ความหลากหลายทางพันธุกรรมของประชากรปลาทู *Rastrelliger brachysoma* ในอ่าวไทยและทะเลอันดามัน

นายธีระรักษ์ ศรีนวลกราย

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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GENETIC DIVERSITY OF SHORT MACKEREL *Rastrelliger brachysoma*

POPULATIONS IN THE GULF OF THAILAND AND ANDAMAN SEA

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ธีระรักษ์ ศรีนวลกราย: ความหลากหลายทางพันธุกรรมของประชากรปลาทู *Rastrelliger brachysoma* ในอ่าวไทยและทะเลอันดามัน. (GENETIC DIVERSITY OF SHORT MACKEREL *Rastrelliger brachysoma* POPULATIONS IN THE GULF OF THAILAND AND ANDAMAN SEA) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร. ศานิต ปิยพัฒนากร, 112 หน้า.

ปลาทูเป็นปลาผิวน้ำที่มีความสำคัญทางเศรษฐกิจพบกระจายทั่วไปบริเวณชายฝั่งอ่าวไทยและทะเลอันดามัน การศึกษานี้ทำการตรวจสอบความหลากหลายทางพันธุกรรม โครงสร้างพันธุศาสตร์ประชากรและ phylogeographic relationships ของปลาทูในน่านน้ำไทย โดยวิเคราะห์ด้วยวิธี Inter-simple sequence repeat (ISSR) และการหาลำดับเบสของไมโตคอนเดรียลดีเอ็นเอ ตรวจสอบความหลากหลายทางพันธุกรรมและโครงสร้างพันธุศาสตร์ประชากรด้วยการวิเคราะห์ ISSR จากการสำรวจ ISSR primer 49 ตัว มี 5 ตัวที่ให้ผลที่เชื่อถือได้และมีโพลิมอร์ฟิซึม (HB13, HB15, UBC811, UBC840 และ UBC841) หลังจากสำรวจความหลากหลายทางพันธุกรรมของปลาทู 276 ตัวอย่างจาก 8 สถานี ได้แก่ จันทบุรี ระยอง สมุทรสงคราม ประจวบคีรีขันธ์ สุราษฎร์ธานี สงขลา สตูล และกระบี่ พบว่า มีแถบ DNA ที่สามารถเก็บข้อมูลได้ทั้งหมด 52 แถบ มีแถบดีเอ็นเอให้โพลิมอร์ฟิซึม 42 แถบ (80.77%) พบความหลากหลายทางพันธุกรรมของปลาทูมีค่าค่อนข้างสูง (PPB: 80.77%, *H*: 0.1485, *I*: 0.2373) ประชากรที่มีความหลากหลายสูงสุดและต่ำสุดคือประชากรจากจังหวัดสตูล (PPB: 46.15%, *H*: 0.1336, *I*: 0.2064) และจังหวัดสุราษฎร์ธานี (PPB: 28.85%, *H*: 0.0887, *I*: 0.1356) ตามลำดับ ความห่างทางพันธุกรรมระหว่างประชากรแต่ละคู่มีค่าตั้งแต่ 0.0061 ถึง 0.1226 แผนภาพความสัมพันธ์ทางพันธุกรรมของประชากรปลาทูแสดงการแบ่งกลุ่มของประชากรออกเป็น 3 กลุ่มอย่างชัดเจนคือ อ่าวไทยตอนบน (จันทบุรี ระยอง สมุทรสงคราม ประจวบคีรีขันธ์ และสุราษฎร์ธานี) อ่าวไทยตอนล่าง (สงขลา) และทะเลอันดามัน (สตูลและกระบี่) การวิเคราะห์โครงสร้างพันธุศาสตร์ประชากรด้วยวิธี AMOVA แสดงความแตกต่างทางพันธุกรรมอย่างมีนัยสำคัญระหว่างตัวอย่างปลาทูในแต่ละประชากร ($P<0.001$) เมื่อแบ่งประชากรทั้งหมดออกเป็น 2 พื้นที่ (อ่าวไทยและทะเลอันดามัน) แสดงความแตกต่างทางพันธุกรรมอย่างมีนัยสำคัญระหว่างพื้นที่ (F_{ct}: 0.1984, $P=0.0342$) ระหว่างประชากรในแต่ละพื้นที่ (F_{sc}: 0.2223, $P<0.001$) และระหว่างตัวอย่างทั้งหมดในแต่ละประชากร (F_{st}: 0.3766, $P<0.001$) พบความสัมพันธ์ระหว่างความห่างทางพันธุกรรมกับระยะความห่างทางภูมิศาสตร์ของแต่ละประชากรเมื่อวิเคราะห์ด้วยวิธี Mantel test ($r = 0.6925$, $P<0.0003$)

