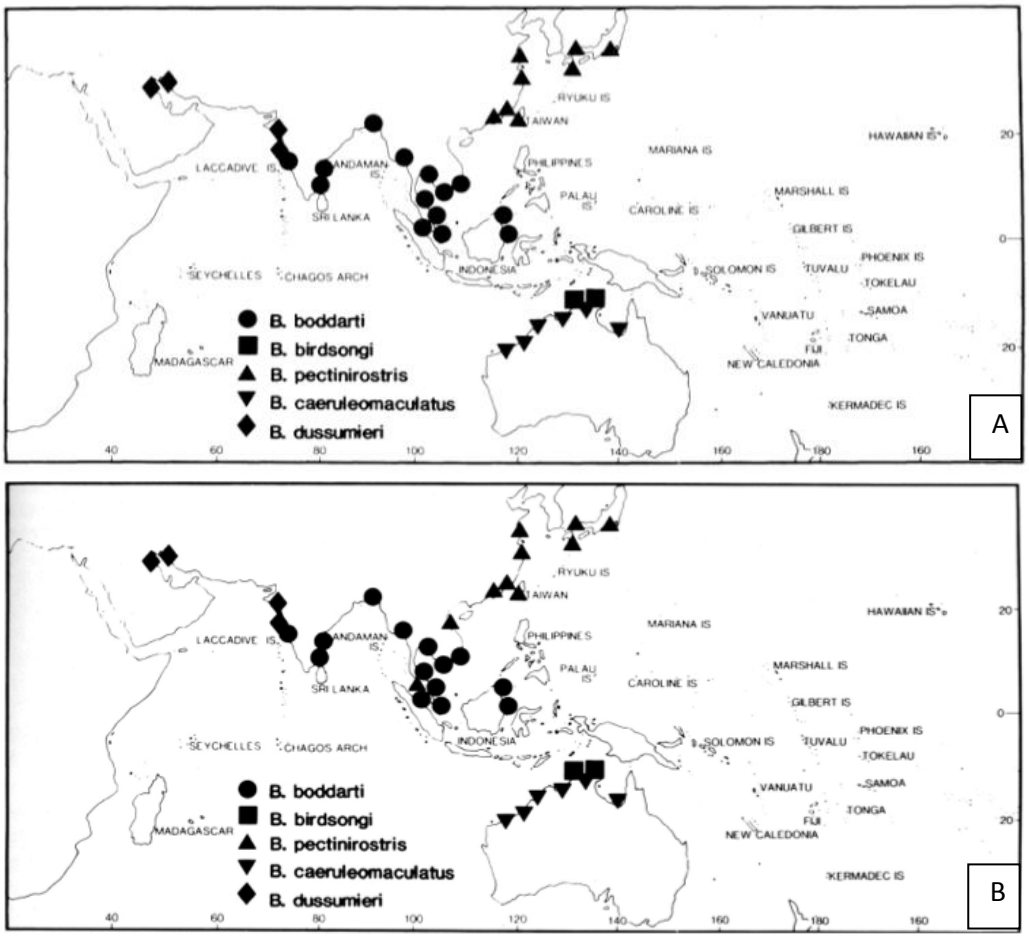


Figure 2.5 Population distribution of Boleophthalmas from known localities based on examined material (A; Murdy (1989)) (B; Chen et al (2015))

2.1.6 Genetic Structure of Mudskipper

High dispersal ability is a common skill of marine organisms, including, gametes shed into the water, larvae released from brooding females, high dispersal adults. The movement of hundreds or thousands of kilometers is a key of life history feature. High gene flow among population is thought to limit opportunities for genetic divergence and to inhibit species formation. Successful larval transport implies gene flow. Moreover, geographic distance and speciation relate the gene flow for population genetics (Palumbi, 1996a). Genetic structure of marine fishes have revealed high population connectivity within ocean basins, consistent with the

assumption that pelagic larvae disperse long distances by oceanic currents (Bowen et al, 2006).

For the population of mudskippers, the investigations on genetic structures were carried out in many species, especially in Boleophthalmus. The various molecular markers were used, for instant Random Amplification of Polymorphic DNA (RAPD) (Ramanadevi et al, 2013), Amplified Fragment Length Polymorphism (AFLP) (Chen et al, 2014), and DNA sequencing (Chen et al, 2015; Kanemori et al, 2006). *Boleophthalmus pectinirostris* populations were studied in area for example North Pacific Ocean. The most studies of *B. pectinirostris* showed the genetic differentiation between China population and Korea & Japan populations because these populations have been separated from China population since the late Pleistocene period (Chen et al, 2014; Chen et al, 2015; Kanemori et al, 2006). However, Liu et al (2009) reported that there was no genetic structure of *B. pectinirostris* in Yangtze River in China because there was no obviously geographical and physical barrier in this area.

2.1.7 Factors Affect the Genetic Structure of Mudskippers

2.1.7.1 Habitat loss (Mangrove Deforestation)

Mangrove is very important ecosystem in coastal area. This ecosystem plays important roles in coastal protection, coastal fishery, and providing timber and other natural products. However, the mangrove deforestation is now increasing and mostly caused by human activities, including shrimp farm, mining, building the ports and dams, and industries. In 2010, there was a report suggested that the mangrove areas around the world will reduce to 50 percent in the 20th century (Polidoro et al, 2010). In Thailand, the mangroves

were decreased more than 54.49 percent in the last 25 years, from 3,679 square kilometers in 1951 to 2,440 square kilometers in 2009 (Figure 2.6 and Table 2.1). This situation can affect the number of flora and fauna inhabiting in the mangrove, including the mudskippers, in term of habitat loss (Whitmore & Sayer, 1992).

2.1.7.2 Current in the Gulf of Thailand

The Gulf of Thailand (GOT) is located on the Eastern part of Thailand. Its width and length is 400 kilometer and 720 kilometer, respectively (rectangular shape). It is quite shallow, with a maximum depth of 80 meters, and semi-enclosed bay. The upper Gulf of Thailand (UGOT) is located at the Northern most end of the Gulf, and occupies about 10,000 square kilometer. GOT currents are mainly affected by the Northeast (NE) and Southwest (SW) monsoons. In each season, its current is different pattern (Figure 2.7).

The ocean current affects to physical parameters that control the water exchange and dispersion of organic and inorganic matters in the water column. It is very important for enhancement of primary productivity (Sojisuporn et al, 2010). Moreover, the prevalent current would help to know better understanding of the organisms' behaviors, in term of, the distribution pattern. Transport of larvae is an important role of the ocean currents. This mechanism force ichtyoplankton larval transportation and retention into the estuary. For the lagoon of Mexican Caribbean, the main mechanism of planktonic fish larvae transportation from spawning grounds to nurseries and control recruitment variability are coastal and estuarine circulation and tidally

Table 2.1 The mangrove area in Thailand between 2000 and 2009 (+ showed increased area, while - showed decreased area from 2000 to 2009)

Provinces	Coastal side	Mangrove area (Rai)		Changing area (Rai)	Changing rate
		2000	2009		
Trat	GOT	60,018.24	61,974.19	+1,892.95	+3.15
Chanthaburi	GOT	77,456.23	75,428.91	-2,027.23	-2.62
Rayong	GOT	12,2279.54	11,283.57	-995.97	-8.11
Chonburi	GOT	4,861.90	5,554.41	+692.51	+14.24
Chachoengsao	GOT	10,476.13	7,309.34	-3,166.79	-30.23
Samut Prakan	GOT	6,936.48	12,524.17	+5,587.69	+80.56
Bangkok	GOT	4,138.30	3,351.79	-786.51	-19.01
Samut Sakhon	GOT	18,590.06	25,257.22	+6,667.16	+35.86
Samut Songkhram	GOT	14,773.72	14,272.75	-500.97	-3.39
Phetchaburi	GOT	20,463.39	18,568.75	-1,894.64	-9.26
Prachuap Khiri Khan	GOT	3,121.88	1,707.58	-1,413.30	-45.27
Chumphon	GOT	45,291.80	32,240.11	-13,051.69	-28.82
Surat Thani	GOT	46,980.59	46,574.20	-406.39	-0.87
Nakhon Si Thammarat	GOT	71,022.29	73,549.60	+2,527.51	+3.56

Table 2.1 (Continued)

Provinces	Coastal side	Mangrove area (Rai)		Changing area (Rai)	Changing rate
		2543	2552		
Nakhon Si Thammarat	GOT	71,022.29	73,549.60	+2,527.51	+3.56
Phatthalung	GOT	698.60	399.98	-298.62	-42.75
Songkhla	GOT	21,910.29	7,991.95	-13,918.34	-63.52
Pattani	GOT	26,990.47	21,993.68	-4,996.79	-18.51
Narathiwat	GOT	0.00	184.49	+184.49	+100.00
Total of the Gulf of Thailand		446,027.71	420,167.69	-25,905.02	-5.81
Ranong	Andaman	170,334.80	154,448.34	-15,886.46	-9.33
Phang Nga	Andaman	263,983.37	275,316.68	+11,333.31	+4.29
Phuket	Andaman	11,848.72	12,327.42	+478.70	+4.04
Krabi	Andaman	221,863.40	218,185.74	-3,677.66	-1.66
Trang	Andaman	228,191.01	220.975.74	-7,215.27	-3.16
Satun	Andaman	237,399.80	223,638.95	-13,760.85	-5.80
Total of Andaman Sea		1,133,621.10	1,104,892.87	-28,728.23	-2.53
Total of coastal area in Thailand		1,579,693.81	1,525,060.56	-54,633.25	-3.46

Modified from the table of land use in the mangrove area, Department of Marine and Coastal Resoucres, Thailand

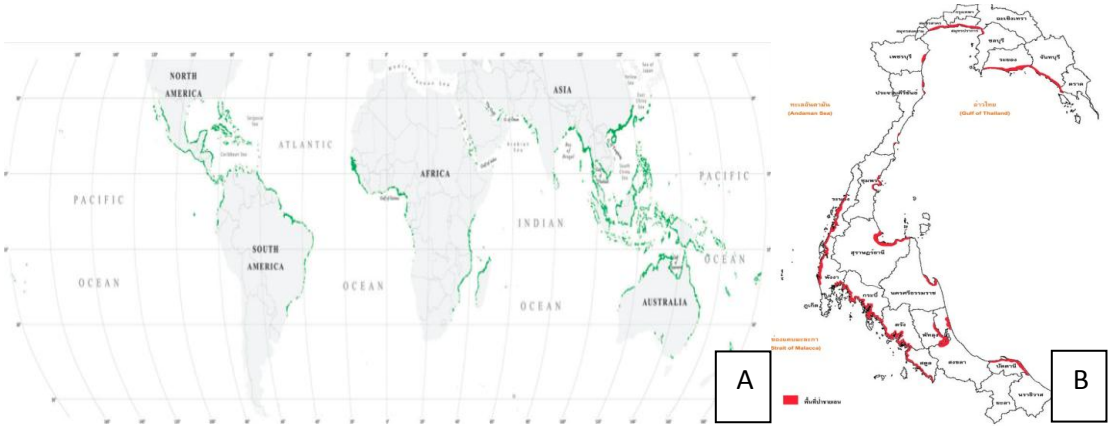


Figure 2.6 Mangrove distribution in the world (A) The mangrove distribution around the world (Giri et al, 2011) (B) the mangrove distribution in Thailand (สนิท อักษรแก้ว, 2545)

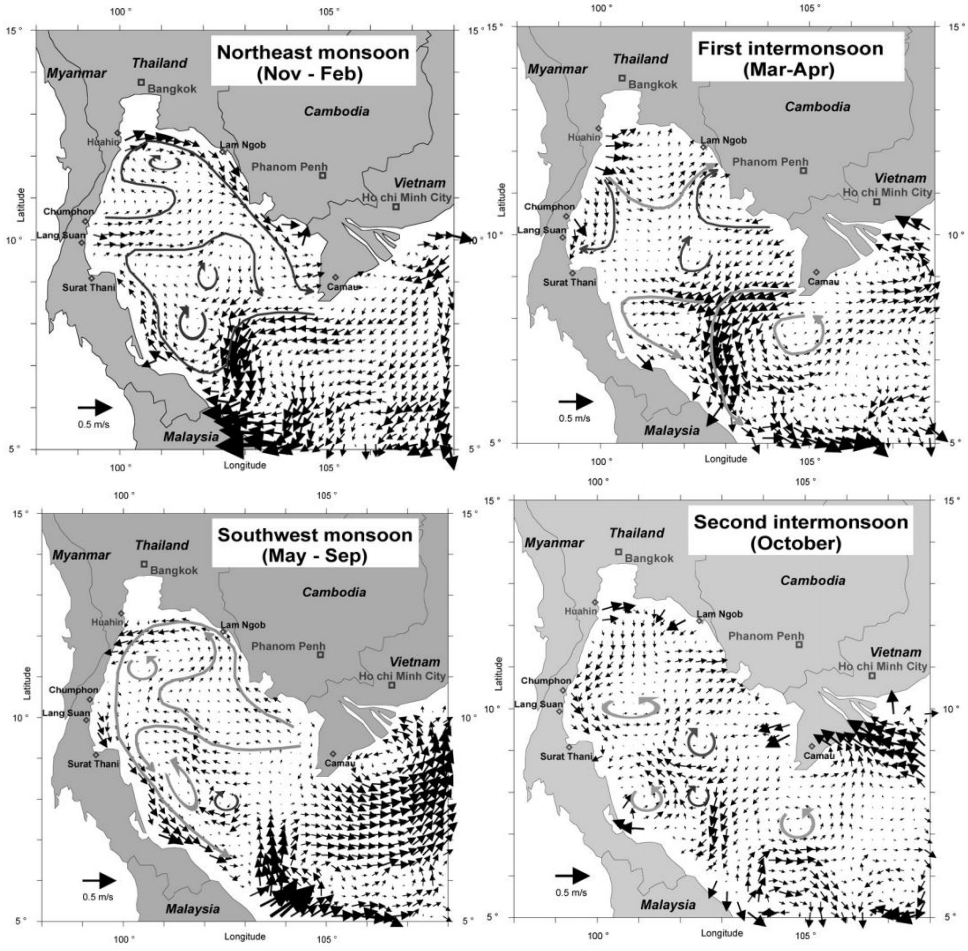


Figure 2.7 Seasonal circulation in the Gulf of Thailand (Sojisuporn et al, 2010)

driven current (Boicourt, 1988). Transport of the ichthyoplankton into and from coastal water is a function of water-mass displacements. However, the salinity gradient may limits the distribution of organisms (Chiappa-Carrara et al, 2003).