ตรวจสอบ phylogeographic relationships ของประชากรปลาทู 40 ตัวอย่างจากน่านน้ำไทยด้วยการวิเคราะห์ลำดับเบสของ partial mtDNA control region และยีน cytochrome *b* พบความความยาวเบสของ partial mtDNA control region และยีน cytochrome *b* ทั้งหมด 549 คู่เบสและ 627 คู่เบสตามลำดับ โดย variable site ของทั้งสองตำแหน่งที่ศึกษาอยู่ในระดับต่ำ วิเคราะห์ลำดับเบสของ partial mtDNA control region พบ variable sites ทั้งหมด 7 ตำแหน่งและรูปแบบ haplotype ทั้งหมด 10 รูปแบบ ไม่พบรูปแบบ haplotype จำเพาะระหว่างอ่าวไทยและทะเลอันดามัน วิเคราะห์ลำดับเบสของยีน cytochrome *b* พบ variable sites ทั้งหมด 17 ตำแหน่งและรูปแบบ haplotype ทั้งหมด 6 รูปแบบ เมื่อนำลำดับเบสของทั้งสองบริเวณนี้มาต่อกัน พบ variable sites ทั้งหมด 24 ตำแหน่งและรูปแบบ haplotype ทั้งหมด 16 รูปแบบ โดยทั้งยีน cytochrome *b* และลำดับเบสของทั้งสองบริเวณที่ต่อกันแสดง haplotype จำเพาะระหว่างอ่าวไทยและทะเลอันดามัน ศึกษา phylogeographic relationships ด้วยวิธี neighbor-joining และ maximum parsimony เมื่อวิเคราะห์ลำดับเบสของ partial mtDNA control region พบว่าไม่แสดงโครงสร้างพันธุศาสตร์ประชากรระหว่างอ่าวไทยและทะเลอันดามัน ส่วนการวิเคราะห์ลำดับเบสของยีน cytochrome *b* และลำดับเบสของทั้งสองตำแหน่งที่นำมาต่อกันแสดงโครงสร้างพันธุศาสตร์ประชากรระหว่างอ่าวไทยและทะเลอันดามัน อย่างไรก็ตามพบว่าประชากรจากสถานีสงขลาน่าจะแยกออกจากประชากรจากสถานีอื่นของอ่าวไทย

ข้อมูลที่ได้จากการศึกษานี้สามารถนำไปใช้ประโยชน์ในการจัดการและการวางแผนอนุรักษ์ประชากรปลาทูในประเทศไทยได้

สาขาวิชา เทคโนโลยีชีวภาพ ลายมือชื่อนิสิต ....

ปีการศึกษา 2551 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก ....

4972331523: MAJOR BIOTECHNOLOGY
KEYWORDS: GENETIC DIVERSITY/ *Rastrelliger brachysoma*

THEERARAK SRINULGRAY: GENETIC DIVERSITY OF SHORT MACKEREL *Rastrelliger brachysoma* POPULATIONS IN THE GULF OF THAILAND AND ANDAMAN SEA. THESIS ADVISOR: ASSIST. PROF. SANIT PIYAPATTANAKORN, Ph.D., 112 pp.

Short mackerel *Rastrelliger brachysoma* is an economical pelagic fish commonly found widely distributed along the coast of the Gulf of Thailand and Andaman Sea. The present study is to use Inter-simple sequence repeat (ISSR) and mitochondrial DNA sequencing methods to investigate genetic diversity, population genetic structure and phylogeographic relationships of *R. brachysoma* in Thai waters. ISSR method was used to investigate genetic diversity and population genetic structure of *R. brachysoma*. Forty-nine primers were screened, five reliable and polymorphic primers (HB13, HB15, UBC811, UBC840 and UBC841) were obtained and used. After the investigation on genetic diversity of two hundred and seventy-six *R. brachysoma* samples from eight sites (Chanthaburi, Rayong, Samut Songkhram, Prachuap Khiri Khan, Surat Thani, Songkhla, Satun and Krabi), fifty-two DNA bands can be scored, of which forty-two were polymorphic (80.77%). High genetic diversity at species level was found (PPB: 80.77%, H: 0.1485, I: 0.2373). The highest and lowest genetic diversity within population were detected in Satun (PPB: 46.15%, H: 0.1336, I: 0.2064) and Surat Thani (PPB: 28.85%, H: 0.0887, I: 0.1356) respectively. Pairwise genetic distances among populations ranged from 0.0061 to 0.1226. UPGMA dendrogram based on Nei's genetic distances divided the populations of *R. brachysoma* into three groups, the upper area of Gulf of Thailand (Chanthaburi, Rayong, Samut Songkhram, Prachuap Khiri Khan, and Surat Thani), the southern area of the Gulf of Thailand (Songkhla) and Andaman Sea (Satun and Krabi). The hierarchical analysis of molecular variance tested by AMOVA showed highly significant genetic differences among populations (P<0.001). When the populations divided into two regions (the Gulf of Thailand and Andaman Sea), which showed significant genetic differentiation among the regions (F_{ct}: 0.1984, P=0.0342), among populations within regions (F_{sc}: 0.2223, P<0.001) and within populations (F_{st}: 0.3766, P<0.001). Mantel test showed correlation between genetic distances and geographic distances (r = 0.6925, P<0.0003).

The partial mtDNA control region and cytochrome *b* gene sequencing method was used to investigate phylogeographic relationships of 40 *R. brachysoma* samples in Thai waters. The sequences of partial mtDNA control region (549 base pairs) and the mitochondrial DNA cytochrome *b* gene (627 base pairs) were obtained. The variable sites of the two sequences were low. The 7 variable sites and 10 haplotypes of partial mtDNA control region sequences were identified and no distinct haplotype between the Gulf of Thailand and Andaman Sea. The 17 variable sites and 6 haplotypes of cytochrome *b* gene sequences and the 24 variable sites and 16 haplotypes of the combined sequence of the two regions showed unique haplotype for the Gulf of Thailand and Andaman Sea. Phylogeographic relationships were established using neighbor-joining and maximum parsimony methods. The partial mtDNA control region sequences did not showed population genetic structure between the Gulf of Thailand and Andaman Sea. Cytochrome *b* gene and the combined sequence of the two regions showed population genetic structure between the Gulf of Thailand and Andaman Sea. However, Songkhla population is likely to be separated from other populations of the Gulf of Thailand.