2.1.7.3 Breeding Season and Pelagic Larval Stage

Breeding season of the mudskipper depends on species and habitat zone (Table 2.2). Breeding season and pelagic larval stage are important for mudskipper's distribution because the distribution of mudskipper is well-performed in the larval stage since mudskippers do not migrate after they settle on mudflats and eggs are attached in the burrow. In other marine fish species, the adults have an ability to migrate for a long distance and spawn pelagic eggs that can be dispersed by water current. For Boleophthalmus, spawning occurs in a spawning chamber located in the burrow prepared by male. Their eggs are attached the ceiling of the chamber until they hatch. The planktonic larvae were then released to water column. Their planktonic stage has only about one month long. Then, they will settle in new suitable mudflats and become the recruitment of the population (Koga et al, 1989a; Takegaki, 2008). The recruitment is the one of important factor in the genetic structure of populations (Caley et al, 1996).

2.1.7.4 Anthropogenic Impacts

The mudskipper populations are affected by anthropogenic activities. The number of some species of mudskipper in China are considered as endanger now because of pollutions, overconsumption, and uncontrolled.

Table 2.2 Breeding seasons of mudskipper depending on species and area

Species	Country	Breeding Season	Reference
Boleophthalmus boddarti	Vietnam	August-October	(Quang et al, 2016)
Boleophthalmus pectinirostris	Kuwait	March-August	(Clayton et al, 1994)
	Japan and Korea	June-August	(Chen et al, 2015)
	Vietnam, China and Taiwan	April-June	
	China	May-June	(Zhao et al, 2002)
	Japan	Early May-Early August	(Takegaki, 2008)
	China	May-August	(Zhang et al, 1989)
Boleophthalmus dussumieri	India	July-September	(Mutsaddi & Bal, 1970)
	Pakistan	April-May and July to September	(Hoda, 1986)
Periophthalmus barbarus	South Africa	February-July	(Etim et al, 2002)
Periophthalmus magnuspinnatus	Korea	May-July	(Baeck et al, 2008)
Periophthalmus schlosseri	Malasia	June-July and October-November	(Mazlan & Rohaya, 2008)
Periophthalmus cantonensis	Hongkong	March-November	(Gordon & Gabaldon, 1985)
Periophthalmus po	Nigeria	November-February	(Lawson, 2011)
Scartelaos gigas	Korea	May-July	(Kim et al, 2011)

fishing (Liu et al, 2009). The decline of number affects the population and genetic structure of marine lives

2.1.8 Benefits

2.1.8.1 Food

The Blue-spotted mudskipper is used for food in many countries, such as Bangladesh, China, Japan, Korea Thailand, Taiwan, Philippines, Vietnam and Indonesia. Moreover, they are an ingredient for traditional medicine and high nutritive value in good lipid and fatty acid including EPA and DHA (Banerjee et al, 1997) in China and India. Several species are considered as a delicacy, and they have been farmed (Polgar & Lim, 2011).

2.1.8.2 Ecological Indicators

Untreated industrial and urban wastes directly discharge in the marine environment. It causes the high concentration of heavy metals, petroleum hydrocarbons, and insecticides. The contaminate concentration in natural environment and tissue sample of mudskipper significantly correlate. The heavy metals accumulate in their gill, skin, digestive system, kidney, liver, brain and muscle. The mudskipper has been considerable resistance to highly polluted habitat. They showed a very high potential for bioaccumulation. Therefore, toxic tolerant ability is used to study the coastal pollutions.

On the other hand, mudskippers cannot tolerate the oil pollution because they live out of water and directly exposed oil unlike the other

fishes. They are very sensitive to polycyclic aromatic hydrocarbon (PAHs), and Dichloro-diphenyl trichloroethane (DDT). (Ansari et al, 2014).

2.2 Molecular Markers

There are two main methods, which are used to study the population, including the morphological and genetic methods. In the recent years, molecular technique has been commonly used to investigate the level of genetic variation of several species (Benzie, 2000). Molecular markers are useful for many genetic studies, especially population genetic. The population genetic studies can define the genetic structure and variation of population. These results of population genetics can infer to gene flow, divergence times and pattern, biogeographical structure (Bohonak & Jenkins, 2003; Eimanifar, 2014; Féral, 2002; Palumbi, 1996b; Slatkin, 1987). The target DNA sequence can be synthesized from a low amount of DNA template within a few hours, using polymerase chain reaction (PCR) procedure (Mullis, 1994).The basis of the PCR technique involves the use of primers (short oligonucleotides) to attach the complementary with DNA sequence on both upstream and downstream line. After that, the target DNA is amplified using DNA polymerase (*Taq* polymerase). The PCR cycle has three steps, including, denaturation, primer annealing, and primer extension (Mullis, 1994). In eukaryotic cell, sources of DNA are composed of nuclear DNA, mitochondrial DNA, and chloroplast DNA. All of these sources of DNA can be found in the plant cells, however, nuclear DNA and mitochondrial DNA can be found in animal cells.

2.2.1 Mitochondrial DNA

Most of animal's mtDNA has closed-circular shape. The genes on mitochondria DNA highly reserve because they are coding region of many genes, except for D-loop or control region. The control region is the only a non-coding region in the mitochondria genome that is the origin of replication of animal mtDNA (Wilson et al, 1985). The conserve coding regions consist of transfer RNA, ribosomal RNA, and protein coding genes because of their functional and structural strain (Simon et al, 1994). The mutations usually occur in mitochondrial DNA because of the free oxygen radicals that are generated by the cellular respirations. Point mutations occur throughout the whole genome, however, the frameshift mutations, for instance, insertion and deletion, usually occur within the control region. Animal mtDNA has been effectively used in term of molecular tool for population and evolution because of small molecule, high mutation rate, maternal inheritance, and no recombination (Wilson et al, 1985).

2.2.1.1 Mitochondrial genome of B. boddarti

The complete mitochondrial genome of blue-spotted mudskipper, *Boleophthalmus boddarti* has been sequence (KF874277) that has 16727 bp in total length. There are two rRNA genes (12S and 16S), twenty two tRNA genes, 14 mRNA, and control region called D-loop (Figure 2.8).

Various effective markers are used for population genetics studies, including non-PCR base techniques (for example, restriction fragment length polymorphism (RFLP)) and PCR base technique (for example, randomly amplified polymorphism DNA (RAPD), amplified fragment length

polymorphism (AFLP), inter-sample sequence repeat (ISSR) and DNA sequencing).

2.2.2 DNA sequencing

DNA sequencing is one of main techniques of genetic information. The structure of DNA molecule is double strands, which attached each other by H-bond among the complementary base, consist of Adenine and Thymine (A-T), and Guanine and Cytosine (G-C) The DNA sequencing's principle track the order of correctly specific DNA bases, consist of adenine (A), thymine (T), guanine (G), and cytosine (C), on extremely long nucleotides. There are two general methods of this process, including the chemical cleavage procedure (Maxam & Gilbert, 1977) and the chain termination procedure (Sanger et al, 1977). The chain termination procedure is the more popular method. The radioactive isotopes, for instance, phosphorus-32, phosphorous-33, and sulfur-35, are required to label each type of nucleotide. The autoradiography technique is used to reading each radioactive labeled nucleotide on the X-ray film. DNA sequencing provides high solution and facilitating interpretation. DNA fragment generated from PCR can be both directly and alternatively sequenced. In recent year, the sequencing technique has been developed to be an automated sequencing method (Figure 2.9). The principle of automate sequencing method is using fluorescence-based system labeling fluorescence dye on sequencing primer or incorporated nucleotides. The automated DNA sequencing is successfully useful because of low-level radioactive and reliable data. This greatly allows wider application of DNA sequencing analysis for molecular genetic studies, including population genetic.

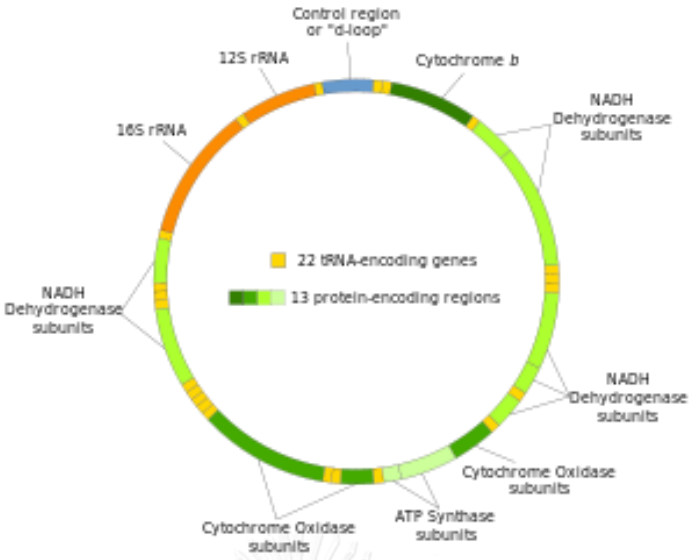


Figure 2.8 Mitochondrial DNA structure

(https://en.wikipedia.org/wiki/Mitochondrial_DNA)

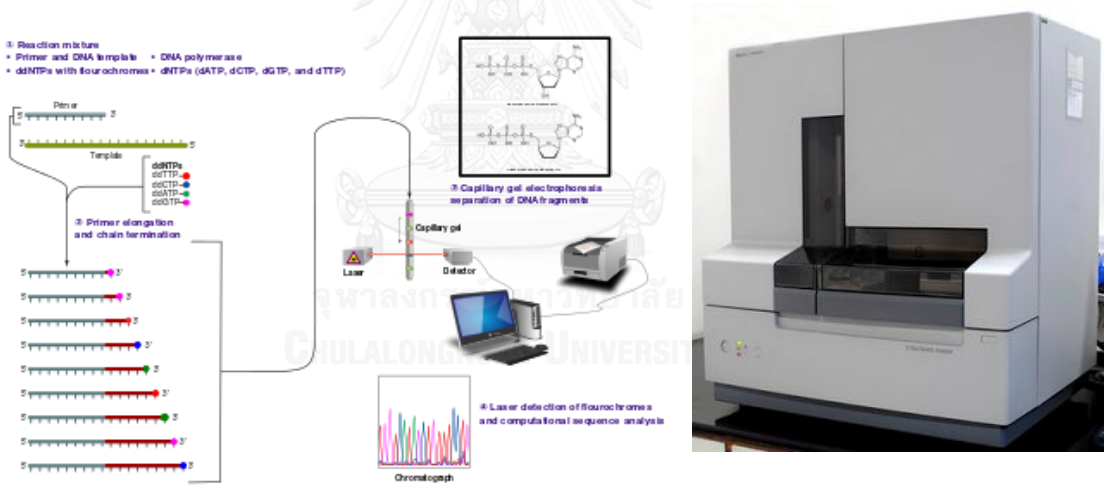


Figure 2.9 Automated DNA sequencing machine

(http://www.srmgenetics.info/2012/05/automated-dna-sequencing-machine_20.html)

(https://en.wikipedia.org/wiki/Sanger_sequencing)

CHAPTER 3
MATERIALS AND METHODS

3.1 Sampling Site and Collection

The samples were collected from 6 sampling sites in the Gulf of Thailand, namely were Rayong, Chachoengsao, Samut Songkram, Petchburi, Nakhon Si Thammarat, and Pattani. In the South of the Gulf, the selected locations were selected following the previous investigation by Darumas (1997), including Nakhon Si Thammarat and Pattani. The other locations were selected by field survey, and interviewing local fishermen. The blue-spotted mudskippers were collected by many methods. The first method is digging their burrows by hand. The dip net was used to cover the other emergency openings while the main burrow was dug by hand. The blue-spotted mudskipper escaped to the emergency opening and was catched by the dip net. This method was used in Rayong, Samut Songkram, and Pattani. The second method was the use of the trawling net. The blue-spotted mudskippers were cornered and trawled. This method was utilized in Nakhon Si Thammarat, since the area has no prop-root trees. Finally, samples from Chachoengsao and Petchburi were collected by local fishermen. The numbers of samples collected from Rayong, Chachoengao, Samut Songkram, Petchburi, Nakhon Si Thammarat, and Pattani were 32, 40, 24, 22, 36, and 24 individuals, respectively (Figure 3.1 and Table 3.1).

The collected blue-spotted mudskippers were euthanized using ice. They were then photographed and measured the standard length. The left pectoral and dorsal fins of each sample were immediately cut and preserved in absolute ethanol.

Table 3.1 Sampling sites, population codes, latitudes and longitudes, sample sizes, and the periods of collection of *B. boddarti* collected in this study

Sampling sites	Population codes	Latitudes	longitudes	Sample sizes	periods of collection
Chachoengao	CH	13°26'36.05"N	100°58'37.47''E	40	September 2014
Nakorn Sri Thammarat	NK	8°35'33.09''N	99°58'57.55''E	36	November 2014
Pattani	PT	6°52'27.03''N	101°13'02.45''E	24	September 2014
Petchbuti	PB	13°15'51.53"N	99°56'47.90''E	22	June 2015
Rayong	RY	12°42'25.65"N	101°44'37.41''E	32	May 2014
Samut Songkram	SS	13°21'44.86"N	99°59'44.33''E	25	March 2015

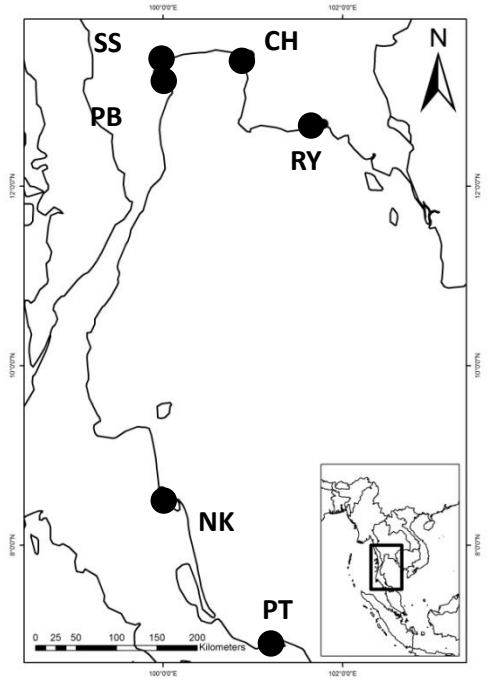


Figure 3.1 Sampling sites of *B. Boddarti* in the Gulf of Thailand; Rayong (RY), Chachoengsao (CH), Samut Songkram (SS), Petchburi (PB), Nakorn Sri Thammarat (NK), and Pattani (PT)

3.2 Sample Analysis

The genetic diversity and genetic population structure of *Boleophthalmus boddarti* were investigated. The control region in mitochondrial DNA was examined by sequencing technique.

3.2.1 DNA Extraction

The genomic DNA was extracted using Phenol-Chloroform method. The tissue that was preserved in absolute ethanol was placed on some tissue paper to drain out any alcohol. The small tissue (about 0.5x0.5 square centimeter) of each sample was cut by sterile scissors and was taken into a 1.5 ml microcentrifuge tube. A 300 µl

of TNES buffer and 5 µl of Proteinase-K were added into the tube. All ingredients were mixed by inverting the tube several times and were incubated in 55 $^{\circ}$C at least 2 hours. After that, 300 ul of Phenol-Chloroform solution (Phenol: Chloroform: isoamyl alcohol is 25: 24: 1, respectively) was added in the tube and the tube was centrifuge at 15,000 rpm for 3 minutes at room temperature. The supernatant was carefully transferred to a new tube, then, 250 µl of absolute ethanol was added into the tube. The new tube was centrifuged at 15,000 rpm for 5 minutes. The absolute ethanol was then removed. Then, 150 µl of 70% ethyl alcohol was added into the tube and centrifuged again at 15,000 rpm for 2 minutes. After that the 70% ethyl alcohol was removed. Finally, the 150 µl of sterile mili-Q water was added into the tube and DNA was stored at -20 $^{\circ}$C.

3.2.2 Agarose Gel Electrophoresis (Genomic DNA Analysis)

The quality and quantity of the extracted DNA was checked by agarose gel electrophoresis, loading mixture consist of 2 ul of extracted DNA, 2 ul of loading dye (standard stain orange G, 40% (v/v) glycerol) and 6 µl of distilled water. A 1% of agarose gel was made, mixing 1.5 g of agarose powder, 150 ml of 0.5X TBE buffer (10X TBE buffer consists of 0.89 M of Tris-base, 0.89 M of boric acid and 0.02 M of EDTA) in 200 ml flask. Then, the mixture was heated in microwave for 1 minute. The mixture solution was added the 3 µl of 0.4% ethidium bromide solution and 2 drops of bubble block solution. The prepared gel was set at room temperature in the gel tray which had the segregated combs for 1 hour. After the gel completely set, the combs were removed and the finished gel was taken place in the horizontal electrophoretic chamber, containing 0.5X TBE buffer over the gel. Each sample was loaded into each well by using an automatic micropipette. The electrophoretic chamber was connected to a power supply that the sample wells were located in

the cathod (negative) site, and was run at 80 voltages for 30 minutes. The DNA bands were visualized and taken photo under UV transilluminator. Concentration of extracted DNA was estimated by comparing with known quantity DNA maker. The quantity of extracted DNA was adjusted into 10-30 ng/µl, for using in PCR amplification.

3.2.3 PCR (Polymerase Chain Reactions) Amplification

The forward and reverse primers of partial part of control region mitochondrail DNA of *B. Boddarti* were designed by Primer3 program, based on *B. boddarti* mitochondrion complete genome (GeneBank: KF874277.1). The forward primer and reverse primer were 5' CAC GAA CCC ATT CAA ACA AG 3' and 5' AGT TTA CGA GTT TAG GGG GG 3' respectively, located in D-loop between $tRNA^{Pro}$ and $tRNA^{Phe}$.

The 20 µl PCR reaction contains 20-30 ng/µl of template DNA, 0.5U of Takara Ex *Taq* DNA Polymerase, 1X Ex *Taq* buffer (containing 20 mM $MgCl_2$), 0.2 mM of dNTPs, and 0.4 µl of each of 10 µM of primer. The negative control was included in all amplifications, by replacing distilled water to template DNA.

The PCR condition were as the following: 95 $^{\circ}C$ for 3 minutes, followed by 35 cycles of 95 $^{\circ}C$ for 30 seconds, 58 $^{\circ}C$ for 30 seconds, and 72 $^{\circ}C$ for 45 seconds, with final extension at 72 $^{\circ}C$ for 7 minutes.

3.3.4 Agarose Gel Electrophoresis (Mt DNA PCR Product Analysis)

PCR product was checked by agarose gel electrophoresis. Loading mixture consists of 2 µl of PCR product, 2 µl of loading dye (standard stain orange G, 40% (v/v) glycerol) and 6 µl of distilled water. A1% (w/v) agarose gel was made by mixing

1.5 g of agarose powder with 150 ml of 0.5X TBE buffer (10X TBE buffer consists of 0.89 M of Tris-base, 0.89 M of boric acid and 0.02 M of EDTA) in 200 ml flask. Then, the mixture was heated in microwave for 1 minute. Three microliters of 0.4% ethidium bromide solution and 2 drops of bubble block solution were added into the mixture solution. The prepared gel was set at room temperature in the gel tray which had the segregated combs for 1 hour. After the gel completely set to be the solid, the combs were removed and the finished gel was taken place in the horizontal electrophoretic chamber, containing 0.5X TBE buffer over the gel. Each sample was loaded into each well using an automatic micropipette, included standard DNA marker called the gene ladder-Fast2 (NIPPON GENE, JAPAN). The electrophoretic chamber was connected a power supply, that the sample wells were located in the cathode (negative) site, and was run at 100 voltages for 45 minutes. The PCR product was visualized and taken photo under UV transilluminator.

3.3.5 PCR Product Purification

The PCR products, which were clear band, were purified by using PEG purification. Ten microliters of PCR product was added with 5 µl of 0.2X TE and 7.5 of 30% PEG (polyethelene glycol) in a tube. The tube was centrifuged 4,000 rpm for 45 minutes. Then, the suspended liquid was removed and 30 µl of cold 70% ethanol was added to the tube. The tube was centrifuge again in 4,000 rpm for 15 minutes. The suspended liquid was removed again. The tube was then placed at room temperature overnight toremove all alcohol. Finally, 10 µl of distilled water was added in the tube.

3.2.6 DNA Sequencing

3.2.6.1 Big Dye Fluoresence Probes

The purified DNA was tagged by dideoxyterminal method of (Sanger et al, 1977) with a Big Dye reagent kit (ABI PrismTM Dye Terminator Cycle Sequencing Reading Reaction). Total 7.5 µl of sequecing reaction was prepared, including 1 µl of purified DNA, 0.75 µl of 1.6 µM forward or reverse primer, 1.4 µl of big dye buffer, 0.2 big dye reagent, and 4.15 distilled water. The mixture was amplified follow $^{\circ}$C for 2 minutes, 30 cycles (including denaturation at 96 $^{\circ}$C for 10 seconds, annealing at 50 $^{\circ}$C for 5 seconds, and extension at 60 $^{\circ}$C for 2 minutes), and cooled down at 20 $^{\circ}$C for 20 minutes.

3.2.6.2 Purify with Ethanol Precipitation

The 7.5 µl of sequencing reaction was purified by adding 0.75 µl of 3M NaOAC, 0.75 µl of 125 µM EDTA, and 25 µl of cold absolute ethanol. The tube was centrifuged 4,000 rpm for 45 minutes. The aqueous was removed and 25 µl of cold 70% ethanol was added and centrifuged at 4,000 rpm for 15 minutes. The liquid was removed and 10 µl formamind was added. Finally, the purified product was run on investigated using automate DNA sequencing machine (Applied Biosystem) to investigate the sequences of DNA.

3.2.7 Data Analysis

All nucleotide sequences were aligned by the parameter ClustalW alignment in the multiple sequence alignment program called MEGA (Tamura et al, 2013) and carefully corrected by eyes. The origin of the sequence was confirmed by comparing the similarity between obtained sequence and the sequences available on GenBank

database using Blast program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Genetic variations among sequences were examined and classified them into haplotypes. Haplotype frequencies were estimated using DnaSP v.5 (Librado & Rozas, 2009). Haplotype (h) and nucleotide (π) diversity (Nei, 1987) were estimated to investigate genetic diversity within the populations using DnaSP v.5 (Librado & Rozas, 2009). The pairwise distances between populations were estimated using phylogenetic program, called PHYLIP; Phylogeny Inference Package v.3.2 (Plotree & Plotgram, 1989). To investigate genetic relationship of the populations, the pairwise genetic distances were used to generate a dendogram under neighbor joining algorithm (NJ) algorithm using PHYLIP; Phylogeny (Plotree & Plotgram, 1989). Mantel test was calculated the correlation between the genetic distance and geographical distance (permute 1,000 times) using IBD (Isolation by distance) program (Bohonak, 2002). Moreover, the Median joining (Bandelt et al, 1999) haplotype network of mtDNA markers was generated using NETWORK v5.0.0.0 (Fluxus Technoogy Ltd., 2004-2016).

For investigation of genetic structure, the 6 populations of *B. boddarti* were divided into 2 groups based on the hypothesis of mangrove' patchy distribution and water currents in the Gulf of Thailand. The first group was the upper Gulf of Thailand, included the samples from Rayong, Chachoengsao, Samut Songkram, and Petchburi. The second group was the lower of the Gulf, included the samples from Nakorn Sri Thammarat and Pattani. The hierarchy of genetic structure was analyzed by the Analysis of Molecular Variance (AMOVA) using Arlequin computer program version 3.5.1.2 (Excoffier & Lischer, 2010). F-statistical analysis, namely F_{CT}, F_{SC}, and F_{ST}, were estimated and the significant test was carried out via permuting the data 10,000 times.