The information obtained from this study will be useful for stock management and conservation plans of *R. brachysoma* in Thailand.

Field of Study : Biotechnology Student's Signature ธีรรักษ์ ศรีนุลเกรย์

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LIST OF ABBREVIATIONS

bp	Basepair
^oC	Degree Celcius
cm	Centimeter
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
EDTA	Ethylenediaminetetraacetic acid
g	Gram
kb	Kilobase
km	Kilometer
m	Meter
M	Molar
mg	Milligram
$MgCl_2$	Magnesium Chloride
ml	Milliliter
mm	Millimeter
mM	Millimolar
mtDNA	Mitochondrial DNA
NaCl	Sodium Chloride
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
rpm	Revolution per minute
SDS	Sodium dodecyl sulfate
TBE	Tris Borate EDTA
TNE	Tris NaCl EDTA
Tris	Tris (hydroxyl methyl) aminomathane
U	Unit
µl	Microliter
µM	Micromolar
UV	Ultraviolet
v/v	Volume by volume
w/v	Weight by volume

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CHAPTER I

GENERAL INTRODUCTION

1.1 General Introduction

Short mackerel *Rastrelliger brachysoma* is a pelagic fish found throughout the Central Indo-West Pacific region. It is one of the most important and highest valued commercial species of pelagic fish exploited in Thailand. The capture fisheries volume of short mackerel in Thai water was increasing from 152.9 to 160.4 thousand tonnes during 2001 to 2004 (Table1.1). In 2004, the capture volume of short mackerel in Andaman Sea (38,328 tons) is smaller than the Gulf of Thailand (122,070 tonnes). As far as the capture volume of pelagic fishes that of Thailand is concerned, short mackerel is the second highest (the first highest is anchovies). However, its value is highest (4,414,624 thousand baht) (Table1.2). For the global capture, the production of short mackerel was highly increased during 1950 to 2006 (Figure1.1) (Department of Fisheries, 2006). Therefore, the high fisheries can be affected to dramatic reducing the natural resource of short mackerel.

Fisheries management can be defined as "the application of scientific knowledge to the problems of providing the optimum yield, which is prescribed on the basic of maximum sustainable yield of commercial fisheries product" (Allendorf *et al*., 1987). Currently, fisheries management of short mackerel in Thailand has been utilized many field methods such as the limitation the used of fishing gear, fishery seasons and areas. However, there is tiny information on stock structure and population dynamics of short mackerel in Thai waters. Those information is an important component of successful and suitable long-term management (Shaklee and Currens, 2003).

The definition of population structure is particularly important for fisheries management of commercial marine fish (Utter, 1994). The hypothesis of population structuring of pelagic fish may explain by environmental factors, including sea level changes and physical barriers such as ocean currents may mix fish populations from

Table1.1. Capture fisheries volume of short mackerel and total pelagic fish in Thailand during 2000-2004

Fish	Capture fisheries volume (x1000 ton)				
	2000	2001	2002	2003	2004
Short mackerel	152.9	141.3	146.4	156.2	160.4
Total pelagic fish	814.5	806.2	833.0	852.1	878.2

Source: Fisheries statistic of Thailand 2004 (Department of Fisheries, 2006).

Table1.2. Capture fisheries volume and value of short mackerel and total pelagic fish by species and fishing area in 2004

Fish	Capture fisheries volume (ton) and value (1000 bath)		
	Gulf of Thailand	Andaman Sea	Total
Short mackerel	122,070 (3,368,310)	38,328 (1,046,314)	160,398 (4,414,624)
Total pelagic fish	695,881 (15,044,308)	182,373 (4,642,579)	878,254 (19,686,887)

Source: Fisheries statistic of Thailand 2004 (Department of Fisheries, 2006).

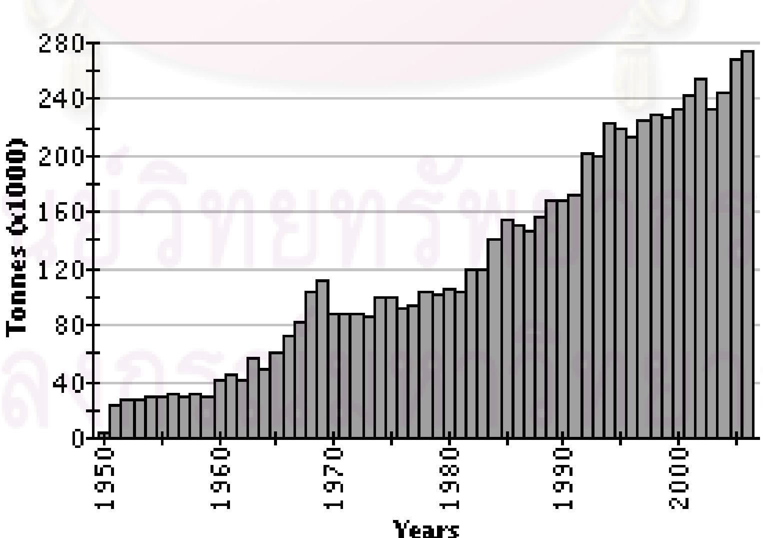

Figure1.1. Global capture production for short mackerel *Rastrelliger brachysoma*