CHAPTER 4
RESULTS

4.1 DNA Extraction

Genomic DNA was successfully extracted from fin of *B. boddarti* using Phenol-Chloroform method. This protocol provided good quality and quantity of genomic DNA. Approximately, 25 ng/µl of the DNA was obtained, which was enough amount to perform PCR reaction. However, some samples provided little amount of the DNA. This might be caused by low quality of the tissues of the samples, especially samples that bought from fishermen. They keep samples in freezer for many days before sending to the laboratory (Figure 4.1).

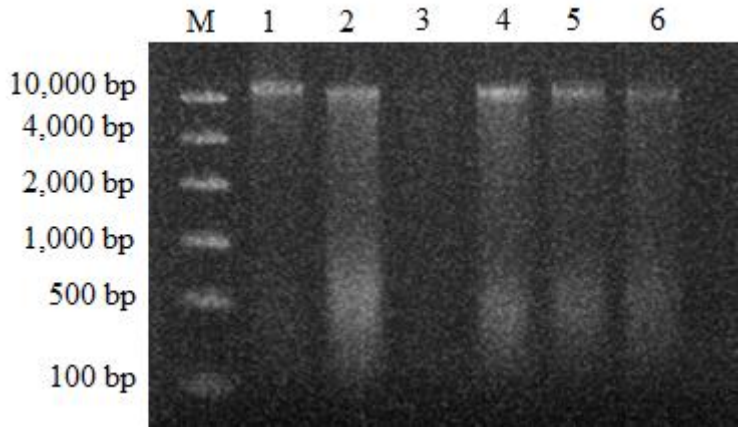


Figure 4.1 Total extracted DNA from fin of each *B. boddarti* individual (1-6) compared with Fast 2 marker (M) (NIPPON GENE, JAPAN) on 1% (w/v) agarose gel stained with ethidium bromide.

4.2 PCR Amplification

The specific sequence in mtDNA control region was successfully amplified. The expect size of the PCR product was approximately 500 base pairs (Figure 4.2).

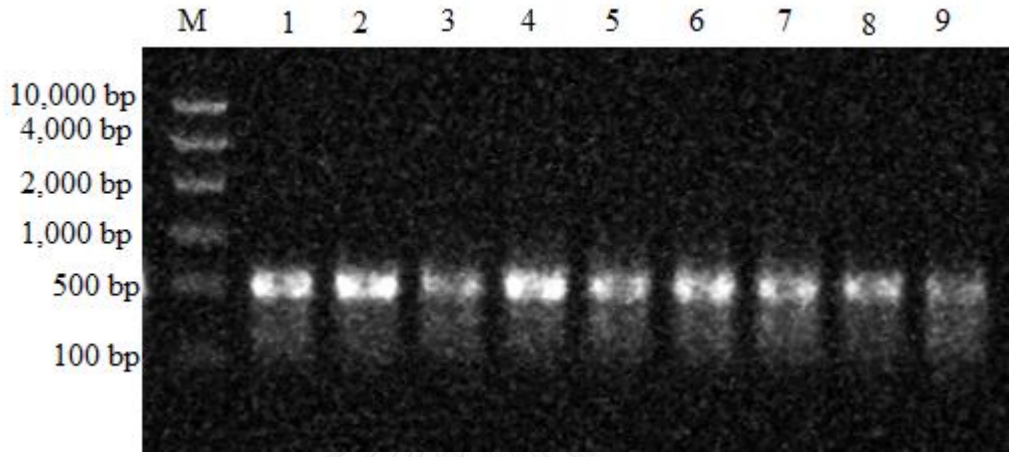


Figure 4.2 PCR products of partial mtDNA control region from each individual of *B. boddarti* in the Gulf of Thailand (1-9) and marker Fast 2 (M) (NIPPON GENE, JAPAN) on 1% (w/v) agarose gel stained with ethidium bromide.

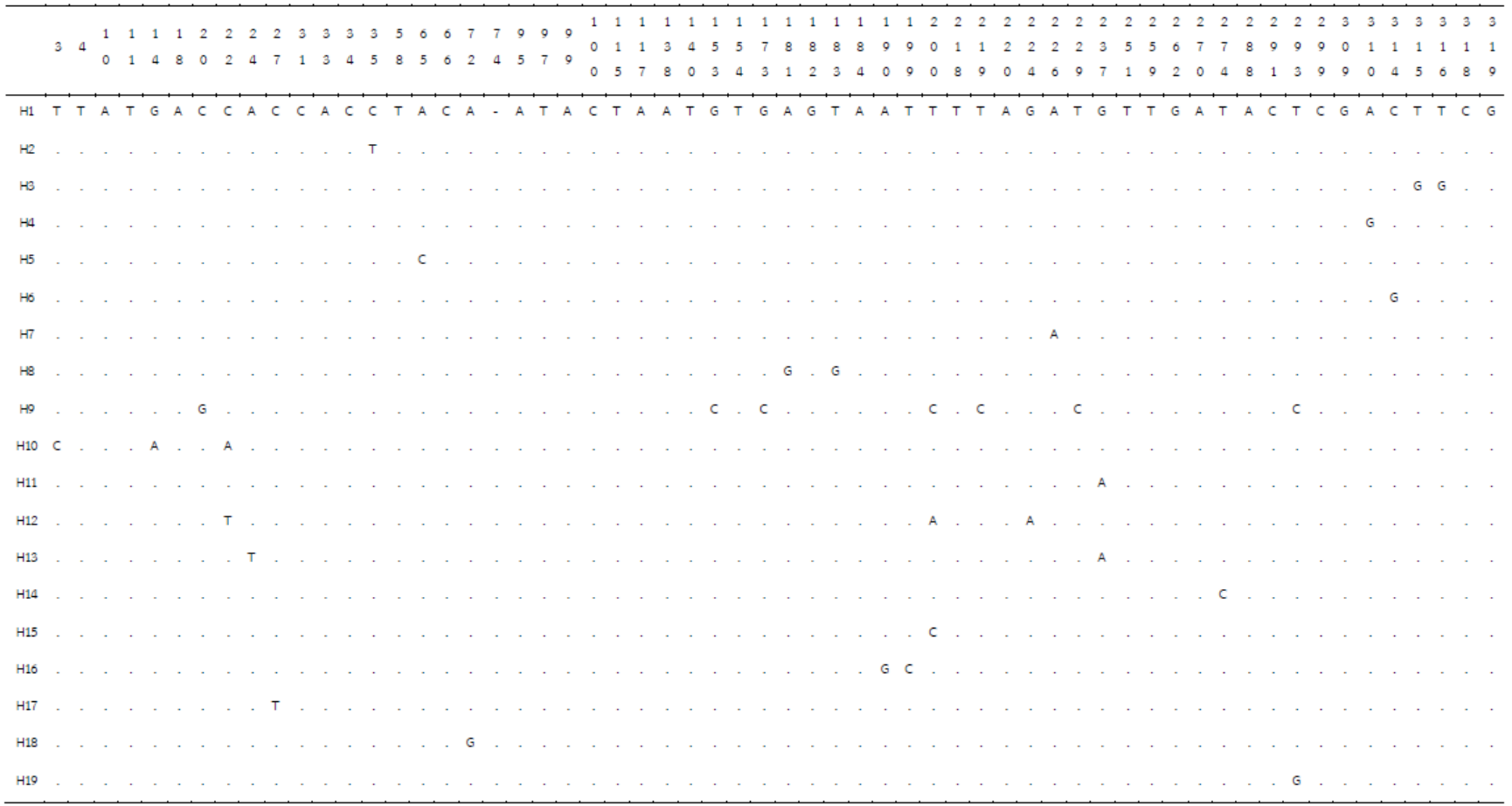
4.3 Data Analysis

The partial sequences of control region obtained in the study were investigated on their origin using Blast in NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The result showed that the sequences were similar to mtDNA control region of this species (KF874277) and another mudskipper, which was *Boleophthalmus pectinirostri* (KF005452). This result confirmed that the sequences are partial sequences of control region of *B. Boddarti*. A total of 320 bp of the sequences from 179 individuals of *B. boddarti* were collected and analyzed. The AT and CG contents were 60.9 % and 39.1 %, respectively. The polymorphic sites and conserved sites were 60 and 260 sites, respectively. The polymorphic sites included an insertion mutation at the 74th position of the sequences.

4.3.1 Genetic Diversity

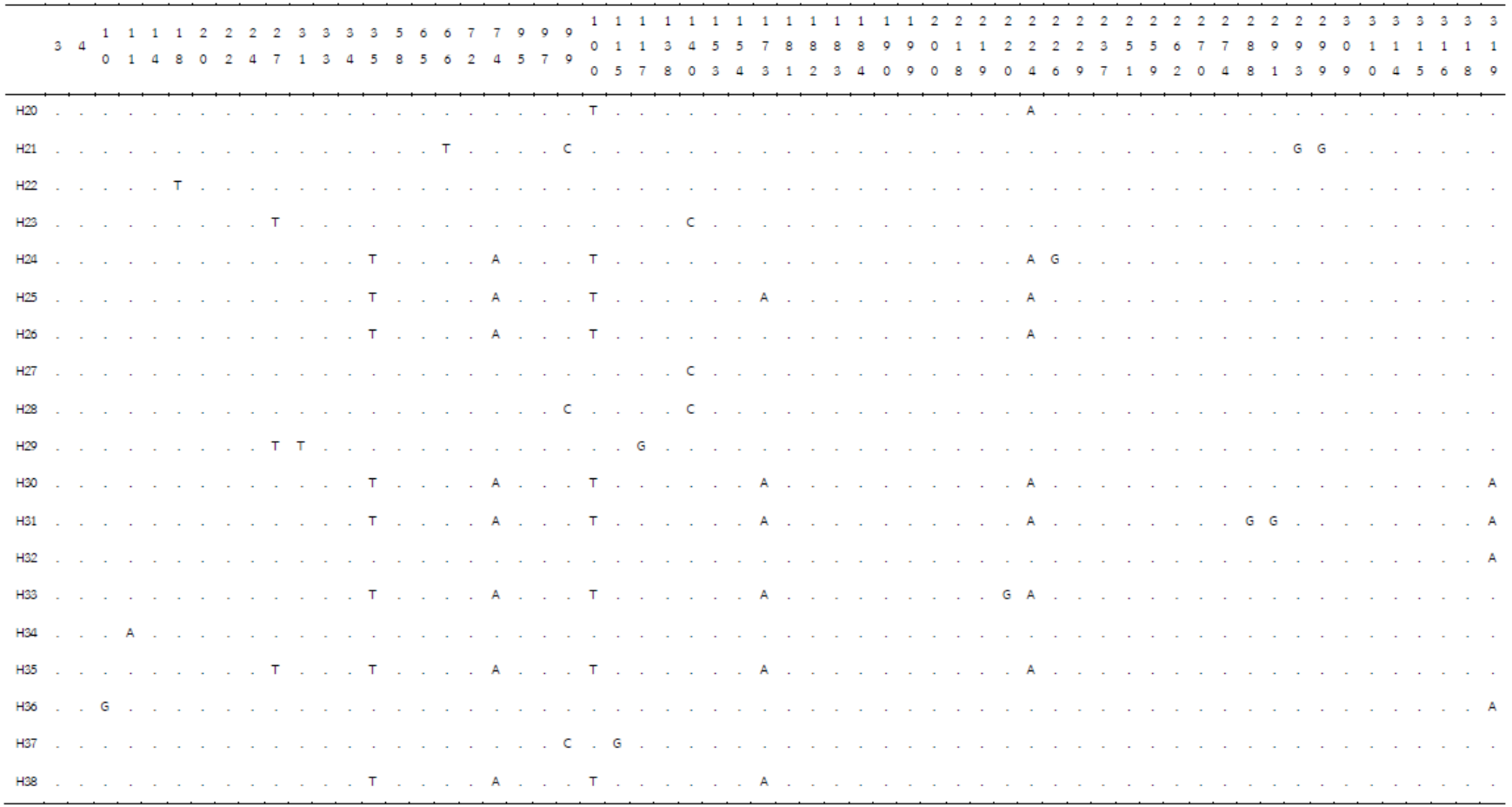
A total of 56 haplotypes were identified from 179 samples in the Gulf of Thailand, consist of 10 shared haplotypes and 46 unique haplotypes. The details of variable nucleotide positions of the partial control region mtDNA (Table 4.1) and the haplotype frequencies of each sampling site from the Gulf of Thailand (Table 4.2) were showed. Haplotype1 (H1) was the most abundant. There were shared by 88 individuals (49.44%) and can be found in all populations (RY, CH, SS, PB, NK, and PT). the frequencies of the other 9 shared haplotypes (H2, H7, H17, H14, H24, H25, H27, H32 and H54) were in range of 2 to 7 individuals.