Source: http://www.fao.org/fishery/species/2477

different geographic locations. Therefore, the increasing in geographic distance is expected to isolate among populations, and the potential for dispersal, homing to spawning zones, and larval retention may play on an important role in population structuring (McMillan and Palumbi, 1995; Johns and Avis, 1998). The first study of short mackerel population was “The investigation of short mackerel 1965”, which showed the used of tagging experiment to examine short mackerel population structure. This study suggested that there were two groups of short mackerel populations, the western and the eastern areas of the Gulf of Thailand. The western group migrated between the upper area of the Gulf of Thailand and the western to southern areas of the Gulf of Thailand, and the eastern group migrated between the eastern area of the Gulf of Thailand and Cambodia Sea. In Andaman Sea, short mackerel populations of Trang to Satun do not move to Phuket, Pang Nga and Krabi (Sutthakorn and Saranakomkul, 1987). However, there is a disadvantage of tagging experiment since the tagged fish were difficult to follow in the sea and small numbers of tagged fish were captured. Therefore, the molecular genetic techniques was a good alternative way for population study because the techniques less complicated in field study than tagging experiment.

The molecular genetic techniques offer the ability to identify and delineate fish stock and population structure where it may not be apparent from phenotypic or behavioral characteristics (Magoulas, 2005). The techniques have been used successfully to understand the mackerel structure such as *Scomber scombrus* by enzyme polymorphism analyses (Jamieson and Smith, 1987) and narrow-barred Spanish mackerel *Scomberomorus commerson* by mitochondrial DNA analyses (Hoolihan *et al*., 2006). However, the population genetic structure of short mackerel from different locations in Thailand is currently unknown.

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1.2 Biology of Short Mackerel *Rastrelliger brachysoma*

1.2.1 Classification of *R. brachysoma*

The Scombridae is a family of 15 genera and about 50 species of epipelagic marine fishes. They possess many morphological and physiological adaptations that are of interest to physiologists and evolutionary biologists. The currently accepted classification for Family Scombridae is largely based on classical morphological studies. Collette and Russo (1985) reports, Genus *Rastrelliger* belong to Family Scombridae, Subfamily Scombrinae and closely related to genus *Scomber* (Figure1.2).

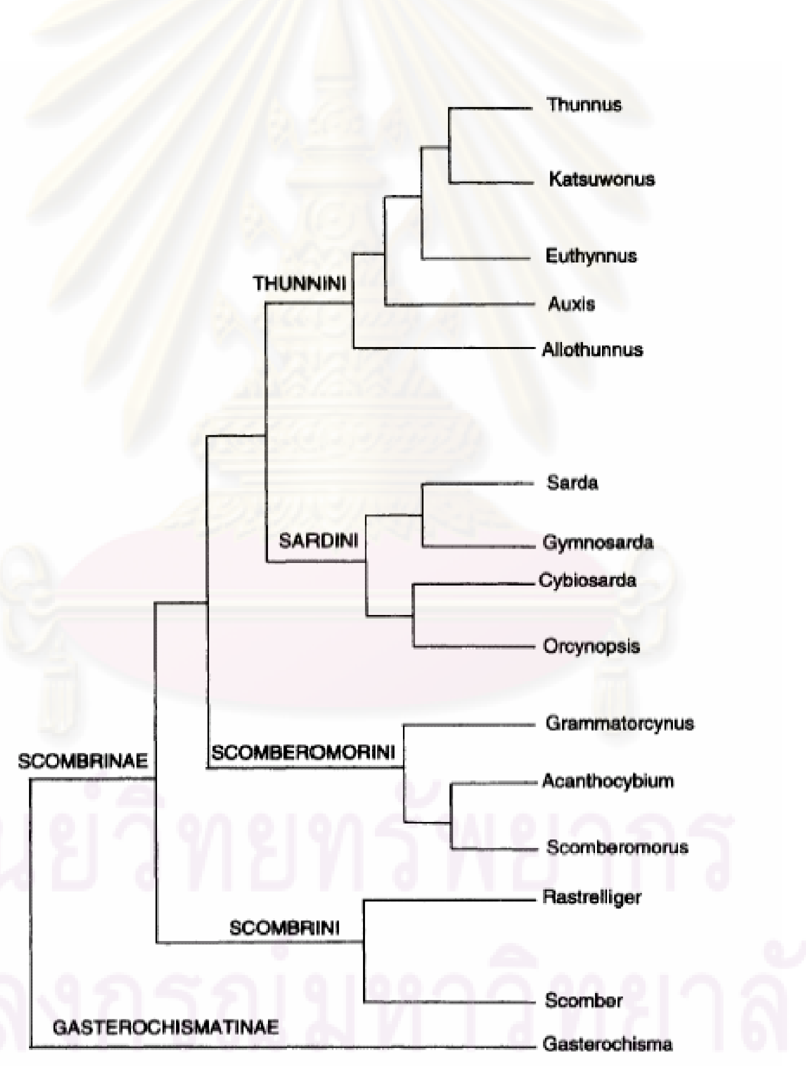

Figure1.2. Morphological tree of the Family Scombridae. (Collette and Russo, 1985).

Genus *Rastrelliger* consists of three species, namely *R. brachysoma*, *R. kanakurta* and *R. faughni*. There are unambiguous synonyms of scientific name for *R. brachysoma* such as *R. brachysomus*, *R. neglectus*, *Scomber brachypomus*, *S. brachysoma* and *S. neglectus*. Moreover, the common name of the fish species vary from location to others such as short mackerel, chub mackerel and short-bodied mackerel. However, in this study, short mackerel and *R. brachysoma* were used as common and scientific names of the fish, respectively.