Table 4.1 Haplotype designation and variable nucleotide positions in 55 *B. boddarti* partial mtDNA Control region sequence haplotypes.



	3	4	10	11	14	18	20	22	24	27	31	33	34	35	58	65	66	72	74	95	97	99	100	105	117	138	140	143	145	153	177	188	189	190	201	211	220	223	226	230	235	251	269	270	274	281	299	304	311	315	316	319
H1	T	T	A	T	G	A	C	C	A	C	C	A	C	C	T	A	C	A	-	A	T	A	C	T	A	A	T	G	T	G	A	G	T	A	A	T	T	T	T	A	G	A	T	G	T	T	G	A	T	A	C	T	C	G	A	C	T	T	C	G
H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	.
H4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
H5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
H7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H9	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	C	.	C	.	.	.	C	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H10	C	.	.	.	A	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H12	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H13	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.
H15	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H17	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H18	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H19	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Table 4.1 (Continued)



	3	4	10	11	14	18	20	22	24	27	31	33	34	35	58	65	66	72	74	95	97	99	100	105	117	138	140	153	154	173	181	183	184	190	199	200	208	211	220	224	226	229	237	251	255	262	270	274	288	289	299	299	299	301	311	311	315	316	318	319
H20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H21	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	.	.	.	.	.	.
H22	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H23	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H25	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	A	.	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H26	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H27	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H28	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H29	.	.	.	.	.	.	.	.	.	T	T	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H30	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	A	.	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
H31	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	A	.	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	G	G	.	.	.	.	.	.	A
H32	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
H33	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	A	.	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H34	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H35	.	.	.	.	.	.	.	.	.	T	.	.	.	.	T	.	.	.	A	.	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H36	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
H37	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H38	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	A	.	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Table 4.1 (Continued)

	3	4	10	11	14	18	20	22	24	27	31	33	34	35	58	65	66	72	74	95	97	99	100	105	117	138	140	153	154	173	181	183	184	190	199	200	208	219	220	222	226	232	237	251	259	262	270	274	288	291	299	299	300	311	314	315	316	318	319
H39	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H40	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	A	C	.	C	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H41	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	A	.	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H42	.	G	.	.	.	T	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	A	.	.
H43	.	.	.	.	.	.	.	.	C	T	.	.	A	T	.	.	.	.	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H44	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.
H45	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	T	.	.	.	.	.	A	.	.	.	.	C	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H46	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H47	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H48	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H49	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H50	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.
H51	.	.	.	.	.	.	.	.	C	T	.	.	.	T	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H52	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	A
H53	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	A
H54	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	A
H55	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
H56	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.

The base at the 74th position in the sequences had a frameshift mutation or insertion. Most of haplotypes did not have the Adenine in that 74th position (Table 4.1). On the other hand, there were 14 haplotypes, consist of 2 shared haplotypes (H24 and H25) and 12 unique haplotype (H26, H30, H31, H33, H35, H38, H40, H41, H43, H45, H50, and H51), were found to have the Adenine insertion. In addition, the result also showed that all samples in the inner Gulf of Thailand, namely Chachoengsao (CH), Samut Songkram (SS), and Petchburi (PB), were not found the insertion except 2 individual from Chachoengsao (CH) (Table 4.2).

Haplotype diversity ($\boldsymbol{h}$) ranges were between 0.54000 (Samut Songkram) to 0.8300 (Pattani) (Table 4.3). Nucleotide diversity ($\boldsymbol{\pi}$) ranges were between 0.002905 and 0.0009417, which are Samut Songkram and Rayong, respectively. Haplotype diversity ($\boldsymbol{h}$) of all population was 0.7879 ± 0.0676 and and nucleotide diversity ($\boldsymbol{\pi}$) was 0.004899 ± 0.003389 (Table 4.3).

Table 4.2 Haplotype frequencies of partial mtDNA control region of 179 *B. boddarti* from six locations (Rayong (RY), Chachoengsao (CH), Samut Songkram (SS), Petchburi (PB), Nakorn Sri Thammarat (NK), and Pattani (PT)), consisting of haplotypes, haplotype frequencies and percentage of haplotype

	The Upper Gulf of Thailand				The Lower Gulf of Thailand		Total	Percentage of haplotype (%)
	RY	CH	SS	PB	NK	PT		
H1	14	18	17	9	21	9	88	49.16
H2	2	3	-	-	-	-	5	2.78
H3	-	1	-	-	-	-	1	0.56
H4	-	1	-	-	-	-	1	0.56
H5	-	1	-	-	-	-	1	0.56
H6	-	1	-	-	-	-	1	0.56
H7	-	4	2	-	-	-	6	3.34
H8	-	1	-	-	-	-	1	0.56
H9	-	1	-	-	-	-	1	0.56
H10	-	1	-	-	-	-	1	0.56
H11	-	1	-	-	-	-	1	0.56
H12	-	1	-	-	-	-	1	0.56
H13	-	1	-	-	-	-	1	0.56
H14	-	1	-	-	-	-	1	0.56
H15	-	1	-	-	-	-	1	0.56
H16	-	1	-	-	-	-	1	0.56

Table 4.2 (continued)

	The Upper Gulf of Thailand				The Lower Gulf of Thailand		Total	Percentage of haplotype (%)
	RY	CH	SS	PB	NK	PT		
H17	2	1	2	-	-	1	6	3.34
H18	-	1	-	1	-	-	2	1.12
H19	-	-	1	-	-	-	1	0.56
H20	-	-	1	-	-	-	1	0.56
H21	-	-	1	-	-	-	1	0.56
H22	-	-	1	-	-	-	1	0.56
H23	-	-	-	-	1	-	1	0.56
H24	2	-	-	-	1	1	4	2.23
H25	-	-	-	-	4	2	6	3.34
H26	-	-	-	-	1	-	1	0.56
H27	-	-	-	-	2	4	6	3.34
H28	-	-	-	-	1	-	1	0.56
H29	-	-	-	-	1	-	1	0.56
H30	-	-	-	-	1	-	1	0.56
H31	-	-	-	-	1	-	1	0.56
H32	1	-	-	5	1	-	7	3.91
H33	-	-	-	-	1	-	1	0.56
H34	-	-	-	-	-	1	1	0.56

Table 4.2. (continued)

	The Upper Gulf of Thailand				The Lower Gulf of Thailand		Total	Percentage of haplotype (%)
	RY	CH	SS	PB	NK	PT		
H35	-	-	-	-	-	1	1	0.56
H36	-	-	-	-	-	1	1	0.56
H37	-	-	-	-	-	1	1	0.56
H38	-	-	-	-	-	1	1	0.56
H39	-	-	-	-	-	1	1	0.56
H40	-	-	-	-	-	1	1	0.56
H41	1	-	-	-	-	-	1	0.56
H42	1	-	-	-	-	-	1	0.56
H43	1	-	-	-	-	-	1	0.56
H44	1	-	-	-	-	-	1	0.56
H45	1	-	-	-	-	-	1	0.56
H46	1	-	-	-	-	-	1	0.56
H47	1	-	-	-	-	-	1	0.56
H48	1	-	-	-	-	-	1	0.56
H49	1	-	-	-	-	-	1	0.56
H50	1	-	-	-	-	-	1	0.56
H51	1	-	-	-	-	-	1	0.56
H52	-	-	-	1	-	-	1	0.56

Table 4.2 (continued)

	The Upper Gulf of Thailand				The Lower Gulf of Thailand		Total	Percentage of haplotype (%)
	RY	CH	SS	PB	NK	PT		
H53	-	-	-	1	-	-	1	0.56
H54	-	-	-	3	-	-	3	1.68
H55	-	-	-	1	-	-	1	0.56
H56	-	-	-	1	-	-	1	0.56
Total	32	40	25	22	36	24	179	100

Table 4.3 Genetic diversity indices of *B. boddarti* samples from the Gulf of Thailand

Sampling site	Code	Number	Number of haplotypes	Haplotye diversity (h)	Nucleotide diversity (π)
Chachoengsao	CH	40	18	0.7923 ± 0.0650	0.005972 ± 0.003861
Nakorn Sir Tammarat	NK	36	12	0.6556 ± 0.0882	0.007324 ± 0.004552
Pattani	PT	24	10	0.8300 ± 0.0681	0.008872 ± 0.005413
Petchburi	PB	22	8	0.7879 ± 0.0676	0.004899 ± 0.003389
Rayong	RY	32	16	0.8105 ± 0.0708	0.009417 ± 0.005614
Samut Songkram	SS	25	7	0.5400 ± 0.1170	0.002905 ± 0.002309
All population		179	56	0.788±0.033	0.00858±0.00122

4.3.2 Genetic Structure of Populations

Most of pairwise genetic distances were significant between the populations of the inner Gulf of Thailand and lower Gulf of Thailand. However, there were no significant genetic differentiation between Rayong (RY) and Nakorn Sri Thammarat (NK), and between Rayong (RY) and Pattani (PT) (Table 4.4). As far as the cladogram obtained from pairwise genetic was concerned, the populations of *B. boddarti* in the inner Gulf of Thailand, namely, Chachoengsao (CH), Samut Songkram (SS), and Petchburi (PB), were grouped together. Rayong (RY), which is located in the upper Gulf of Thailand, was not in the group. However, there was unclear genetic structure among the populations under this study (Figure 4.3). The result of Mantel test also showed no correlation between genetic and geographical distances ($p>0.05$).

Median joining haplotype network showed the star-liked form and can be defined into two groups. In group I, there were a common haplotype (H01) found in all sampling sites and unique haplotypes having few mutation points compared with the common haplotype (H01) sequences (Figure 4.4). However, the haplotype (H9) has the highest number of point mutations in the group and found only one sample from Nakorn Sri Thammarat (NK). Most of the samples from the inner Gulf of Thailand, namely Chachoengsqo (CH), Samut Songkram (SS), and Petchburi (PB), were the members of group I. However, there was a small group, called group II, consisting the samples from Chachoengsao (CH), Rayong (RY), Nakorn Sri Thammarat (NK), and Pattani (PT) (Figure 4.4).

Table 4.4 Pairwise difference of F_{ST} (below diagonal) and associated P-values (above diagonal) among blue-spotted mudskipper populations in the Gulf of Thailand. (* indicated statistical significance P<0.05)

Distance method: Pairwise difference

	NK	PT	RY	CH	SS	PB
NK		0.811	0.117	0.000*	0.045*	0.000*
PT	-0.01636		0.072	0.000*	0.009*	0.000*
RY	0.01554	0.02892		0.000*	0.018*	0.000*
CH	0.05855	0.05367	0.05570		0.613	0.000*
SS	0.07231	0.06123	0.06221	-0.00548		0.000*
PB	0.16159	0.14710	0.13457	0.13299	0.17158	

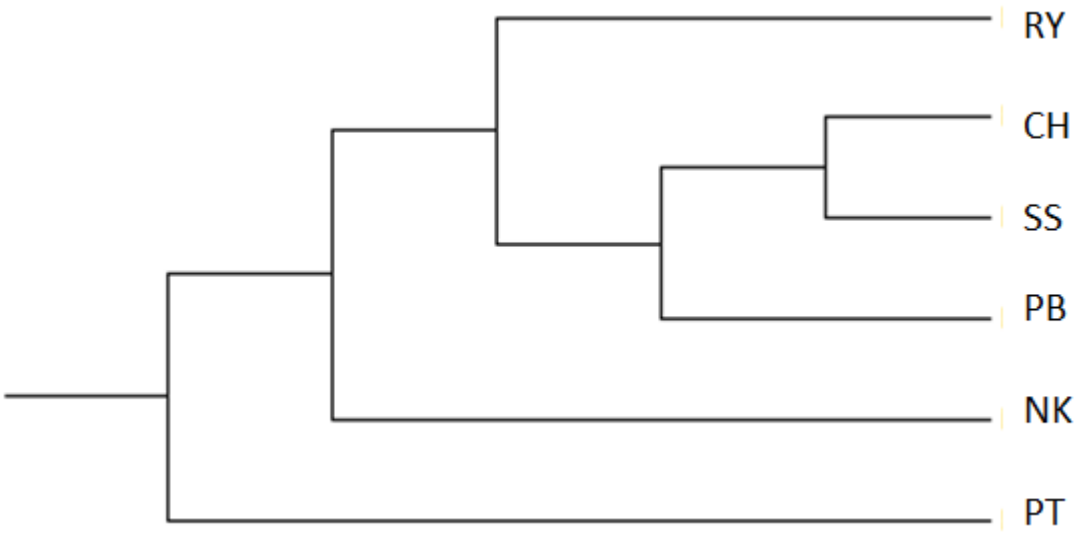


Figure 4.3 Neighbour-Joining (NJ) tree generated by pairwise genetic distances among six sampling sites within the Gulf of Thailand (Rayong (RY), Chachoengsao (CH), Samut Songkram (SS), Petchburi (PB), Nakorn Sri Thammarat (NK), and Pattani (PT)).