1.2.2 Morphology

Externally, the short mackerel can be divided into three parts; head, body and fork. Diagnostic characters are body very deep, its depth at posterior margin of opercle 3.7 to 4.3 times in fork length and head equal or less than body depth. It has 8 to11 of dorsal spines, 12 of dorsal soft-rays, no anal spines and 12 of anal soft-rays. In addition, the spinous dorsal fin is yellowish with a black edge, pectoral and pelvic fins dusky, other fins are yellowish. The maximum fork length is 34.5 cm, commonly between 15 and 20 cm (Collette and Nauen, 1983) (Figure1.3).

Internally, the maxilla covered by lacrimal bone but extending nearly to end of lacrimal. Gill raker very long, visible when mount is opened, 30 to 48 on lower limb of first gill arch. Interpelvic process small and single. Swim bladder present. Intestine very long, 3.2 to 3.6 time fork length (Collette and Nauen, 1983).

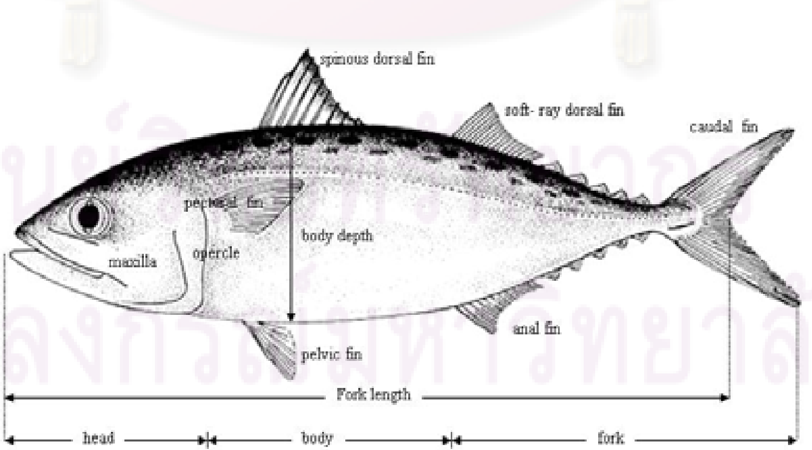

Figure1.3. External morphology of short mackerel *Rastrelliger brachysoma*

Source: http://www.fao.org/fishery/species/2477

1.2.3 Habitat and Distribution

Short mackerel *R. brachysoma* is a pelagic, neriticspecies that tolerates slightly reduced salinities in estuarine habitats and occurs in areas where surface temperatures ranging from 20 to 30°C. It forms schools of equally sized individuals. This marine fish species found disperse along the coast with the depth less than 50 m in the Gulf of Thailand and Andaman Sea (Boonprakob, 1965). Short mackerel is a pelagic fish found throughout the major part of the Central Indo-West Pacific region, including Thailand, Indonesia, Papua New Guinea, Philippines, Solomon islands and Fiji (Somjaiwong and Jullasorn, 1968; Collette and Nauen, 1983) (Figure1.4). In the Gulf of Thailand, Angthong islands are supposed to be the center of distribution on the West coast of the Gulf. From the center, short mackerel can migrate up to the coast of Surat Thani, Chumporn, Petchaburi, Samut Songkhram and Samut Sakorn provinces. For the East coast of the Gulf, Chang islands are the center of distribution, and then the short mackerel move to the coast of Trad, Chanthaburi, Rayong, Cholburi, Chachengsoa and Samut Prakarn provinces (Inthong, 1967). In Andaman Sea, short mackerel can be found along the coast, and it is abundant in inshore waters along the east coast of Phuket Island and from the Phang Nga Bay and Krabi Bay downward to the southern end of Thai waters (Sutthakorn and Saranakomkul, 1987).

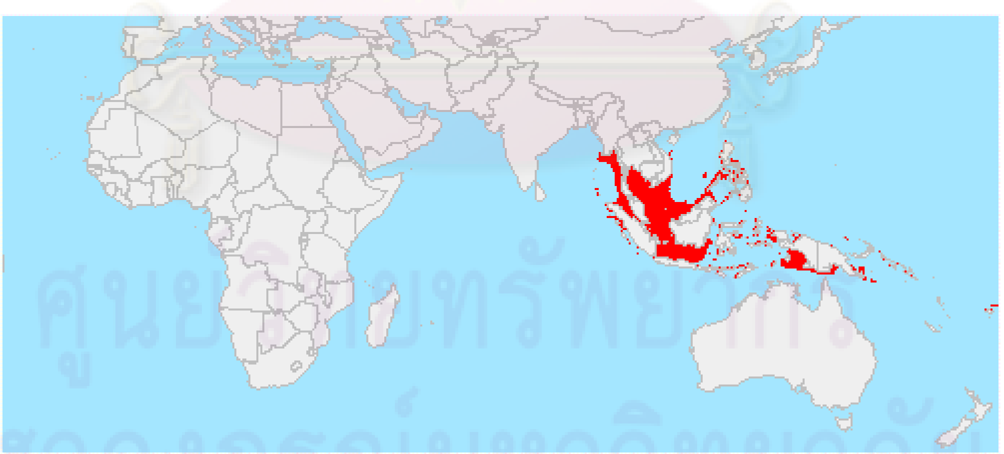

Figure1.4. Species distribution map for short mackerel *Rastrelliger brachysoma*.