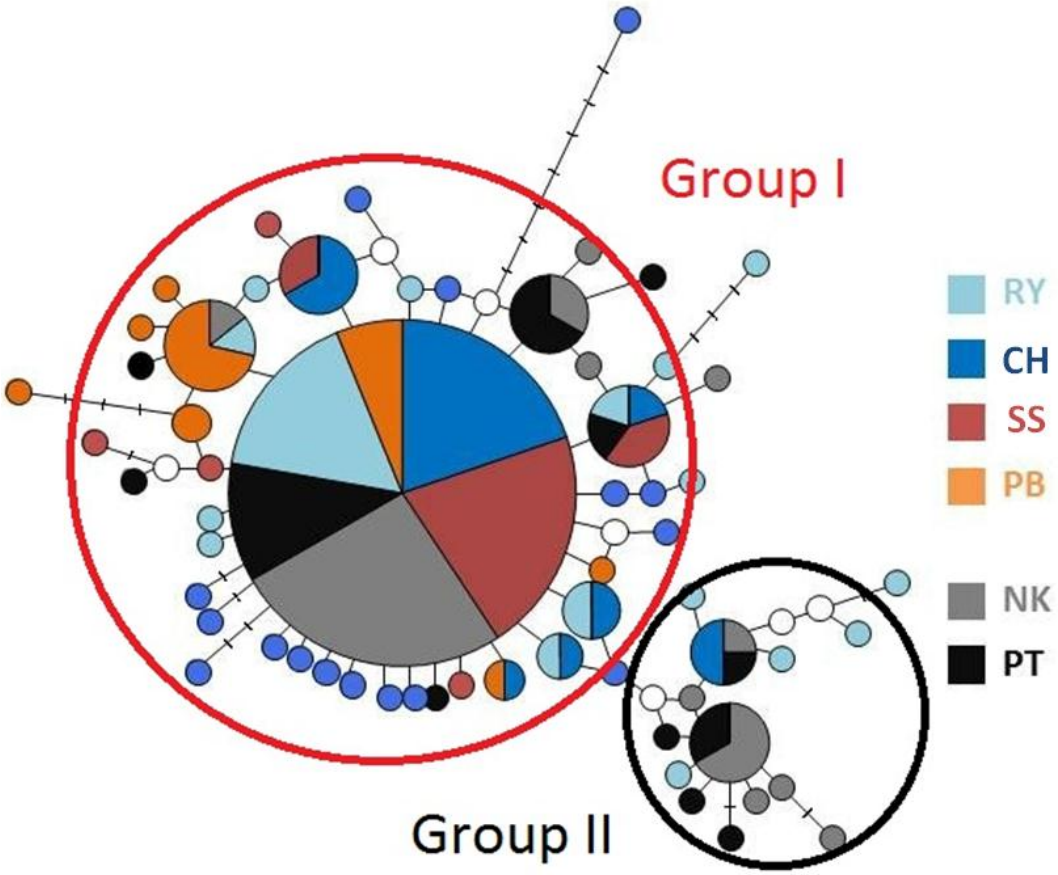


Figure 4.4 Median joining haplotype networks of partial control region in mtDNA of *B. boddarti* in the Gulf of Thailand. Haplotypes are represented as circles, with the size of each circle proportion of haplotype's frequency. Short line strokes represent the number of nucleotide sequence changes between the individual haplotypes. White circles represent missing haplotype.

The Analysis of Molecular Variance (AMOVA) showed that there was no significant difference between the populations of upper and lower Gulf of Thailand (F_{CT}=0.02461 (p=0.13099)). However, there were significant genetic differences of the populations within group (F_{ST} = 0.07800, P-value <0.001) and within population (F_{SC} = 0.05474, P-value < 0.001) was significantly high (Table 4.5). Most of the variation was contributed to within populations (92.20%), while the percentages of variation among the groups and among populations within groups were only 2.46.and 5.35, respectively. The percentages of each variation were the same trend with the variance components and fixation indices. The variance components of among groups, among populations within group, and within population were 0.02844, 0.06170, and 183.261, respectively.

Table 4.5 Hierarchical analysis of Molecular variance (AMOVA) of mtDNA haplotypes among blue-spotted mudskipper from two groups , which are the upper Gulf of Thailand (Rayong (RY), Chachoengsao (CH), Samut Songkram (SS), and Petchburi (PB)) and the lower Gulf of Thailand (Nakorn Sri Thammarat (NK) and Pattani (PT)). The P-value is based on 1000 random permutations of the data matrix.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation Indices	P-value
Among groups	1	5.226	0.02844	2.46	F_{CT} : 0.02461	0.13099
Among populations within group	4	11.401	0.06170	5.34	F_{SC} : 0.05474	0.00098
Within population	172	183.261	1.06547	92.20	F_{ST} : 0.07800	0.00000
Total	177	199.888	1.15561			

CHAPTER 5
DISCUSSION

5.1 Genetic Diversity

The genetic diversity of *B. Boddarti* from 6 locations in the Gulf of Thailand was investigated using partial mtDNA control region sequencing analysis. Two diversity indices, namely, haplotype diversity ($\boldsymbol{h}$) and nucleotide diversity ($\boldsymbol{\pi}$), were estimated (Nei & Li, 1979). The average haplotype diversity and nucleotide diversity values were 0.788±0.033 and 0.00858±0.00122, respectively. These values indicated high haplotype diversity and moderate nucleotide diversity of the populations in the Gulf of Thailand as reported in *B. pectinirostris* from the North-Western Pacific coast. The average haplotype diversity and nucleotide diversity of *B. pectinirostris* populations estimated from partial *Cyt b* sequences were 0.9645±0.006 and 0.00636±0.00021, respectively (Chen et al, 2015). The similar results of genetic diversity were also found on the estuarine tapertail anchovy *Colila ectenes* (Ma et al, 2010), Cyprinid *Opsariichthys bidens* (Perdices et al, 2005), and the white croaker *Pennnahia argentata* (Han et al, 2008). The high haplotype diversity and moderate nucleotide diversity values indicated the pattern of the large population that has rapidly been expanded from a small one which permits retention of the new mutations (Grant & Bowen, 1998; Stepien, 1999).

The results from this study showed that the genetic diversity of the the inner Gulf of Thailand populations, namely Petchburi (PB), Chachoengsao (CH), especially in Samut Songkram (SS), was lower than the other populations in the Gulf (Table 4.3). There are many physical and biological factors that can affect the genetic diversity of marine fishes, such as physical barrier (Boicourt, 1988), habitats loss

(Whitmore & Sayer, 1992), anthropogenic activities (Liu et al, 2009) , and migration and dispersal ability (Caley et al, 1996) (see also section 2.1.6). In the past two decades, industrialization and economic development is rapidly increased in the inner Gulf of Thailand. There have been many anthropogenic activities, such as aquacultures, ports, and domestic, agricultural, and industrial wastes (Wattayakorn, 2006). The coastal development has exerted stress on the marine environment and the habitats, especially mangrove areas, which are rapidly decreased (Cheevaporn & Menasveta, 2003). The habitat reduction can cause a demographic bottleneck and a decrease in genetic variation (De Jong et al, 2011). In Samut Songkram population, the lowest genetic diversity was found. The area is mostly covered by the mud flat and has no mangrove area. Mangrove swamps play a vital role as nursery grounds for fish. Lack of mangrove could then affect the larval supplies and recruitment of the area (Robertson & Duke, 1990). In addition, the anthropogenic activities, such as shellfish farming, shrimp farming, short mackerel fishing, and tourisms can also have an effect on the reduction of the habitats and populations of mudskipper in the area. For these reason, low genetic diversity of Samut Songkram population was found. There were haplotypes found mostly on the samples from lower Gulf of Thailand (Nakorn Sri Thammarat and Pattani) and the East of the Gulf (Rayong). The haplotypes had Adenine insertion at 74th position on the sequences (Table 4.1 and Table 4.2). The occurrence of these haplotypes was a reason that caused the overall high genetic diversity of the populations.

There were 46 unique haplotypes (82.14%) from a total of 56 haplotypes found in this study. The high percentage of unique haplotypes was also reported in *B. pectinirostris* which were 80% in *Cyt b* (Chen et al, 2015) and 88% in control region (Kanemori et al, 2006). These indicated that the blue-spotted mudskipper in the Gulf of Thailand had high genetic diversity and showed that the reproductive ability of

female's mudskipper have been efficiently performed in the Gulf of Thailand and the anthropogenic activities have less effect on the genetic diversity of the mudskipper populations in the present. The H1 haplotype was the common haplotype (around 50 percent of the total 179 samples). It was shared in all of populations, suggested that it could be the ancestral haplotype (Figure 4.4).

5.2 Genetic Structure

Most marine organisms have migratory adults and dispersal ability of the pelagic larval stage, which can play an important role in gene flow and genetic structure of the populations (Taylor & Hellberg, 2003). However, for the mudskippers, juveniles and adults are more likely to stay in their habitats after settling (Hong et al, 2007; Murdy, 1989). Therefore, gene flow between populations should be mostly dependent on the dispersal ability of their pelagic larvae (Chen et al, 2015). In some cases, the life history of mudskipper should be considered as those of marine sessile animals. In this study, there was unclear genetic structure between the populations of *B. boddarti* in the upper and lower Gulf of Thailand. As far as the genetic structure of marine organisms in the Gulf is concerned, similar results were reported in the studies on other marine fishes in the area, such as short mackerel *Rastrelliger brachysoma (*Srinulgray*,* 2008*)*, and Greenback mullet *Liza subviridis* (Suppapan, 2015). However, the adults of those fishes are pelagic. For sessile animals populations in the Gulf of Thailand, there were reported on green mussel *Perna viridis* (Prakoon et al, 2010), and the surf clam *Paphia undulata* (Donrung et al, 2011). Both studies showed the genetic structure among the populations of the species in the Gulf of Thailand. The short planktonic larval period (about 2 weeks) and the currents might be the cause of the genetic differentiation of the two species. However, Zhang et al (1989) and Chen et al (2015) reported that the planktonic stage

of mudskippers in genus Boleophthalmus was approximately 30-42 days before they develop to be the juvenile and settle on the mudflats. This indicates that the larval dispersal ability of mudskipper is much longer than those mollusk species and it could play an important role in the genetic homogeneity of *B. boddarti* populations in the Gulf of Thailand. Furthermore, *B. boddarti* have high fecundity (about 10,000 to over 30,000 eggs) (Quang et al, 2016). High fecundity strategy of marine species with pelagic larvae can appear to cope the large-scale marine environment by producing many offspring (Winemiller & Rose, 1992).

In the previous studies on the mudskipper (*B. pectinirostris*) in the North-Western Pacific coast using complete sequence of *Cyt b* gene (Chen et al, 2015) and partial sequences of control region (Kanemori et al, 2006), the results showed that there were significant genetic differentiation among the populations in Japan and the other parts of North-Western Pacific coasts. The different results between this study and the previous studies may be caused by the scale of study areas. The previous researches were studied in the board scale geography, covering the North-Western Pacific coasts, covering many countries, namely Japan, Korea, China, and Vietnam. Therefore, the fish or the larvae might not migrate or disperse throughout the area. However, they suggested the populations in the North-Western Pacific coast were isolated during the late Pleistocene period and the different temperature zones in the area might be the physical barrier of the species. In contrast with this study, the Mantel's test showed no correlation between geographical and genetic distances. This suggested the fish or the larvae could migrate or disperse throughout the area, correspondent to the result of AMOVA that showed no genetic differentiation between the group of populations in the upper and the lower Gulf of Thailand. The high percentage of variation within populations (92.20%) and low percentage of variation among group (2.46%) also inferred no genetic structure in the study area.

Although, no significant genetic structure of *B. boddarti* populations in the Gulf of Thailand was found in this study, the Neighbour-Joining (NJ) tree generated from pairwise genetic distances , showed a group of the populations in the inner Gulf of Thailand, namely, Chachoengsao, Samut Songkram, and Petchburi.