Source: http://www.fao.org/fishery/species/2477

1.2.4 Reproductive Biology and Spawning Season

The estimated fecundity of female *R. brachysoma* ranging from 190 to 208 mm in length, would release approximately 20,000-30,000 eggs per batch (Boonprakob, 1965). In the Gulf of Thailand, spawning characteristics and spawning season of *R. brachysoma* between the upper area and the southern area of the Gulf were slightly difference.

In the upper area of Gulf of Thailand (Cholburi, Samut Prakarn, Samut Songkhram, Petchaburi and Prachuap Khiri Khan), the sizes at first maturity of male and female of *R. brachysoma* were 18.30 and 17.25 cm, respectively. Annual sex ratio of male and female was 1:1.21. *R. brachysoma* was partial or heterocronal spawners which can found ripen stage in every month of year but peak of Gonadosomatic Index (GSI) of was found during February to May and November. Therefore, the spawning season of *R. brachysoma* in this area could be in the period of February to May and August to October, while there was higher peak in the first period (Maila-iad *et al*., 2006). In addition, the spawning grounds of the western area of the Gulf are Prachuab Khiri Khan, Chumporn and Surat Thani (Watthanakul, 1999) and the spawning ground of the eastern area of the Gulf is Chang Island (Chomjurai *et al*., 1965).

In the southern area of the Gulf of Thailand (Nakorn Si Thammarat, Songkhla and Pattani), the sizes at first maturity of male and female *R. brachysoma* was 16.02 and 16.84 cm, respectively. Their monthly sex ratio of male and female ranged from 1:0.83 to 1:2.39. Their spawning season was found all year round with 2 peaks: December to February and May to August (Sritakon *et al*., 2006).

In Andaman Sea, the sizes at first maturity of female of *R. brachysoma* was 19.33, 16.95 and 16.79 cm in Area I, II and III, respectively (Area I: Ranong to the western area of Phuket, Area II: the eastern area of Phuket, Pang Nga to Krabi and Area III: Trang to Satun). Sex ratios were approximately 1:1 in all of three areas. Generally, *R. brachysoma* can spawn all year, but there were two peaks in three areas. In Area I, the peaks were November or December to May (with a peak in March) and July to October (with a peak in August). In Area II, the peaks were December to June (with a peak in April) and July to December (with a peak in August). In Area III, the peaks were November to March (with a peak in December) and April to September

(with a peak in May). In addition, the spawning grounds in Area II were around Yao Yai Island, Kai Island, Phi Phi Island, Lanta Yai Island, Ha Island, Rok Island and Talibong Island. In Area III, the spawning grounds were around the western part of Liang Island, Phetra Island, Ta Bai Island, Bulon Le Island, Talutao Island, Tanga Island and Adang-Rawi Islands. Gonad index (GI) and the condition factor (CF) were positively related to air temperature. However, only the gonad index was negatively related to rainfall and lavae abundance showed positive relation with sea surface temperature (Sutthakorn, 1998). The spawning season of *R. brachysoma* between the Gulf of Thailand and Andaman Sea areas was showed (see Table1.3).

Table1.3 The spawning season of *R. brachysoma* in the Gulf of Thailand and Andaman Sea areas

Spawning Areas		**Months**											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
The Gulf of Thailand	The upper		—	—	—	—			—	—	—		
	The southern	—	—	—	—	—	—	—	—	—	—	—	—
Andaman Sea	Area I	—	—	—	—			—	—	—	—	—	—
	Area II	—	—	—	—	—	—	—	—	—	—	—	—
	Area III	—	—	—	—	—	—	—	—	—		—	—

1.2.5 Fishery Area and Season

The fishery area of Thailand can be divided into four areas, namely the western, the eastern and the inner areas of the Gulf of Thailand, and Andaman Sea area. In each area the fisheries season slightly different that the western area of the Gulf of Thailand was May to March, the eastern area of the Gulf of Thailand was November to October, and the inner area of the Gulf of Thailand was August to February (Chomjurai *et al*., 1965). The fisheries season in Andaman Sea was all the year, except in South-East monsoon season (Aosomboon *et al*., 2000) (see Table1.4). The over exploitation of fisheries could be reduced short mackerel populations in the Gulf of Thailand and Andaman Sea. Therefore, no fishing in spawning season is

promoted important for control the fisheries when hatching and spawning season. The no fishing in spawning season in the period of February, 15th to May, 15th for the Gulf of Thailand and April 15th to June 15th for Andaman Sea (including Pang Nga bay, Krabi, Phuket, Trang and Satun).

Table1.4 The fishery season of *R. brachysoma* in the Gulf of Thailand and Andaman Sea areas

Fishery Areas		Months											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
The Gulf of Thailand	The western	—	—	—		—	—	—	—	—	—	—	—
	The eastern	—	—	—	—	—	—	—	—	—	—	—	—
	The inner Gulf	—	—						—	—	—	—	—
Andaman Sea		—	—	—	—	—	—	—	—	—	—	—	—

1.3 The Use of Molecular Techniques to Examine Genetic Variations

In recent years, DNA techniques have been commonly used to determine the level of genetic variation of several species (Benzie, 2000), and molecular techniques have been widely used in genetic studies. Most molecular methods on polymerase chain reaction (PCR) procedure. The introduction of PCR has opened a new approach for molecular genetic studies (Mullis and Faloona, 1987). PCR is a method for amplification of specific DNA sequences by the simultaneous primer extension of complementary strands of DNA by two oligonucleotide primers. The target DNA sequence can be synthesized from a low amount of DNA template within a few hours. Current techniques are usually easy to use, saving in time, inexpensive, and provide more genetic information. Therefore, in the following sections, molecular techniques used to detect genetic variation studies will be discussed.