Oceanographic characteristics can influence dispersal ability of marine fishes (Giovannotti et al, 2009; McManus & Woodson, 2012). The inner Gulf of Thailand has been affected by the rivers discharge, including Bang Pakong, Chao Phraya, Tha Chin, and Mae Klong. The four major rivers create complex gyre patterns. They are counterclockwise between April to September, but clockwise between October to March. (Sojisuporn et al, 2010). The gyre might retain the larvae in the inner Gulf of Thailand and be the cause that the populations of the inner Gulf of Thailand were grouped together. There was also the difference of current circulation patterns between the upper and lower Gulf of Thailand. These could be presented as a natural barrier for the larval dispersals between the two areas. In addition, the difference of salinity and temperature between the areas might cause of the genetic difference. The discharges of four major rivers create a two-layered estuary that the lower-salinity shallow layer is affected by these discharges. In the contrary, the other parts of the Gulf of Thailand got the high salinity and cold water from the South China Sea (Robinson, 1974). The different of salinity can be a physical barrier to separates the population of mudskipper (Chen et al, 2015). Despite, the complexity of water current patterns in the Gulf of Thailand, no significant genetic differences between the populations of the upper and lower of the Gulf might be result of the speed of water current. The water velocity is influenced by the North-East and South-West monsoons. The average velocity of current in the spawning period of *B. boddarti* was about 10 km/day or 300 km/month during the monsoon season (Snidvongs & Sojisuporn, 1997). The high velocity of the current in the spawning time

can facilitate the larval dispersals of *B. boddarti*. It allows the gene flow among the populations in the Gulf of Thailand and causes no genetic structure of *B. boddarti* in the area.

The occurrence of haplotypes having A (Adenine base) insertion at 74th position of the sequences was found on the samples from the East (Rayong (RY)) and the South (Nakorn Sri Thammarat (NK) and Pattani (PT)) of the Gulf of Thailand (Figure 4.4 (Group II)). There should be the recruitments of Rayong (RY) obtained from Nakorn Sri Thammarat (NK) and Pattani (PT). The currents and ballast water could be the factors that bring the recruitment from the South to the East populations of the Gulf. The currents in the Gulf of Thailand move from the southern to the eastern part during the spawning season of mudskipper (August to October) (Quang et al, 2016). The planktonic larvae might be dispersed by the current from the southern part to the eastern part. Moreover, the planktonic larva of this fish may be transported by ballast water from the ships (Gollasch et al, 2000), that cruise from the Songkla port (the southern part of the Gulf of Thailand) to the Map Ta Put and Laem Chabang ports (the eastern part of the Gulf of Thailand). Zhang et al (2016) had investigated the complete mtDNA genome of blue-spotted mudskipper, *Boleophthalmus boddarti* (KF874277), in Malaysia. The sequence in D-loop had an additional Adenine base (A) at the same position as the sequences in Group II found in this study. This result suggested that the original of these haplotypes might be from the Southern area of the Gulf of Thailand and the Malay Peninsula.

5.3 Implication on Conservation and Stock Management

The level of genetic variation and population genetic structure, determined by DNA analysis (Benzie, 2000), are fundamental information used for management plan of natural resources (Avise, 1994). The exploratory research of

population genetics, especially focusing on genetic diversity and structure, would provide a basic knowledge for future management and conservation program. An assessment of the genetic diversity would assist in modeling specific populations for the current status (Avise, 1989). In this study, the average genetic diversity of *B. boddarti* populations in the inner Gulf of Thailand was lower than the others in the Gulf. It could be the result of the reduction of mangrove areas and pollution, which are mainly caused by anthropogenic activities in the area (Wattayakorn, 2006). Therefore, to protect the populations of mudskipper in this area, the conservation of mangrove forest and environmental protection are needed. There was no significant genetic differentiation between the populations in the upper and lower Gulf of Thailand and the mantle's test also show no correlation between genetic and geographical distances. The results indicated that the blue-spotted mudskipper has gene flow throughout the area and the populations of *B. boddarti* in the Gulf of Thailand could be treated as one stock, when the conservation of the species is required.

Therefore, the results of population genetic structure and their genetic diversity information from this study might be used for the purpose of effective conservation and management plans of *B. boddarti* in Thailand. However, for the efficient and sustainable use of *B. Boddarti* in Thailand, more basic knowledge are needed, for example, biology of fish (reproductive characteristics and periods), genetic structure, and population dynamics (migration and recruitment).

REFERENCES

Ansari AA, Trivedi S, Saggu S, Rehman H (2014) Mudskipper: A biological indicator for environmental monitoring and assessment of coastal waters. *Journal of Entomology and Zoology Studies* **2:** 22-33

Avise JC (1989) A role for molecular genetics in the recognition and conservation of endangered species. *Trends in Ecology & Evolution* **4:** 279-281

Avise JC (1994) *Molecular markers, natural history and evolution*, New York: Chapman and Hall.

Baeck GW, Takita T, Yoon YH (2008) Lifestyle of Korean mudskipper *Periophthalmus magnuspinnatus* with reference to a congeneric species *Periophthalmus modestus*. *Ichthyological Research* **55:** 43-52

Bandelt H-J, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular biology and evolution* **16:** 37-48

Banerjee D, Pal D, Patra TK, Misra S, Ghosh A (1997) Lipids and fatty acids of air breathing fish *Boleophthalmus boddaerti*. *Food chemistry* **60:** 303-309

Benzie J (2000) Population genetic structure in penaeid prawns. *Aquaculture Research* **31:** 95-119

Bohonak A (2002) IBD (isolation by distance): a program for analyses of isolation by distance. *Journal of Heredity* **93:** 153-154

Bohonak AJ, Jenkins DG (2003) Ecological and evolutionary significance of dispersal by freshwater invertebrates. *Ecology letters* **6:** 783-796

Boicourt WC (1988) Recruitment dependence on planktonic transport in coastal waters. In *Toward a theory on biological-physical interactions in the world ocean*, B. J. Rothschild edn, pp 183-202. Springer

Bowen BW, Bass AL, Muss A, Carlin J, Robertson DR (2006) Phylogeography of two Atlantic squirrelfishes (Family Holocentridae): exploring links between pelagic larval duration and population connectivity. *Marine biology* **149:** 899-913

Caley M, Carr M, Hixon M, Hughes T, Jones G, Menge B (1996) Recruitment and the local dynamics of open marine populations. *Annual Review of Ecology and Systematics***:** 477-500

Cheevaporn V, Menasveta P (2003) Water pollution and habitat degradation in the Gulf of Thailand. *Marine Pollution Bulletin* **47:** 43-51

Chen W, Hong W, Chen S, Wang Q, Zhang Q (2014) AFLP analysis of genetic structure of *Boleophthalmus pectinirostris* populations from the coastal waters of Northwest Pacific. *Journal of Fishery Sciences of China* **5:** 1020-1028

Chen W, Hong W, Chen S, Wang Q, Zhang Q (2015) Population genetic structure and demographic history of the mudskipper *Boleophthalmus pectinirostris* on the northwestern pacific coast. *Environmental Biology of Fishes* **98:** 845-856

Chew S, Ip Y (1990) Differences in the responses of two mudskippers, *Boleophthalmus boddaerti* and *Periophthalmus chrysospilos* to changes in salinity. *Journal of Experimental Zoology* **256:** 227-231

Chiappa-Carrara X, Sanvicente-Añorve L, Monreal-Gómez A, De León DS (2003) Ichthyoplankton distribution as an indicator of hydrodynamic conditions of a lagoon system in the Mexican Caribbean. *Journal of plankton Research* **25:** 687-696

Clayton D (1993) Mudskippers. *Oceanography and Marine Biology: an annual review* **31:** 507-577

Clayton D, Vaughan T (1988) Ethogram of *Boleophthalmus boddarti*(Pallas)(Teleostei, Gobiidae), a mudskipper found on the mudflats of Kuwait. *Kuwait Journal of Science* **15:** 115-140

Clayton D, Wells K, Al-Hashem A, Stuart B (1994) *Discovering Kuwait's wildlife*: Fahad Al-Marzouk.

Colombini I, Berti R, Nocita A, Chelazzi L (1996) Foraging strategy of the mudskipper *Periophthalmus sobrinus* Eggert in a Kenyan mangrove. *Journal of Experimental Marine Biology and Ecology* **197:** 219-235

Cuvier G, Valenciennes A (1829) Histoire naturelle des poissons. Tome troisième. Suite du Livre troisième. Des percoïdes à dorsale unique à sept rayons branchiaux et à dents en velours ou en cardes. *Histoire Naturelle des Poissons* **3:** 500

Darumas U (1997) Taxonomy and Ecology of mudskipper (Gobiidae: Oxudercinae) in Southern Thailand. Master Thesis, Ecology, Faculty of Science, Prince of Songkla University, Prince of Songkla University

Darumas U, Tantichodok P (2002) A new species of mudskipper (Gobiidae: Oxudercinae) from southern Thailand. *Phuket Marine Biological Center Research Bulletin* **64:** 101-107

De J, Nandi N (1984) Note on the locomotory behaviour of the mudskipper *Boleophthalmus boddarti*. *Indian Journal of Fisheries* **31:** 407-409

De Jong MA, Wahlberg N, Van Eijk M, Brakefield PM, Zwaan BJ (2011) Mitochondrial DNA signature for range-wide populations of *Bicyclus anynana* suggests a rapid expansion from recent refugia. *PloS one* **6:** e21385

Donrung P, Tunkijjanukij S, Jarayabhand P, Poompuang S (2011) Spatial genetic structure of the surf clam *Paphia undulata* in Thailand waters. *Zoological Studies* **50:** 211-219

Dotsu Y, Matoba M (1977) Behaviours of the mudskipper *Boleophthalmus pectinirostris* and *Periophthalmus modestus* in Arak Sound. *Animal* **5:** 15-23

Eimanifar A (2014) Molecular phylogeography and population genetics of the brine shrimp *Artemia* (Crustacea, Branchiopoda, Anostraca). Doctor Thesis, Natural Sciences, Ruperto-Carola University of Heidelberg, Germany, Ruperto-Carola University of Heidelberg, Germany

Etim L, King RP, Udo MT (2002) Breeding, growth, mortality and yield of the mudskipper *Periophthalmus barbarus* (Linneaus 1766)(Teleostei: Gobiidae) in the Imo River estuary, Nigeria. *Fisheries research* **56:** 227-238

Excoffier L, Lischer HE (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10:** 564-567

Féral J-P (2002) How useful are the genetic markers in attempts to understand and manage marine biodiversity? *Journal of Experimental Marine Biology and Ecology* **268:** 121-145

Fluxus Technoogy Ltd. (2004-2016) Network http://www.fluxus-engineering.com/.

Gee JH, Gee PA (1991) Reactions of gobioid fishes to hypoxia: buoyancy control and aquatic surface respiration. *Copeia***:** 17-28

Giovannotti M, La Mesa M, Caputo V (2009) Life style and genetic variation in teleosts: the case of pelagic (*Aphia minuta*) and benthic (*Gobius niger*) gobies (Perciformes: Gobiidae). *Marine biology* **156:** 239-252

Giri C, Ochieng E, Tieszen LL, Zhu Z, Singh A, Loveland T, Masek J, Duke N (2011) Status and distribution of mangrove forests of the world using earth observation satellite data. *Global Ecology and Biogeography* **20:** 154-159

Gjedrem T, Gjerde B, Refstie T (1988) A review of quantitative genetic research in salmonids at Akvaforsk. In *Proceedings of the second international conference on quantitative genetics. Sinauer, Sunderland, MA*, pp 527-535.

Gollasch S, Lenz J, Dammer M, Andres H-G (2000) Survival of tropical ballast water organisms during a cruise from the Indian Ocean to the North Sea. *Journal of Plankton Research* **22:** 923-937

Gordon M, Gabaldon D (1985) Exploratory observations on microhabitat selection within the intertidal zone by the Chinese mudskipper fish *Periophthalmus cantonensis*. *Marine biology* **85:** 209-215

Graham JB (1997) *Air-breathing fishes: evolution, diversity, and adaptation*: Academic Press.

Grant W, Bowen B (1998) Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *Journal of Heredity* **89:** 415-426

Han ZQ, Gao TX, Yanagimoto T, Sakurai Y (2008) Deep phylogeographic break among white croaker *Pennahia argentata* (Sciaenidae, Perciformes) populations in North**-**western Pacific. *Fisheries Science* **74:** 770-780

Herre AW (1953) *Checklist of Philippine fishes*: US Fish and Wildlife Service.