At the DNA levels, source of DNA from multicellular organisms (animals) are composed of nuclear DNA and mitochondrial DNA. Molecular markers are useful for various genetic studies. Many effective markers were used for population genetic

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studies. The polymorphism from natural selection is assumed to be generated by mutation, migration, gene flow and genetic drift. Natural and artificial selection and genetic drift promote levels of genetic variation within and among individuals and species.

The molecular techniques have been developed for population genetic studies, including non-PCR base techniques or hybridization techniques (e.g. restriction fragment length polymorphism (RFLP) and Variation number of tandem repeats (VNTR)) and PCR base techniques (e.g. randomly amplified polymorphism DNA (RAPD), Amplified fragment length polymorphism (AFLP), Inter-simple sequence repeat (ISSR) and DNA sequencing).

1.3.1 Restriction Fragment Range Polymorphism (RFLP)

Restriction fragment range polymorphism (RFLP) analysis is the one of several techniques is based on the digestion of genomic DNA with restriction endonuclease to determine DNA variation. The restriction fragments were separated by electrophoresis, the same restriction fragments resulted from the homologous fragment and transferring fragments to the suitable membrane, hybridization of a labeled fragment to the target fragment and detection hybridizing fragments with autoradiography or nonradioactive approach (Weising *et al*., 1995), nonradioactive method have been developed to be an alternative method or the investigated fragments are identified by hybridization with specific radiolabeled probe (Karp *et al*., 1997).

The restriction analysis for genetic population of animal taxa has emphasized surveys of genotype frequencies, diversity and population differentiation based on polymorphism of genome. In more comprehensive studies, restriction sites, rather than of fragment length is scored. Genotype (or haplotype) frequencies can be determined by presence or absence fragments among individuals. The digested fragments are labeled with DNA probe for hybridization. Accordingly, RFLP markers have been used for develop genetic maps and phylogenetic trees. The limitations of RFLP method are laborious and expensive, used of radioactive isotopes are hazardous and require many safety precautions.

1.3.2 Variation Number of Tandem Repeats (VNTR)

Variation number of tandem repeats (VNTR) is characterized by the variable the number of repeat core sequences at specific loci in the genome. Variation in the length of the alleles patterned from the repeats provided the basis for detected the polymorphism. VNTR can be divided into three major group based on detecting the repeat length; satellites, minisatellites, and microsatellites (O'Reilly and Wright, 1995).

Multilocus DNA fingerprinting as conventional RFLP analysis, the minisatellite probe is used for detection simultaneous loci. The product is a pattern of band, this pattern is specific to an individual but it is not possible to identify alleles of the same loci or estimate levels of heterozygosity. Minisatellites is a repeating DNA sequence ranging between 15-70 bp per unit and 0.5-30 kb in size (Koreth *et al*., 1996). Minisatellites are found within noncoding region of the chromosome. The variation of this DNA can be detected which is due to differences in length between conserved restriction sites, number of copies on different chromosomes are variable, when cut by restriction enzymes produced fragments in different sizes. Single-locus minisatellites, a single locus probe is used flanking sequences as a part of probe to identify allelic products at a single locus. The banding patterns consist of homozygote or heterozygote DNA fragments. This technique is a powerful tool for genetic population studies.

1.3.3 Randomly Amplified Polymorphism DNA (RAPD)

Randomly amplified polymorphism DNA (RAPD) analysis is amplification of genomic DNA by PCR, which was developed (Williams *et al*., 1990) to analyze by polymorphism. This method use the single short arbitrary oligonucleotides sequence, usually 10 bp long with GC content at least 50% and do not contain palindromic sequences (Ellsworth *et al*., 1993) and this sequences acting as both a forward and reverse primers at low stringency (Welsh *et al*., 1991).

Accordingly, the primer is used to scan genome for small inverted sequences resulting in amplification of DNA segments of variable length (Bowditch *et al*., 1993). The products of RAPD amplification are detected as DNA fragment length

polymorphism for multiple loci by the presence or absence of band at various positions (Mullis *et al*., 1994).

RAPD can be used and has been increasingly used for population genetic study because RAPD analysis is a simple and rapid method, RAPD is unlimited number of primers available commercially, RAPD requires tiny amount of DNA for reaction. RAPD does not require probes, DNA library, and radioactive chemicals obviating complicated processes and the use of hazardous chemicals. It is method to generate genetic marker and genetic markers and DNA fingerprinting patterns without requiring any prior DNA sequence information.

RAPD-PCR method has many disadvantages such as many fragments (especially those arising from mispairing of a primer with the genomic DNA) may not be reproducible among different laboratories because amplification is sensitive to slight changes in temperature cycles, most of the amplified fragments are inherited in the dominant fashion (homozygotes and herozygotes cannot be differentiated) and RAPD bands of the same size may not actually identical, therefore, comigrating RAPD bands may not be allelic.

1.3.4 Amplified Fragment Length Polymorphism (AFLP)

AFLP is a PCR-based, that combines the strengths and overcomes the weaknesses of the RFLP and RAPD methods to generation of multi-locus fingerprinting of organisms (Vos *et al*, 1995). It is a powerful technique, especially when combined with bulked segregrant analysis (BSA), for isolation of phenotypes affected by single locus markers. In addition, fingerprinting-band patterns of AFLP are effectively used to evaluate DNA polymorphism between samples. The major strengths of the AFLP method include large (>100) numbers of polymorphic loci screened, high reproducibility due to high PCR annealing temperatures, and relatively cost effectiveness.