Hindar K, Ryman N, Utter F (1991) Genetic effects of cultured fish on natural fish populations. *Canadian Journal of Fisheries and Aquatic Sciences* **48:** 945-957

Hoda SS (1986) Maturation and fecundity of the mudskipper *Boleophthalmus dussumieri* Cuv. Val. from the Karachi Coast. *Mahasagar* **19:** 73-78

Hong W, Chen S, Zhang Q, Qiong W (2007) Reproductive ecology of the mudskipper *Bolephthalmus pectinirostris*. *Acta Oceanologica Sinica* **26:** 72-81

Ishimatsu A, Yoshida Y, Itoki N, Takeda T, Lee HJ, Graham JB (2007) Mudskippers brood their eggs in air but submerge them for hatching. *Journal of experimental biology* **210:** 3946-3954

Kanemori Y, Takegaki T, Natsukari Y (2006) Genetic population structure of the mudskiper *Boleophthalmus pectinirostris* inferred from mitochondrial DNA sequences. *Japanese Journal of Ichthyology (Japan)* **52:** 133—141

Kim J, Baek H, Kim J, Chang D, Kim J (2011) Sexual maturity and early life history of the mudskipper *Scartelaos gigas* (Pisces, Gobiidae): implications for conservation. *Fisheries and aquatic sciences* **14:** 403-410

Koga H, Noda S, Noguchi T, Aoto I (1989a) Studies on artificial propagation of mud skipper *Boleophthalmus pectinirostris* (Linnaeus)––III: hatching and larval rearing. *Bulletin of Saga Prefectural Ariake Fisheries Experimental Station* **11:** 17-28

Koga H, Noguchi T, Kinoshita K (1989b) Studies on artificial propagation of mudskipper *Boleophthalmus pectinirostris* (Linnaeus)-II Collecting eggs by artificial spawning nest and spawning conditions. *Bulletin of Saga Prefectural Ariake Fisheries Experimental Station* **11:** 9-16

Koumans FP (1953) *The Fishes of the Indo-Australian Archipelago: Gobioidea*: EJ Brill.

Lawson EO (2011) Testicular maturation and reproductive cycle in mudskipper, *Periophthalmus papilio* (Bloch and Schneider 1801) from Lagos lagoon, Nigeria. *Journal of American Science* **7:** 48-59

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25:** 1451-1452

Liu ZZ, Yang JQ, Wang ZQ, Tang WQ (2009) Genetic structure and population history of *Beleophthalmus petinirostris* in Yangtze river estuary and its southern adjacent regions. *Zoological research* **30:** 1-10

Ma C, Cheng Q, Zhang Q, Zhuang P, Zhao Y (2010) Genetic variation of Coilia ectenes (Clupeiformes: Engraulidae) revealed by the complete cytochrome b sequences of mitochondrial DNA. *Journal of Experimental Marine Biology and Ecology* **385:** 14-19

Maxam AM, Gilbert W (1977) A new method for sequencing DNA. *Proceedings of the National Academy of Sciences* **74:** 560-564

Mazlan A, Rohaya M (2008) Size, growth and reproductive biology of the giant mudskipper, *Periophthalmodon schlosseri* (Pallas, 1770), in Malaysian waters. *Journal of Applied Ichthyology* **24:** 290-296

McManus MA, Woodson CB (2012) Plankton distribution and ocean dispersal. *Journal of Experimental Biology* **215:** 1008-1016

Milward NE (1974) *Studies on the taxonomy, ecology, and physiology of Queensland mudskippers*: University of Queensland.

Mullis KB (1994) The polymerase chain reaction (Nobel lecture). *Angewandte Chemie International Edition in English* **33:** 1209-1213

Murdy EO (1989) *A taxonomic revision and cladistic analysis of the oxudercine gobies (Gobiidae: Oxudercinae)*: Australian Museum.

Mutsaddi K, Bal D (1970) Maturation and spawning of *Boleophthalmus dussumieri* (Cuv. & Val.). *Journal of the University of Bombay* **39:** 58-76

Nei M, Li W-H (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences* **76:** 5269-5273

Palumbi SR (1996a) Macrospatial genetic structure and speciation in marine taxa with high dispersal abilities. *Molecular zoology: advances, strategies and protocols Wiley, New York*: 101-117

Palumbi SR (1996b) What can molecular genetics contribute to marine biogeography? An urchin's tale. *Journal of Experimental Marine Biology and Ecology* **203:** 75-92

Perdices A, Sayanda D, Coelho M (2005) Mitochondrial diversity of *Opsariichthys bidens* (Teleostei, Cyprinidae) in three Chinese drainages. *Molecular phylogenetics and evolution* **37:** 920-927

Piper R (2007) *Extraordinary animals: An encyclopedia of curious and unusual animals*: Greenwood Publishing Group.

Plotree D, Plotgram D (1989) PHYLIP-phylogeny inference package (version 3.2). *cladistics* **5:** 163-166

Polgar G, Crosa G (2009) Multivariate characterisation of the habitats of seven species of Malayan mudskippers (Gobiidae: Oxudercinae). *Marine biology* **156:** 1475-1486

Polgar G, Lim R (2011) Mudskippers: Human use, ecotoxicology and biomonitoring of mangrove and other soft bottom intertidal ecosystems. *Mangroves: Ecology, Biology and Taxonomy Nova Science Publishers, Hauppauge***:** 51-86

Polidoro BA, Carpenter KE, Collins L, Duke NC, Ellison AM, Ellison JC, Farnsworth EJ, Fernando ES, Kathiresan K, Koedam NE (2010) The loss of species: mangrove extinction risk and geographic areas of global concern. *PloS one* **5:** 1-10

Prakoon W, Tunkijjanukij S, Nguyen TT, Na-Nakorn U (2010) Spatial and temporal genetic variation of green mussel, *Perna viridis* in the Gulf of Thailand and implication for aquaculture. *Marine biotechnology* **12:** 506-515

Qiyong HW, Dai QZ, Youyi ZJC (1988) Observations on the early development of the mud skipper, *Boleophthalmus pectinirostris* (Linnaeus). *Tropic Oceanology* **2:** 1985-2004

Quang DM, Tra Giang NT, Kieu Tien NT (2016) Reproductive biology of the mudskipper Boleophthalmus boddarti in Soc Trang. *TAP CHI SINH HOC (Vietnam journal)* **37:** 362-369

Ramanadevi V, Thangaraj M, Sureshkumar A, Subburaj J (2013) Detection of Genetic Variation and Genetic Diversity in Two Indian Mudskipper Species (*Boleophthalmus boddarti*, *B. dussumieri*) using RAPD Marker. *Notulae Scientia Biologicae* **5:** 139

Ravi V (2013) Food and feeding habits of the mudskipper, *Boleophthalmus boddarti* (Pallas, 1770) from Pichavaram Mangroves, Southeast Coast of India. *International Journal of Marine Science* **3:** 98-104

Ravi V, Rajagopal S (2007) Age and growth of the mudskipper, *Boleophthalmus boddarti* (Pallas, 1770). *Journal of Aquatic Biology* **22:** 123-128

Robertson AI, Duke NC (1990) Recruitment, growth and residence time of fishes in a tropical Australian mangrove system. *Estuarine, Coastal and Shelf Science* **31:** 723-743

Robinson MK (1974) The physical oceanography of the Gulf of Thailand, Naga Expedition; Bathythermograph (BT) temperature observations in the Timor sea, Naga Expedition, Cruise S11.

Ryman N, Utter F (1987) *Population genetics and fishery management*: University of Washington Press.

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences* **74:** 5463-5467

Sasekumar A, Chong V, Lim K, Singh H (1994) The fish community of Matang mangrove waters, Malaysia. In *Proc. Third ASEAN-Australia Symposium on Living Coastal Resources, Bangkok, Chulalonghorn University, Thailand*, Vol. 2, pp 457-464.

Shulman MJ (1998) What can population genetics tell us about dispersal and biogeographic history of coral-reef fishes? *Australian Journal of Ecology* **23:** 216-225

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the entomological Society of America* **87:** 651-701

Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science* **236:** 787-792

Snidvongs A, Sojisuporn P (1997) Numerical simulations of the net current in the Gulf of Thailand under different monsoon regimes. In *the 1st Technical Seminar of the Interdepartment Collaborative Research Program*, pp 24-26.

Sojisuporn P, Morimoto A, Yanagi T (2010) Seasonal variation of sea surface current in the Gulf of Thailand. *Coastal Marine Science* **34:** 91-102

Srinulgray T (2008) Genetic Diversity of Short Mackerel *Rastrelliger brachysoma* Population in the Gulf of Thailand and Andaman Sea. Master Thesis, Biotechnology, Science, Chulalongkorn University, Chulalongkorn University

Stepien C (1999) Phylogeographical structure of the Dover sole *Microstomus pacificus*: the larval retention hypothesis and genetic divergence along the deep continental slope of the northeastern Pacific Ocean. *Molecular Ecology* **8:** 923-939

Suppapan J (2015) Population genetic structure of greenback mullet (*Liza subviridis*) in Gulf of Thailand : implication for conservation. *Phranakon Rajabhat Research* **10:** 157-175

Swanson BO, Gibb AC (2004) Kinematics of aquatic and terrestrial escape responses in mudskippers. *Journal of experimental biology* **207:** 4037-4044

Swennen C, Ruttanadakul N, Haver M, Piummongkol S, Prasertsongscum S, Intanai I, Chaipakdi W, Yeesin P, Horpet P, Detsathit S (1995) The five sympatric mudskippers (Teleostei: Gobioidea) of Pattani area, southern Thailand. *Natural History Bulletin of the Siam Society* **42:** 109-129

Takegaki T (2008) Threatened fishes of the world: *Boleophthalmus pectinirostris* (Linnaeus 1758)(Gobiidae). *Environmental Biology of Fishes* **81:** 373-374

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular biology and evolution* **30:** 2725-2729

Taylor MS, Hellberg ME (2003) Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. *Science* **299:** 107-109

Tytler P, Vaughan T (1983) Thermal ecology of the mudskippers, *Periophthalmus koelreuteri* (Pallas) and *Boleophthalmus boddarti* (Pallas) of Kuwait Bay. *Journal of Fish Biology* **23:** 327-337

Waples RS (1998) Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *Journal of Heredity* **89:** 438-450

Wattayakorn G (2006) Environmental issues in the Gulf of Thailand. In *The Environment in Asia Pacific Harbours*, Eric Wolanski PhD D, FTSE, FIE Aust (ed), pp 249-259. Springer Netherlands

Weersing KA, Toonen RJ (2009) Population genetics, larval dispersal, and demographic connectivity in marine systems. *Marine Ecoogy Progress Series* **393:** 1-12

Whitmore TC, Sayer JA. (1992) Tropical deforestation and species extinction. Chapman & Hall.

Wilson AC, Cann RL, Carr SM, George M, Gyllensten UB, Helm-Bychowski KM, Russell G, Palumbi SR, Prager EM, Sage RD (1985) Mitochondrial DNA and two

perspectives on evolutionary genetics. *Biological Journal of the Linnean Society* **26:** 375-400

Winemiller KO, Rose KA (1992) Patterns of life-history diversification in North American fishes: implications for population regulation. *Canadian Journal of Fisheries and Aquatic Sciences* **49:** 2196-2218

Zhang Q, Hong W, Dai Q, Zhang J, Cai Y, Huang J (1989) Studies on induced ovulation, embryonic development and larval rearing of the mudskipper (*Boleophthalmus pectinirostris*). *Aquaculture* **83:** 375-385

Zhang YT, Ghaffar MA, Li Z, Chen W, Chen SX, Hong WS (2016) Complete mitochondrial genome of the mudskipper *Boleophthalmus boddarti* (Perciformes, Gobiidae). *Mitochondrial DNA* **27:** 62-64

Zhao WH, Hong WS, Wu DX, Jiang XX, Zhang QY (2002) Induced maturation and spawning by pheromones in female mudskipper (*Boleophthalmus pectinirostris*). *Journal of Oceanography In Taiwan Strait* **21:** 284-291

สนิท อักษรแก้ว (2545) ประชากรและทรัพยากรชายฝั่งทะเล (รวมบทความทางวิชาการ). ทุนเมธีวิจัยอาวุโส สำนักงานกองทุนสนับสนุนการวิจัย (สกว.) Vol. 1, วิทยาลัยประชากรศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย: วิทยาลัยประชากรศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย.

APPENDIX

VITA

Mr. Pachoensuk Theeranukul was born on April 30th, 1990 in Chanthaburi, Thailand. He finished his high school from Benchamarachuthit chanthaburi in 2007. He received the Bachelor's Degree of Science from Department of Biology, Faculty of Science, Mahidol University in 2011. At present, he is graduate candidate in the Master's Degree in Marine science, Faculty of Science, Chulalongkorn University.

Schorlarship

Present – 2008 Development and Promotion of Science and Technology Talents Project(DPST) Scholarship from National Science and Technology Development Agency (NSTDA), Thailand, to study B.Sc. -Ph.D.

2015 Scholarship from Development and Promotion of Science and Technology Talents Project (DPST) to conduct 6-month research at school of Marine and Bioscience, Kitasato University, Kanagawa, Japan

Academic proceeding

Pachoensuk Theeranukul, Jes Kettratad, and Sanit Piyapattanakorn. GENETIC STRUCTURE OF BLUE-SPOTTED MUDSKIPPER, Boleophthalmus boddarti, IN THE GULF OF THAILAND, REVEALED BY MITOCHONDRIAL DNA. The 26th national conference of Thaksin University-Intergrating Research into the Society. May 26th - 29th ,2016. Buri sriphu boutique hotel, Hatyai, Songkla, Thailand. page 724-749.