The molecular basis of AFLP polymorphism includes indels between restriction sites and base substitutions at restriction sits for RFLP as well as indels in the amplification regions and base substitutions at PCR primer binding site for RAPD analyses (Liu and Cordes, 2004). The unique feature of the technique is the addition

of adaptors of known sequence to DNA fragment generated by digestion of whole genomic DNA. This allows for the subsequent PCR amplification of a subset of the total fragments separated by gel electrophoresis.

AFLP begins with digestion of the whole genomic DNA with two restriction enzymes. Since sequences for the resulting DNA fragments are unknown, adaptor of known sequence are ligated to the end of the fragments and used as primer sites for PCR amplification. Since these would result in the production of millions of PCR fragments is reduced by selective amplification. The subset of amplified fragments is then analyzed by denaturing polyacrylamide gel electrophoresis followed by radioactive or non-radioactive detection.

1.3.5 Inter-Simple Sequence Repeat (ISSR)

Inter-simple sequence repeat (ISSR) is one of dominant marker that generated from single-primer polymerase chain reaction (PCR) amplifications in which the primers are based on dinucleotide and trinucleotide repeat motifs. ISSR method relies on the amplification of DNA regions located between closely-spaced, inversely oriented simple sequence repeats (SSRs or microsatellites) at multiple loci throughout the genome by mean of a single primer composed of a short microsatellite sequence (typically, 18-20 base pairs) with one to four degenerate nucleotides anchored at the 5' or 3' end of the oligonucleotide (Zietckiewicz *et al*., 1994; Salimath *et al*., 1995)

ISSR makers have many advantages that no requirement of prior information or mapping studies, development cost was inexpensive and save time. Besides, ISSR markers are nearly identical to RAPD markers but it has many advantages in overcoming limitations of RAPD techniques (Wolfe *et al*., 1998; Esselman *et al*., 1999). ISSR marker may reveal a much higher number of polymorphic fragments from every primer than RAPD (Fang and Roose, 1997; Esselman *et al*., 1999), it could be able to produce more reliable and reproducible bands because of higher annealing temperature by long sequence of ISSR primers and enabling higher stringency DNA amplification (Tsumura *et al*., 1996; Nagaoka and Ogihara, 1997; Wolfe *et al*., 1998; Qian *et al*., 2001). ISSR markers were still some significant limitation for genetic detecting as a dominant marker with less efficiency than co-dominant markers such as isozyme and microsatellite because the dominant

inheritance reduces their suitability for most relevant population inferences. However, it can be compensated by a high number of loci and a large size of sample.

1.3.6 DNA Sequencing

DNA is the main carrier of genetic information in living organisms. DNA molecules are extremely long, large, and consist of repeating nucleotides. Nucleotides are the bases of DNA and consist of adenine (A), thymine (T), guanine (G), and cytosine (C). The structure of a DNA molecule is double stranded, consisting of two DNA strands wound around each other to form a double helix. The nucleotides of the two strands are complementary to each other such that adenine cross-links with thymine (A-T), and guanine cross-links with cytosine (G-C). The goal of DNA sequencing is to determine the order of bases for a specific piece of DNA.

DNA sequencing is the process of determining the exact order of the bases A, T, C and G in a piece of DNA. There are two general methods for sequencing of DNA segment: the chemical cleavage procedure (Maxam and Gilbert, 1977) and the chain termination procedure (Sanger and Nicklen, 1977). Nevertheless, the latter method is the more popular because chemical cleavage procedure requires the use of several hazardous substances. Traditional methods of manual DNA sequencing utilize radioactive isotopes such as phosphorous-32, sulfur-35, and phosphorous-33, incorporated into specific nucleotides (A, T, C, G). Radioactive labeled nucleotides allow for reading the sequence by a technique known as autoradiography. The gel that contains the separated DNA segments is exposed to X-ray film for a period of time. The radiation causes dark spots on the film to indicate its location. Next, the film is developed to reveal the pattern of the labeled nucleotides. Since a process does not exist to discriminate the different nucleotides by the spots on the film, each labeled nucleotide must have its own lane on the gel. Therefore, four individual lanes are required for manual sequencing in order to determine the full DNA sequence. An individual must interpret the results of this process and typically the results are entered into a computer for storage and linking to other results.

DNA sequencing provides high resolution and facilitating interpretation. DNA fragments generated from PCR can be directly sequenced or alternatively, those fragments can be cloned and sequenced. This eliminates the need to establish a

genome library and searching a particular gene in the library. However, sequencing of a large number of individuals using conventional method is extremely tedious and prohibitively possible.

The enzymatic sequencing approach has presently been developed to automate method (Figure1.5). DNA sequences can be detected using a fluorescence-based system following labeling of a sequencing primer or incorporated nucleotides with a fluorescence dye. At present, automated DNA sequencing is commonly used Automated DNA sequencing equipment can eliminate the need for radioactive isotopes to label DNA, thereby reducing the volume of low-level radioactive waste generated on campus. Automated DNA sequencing provides more reliable research results than manual DNA sequencing, thus maintaining the integrity of the research. This greatly allows wider application of DNA sequencing analysis for population genetic and systematic studies.

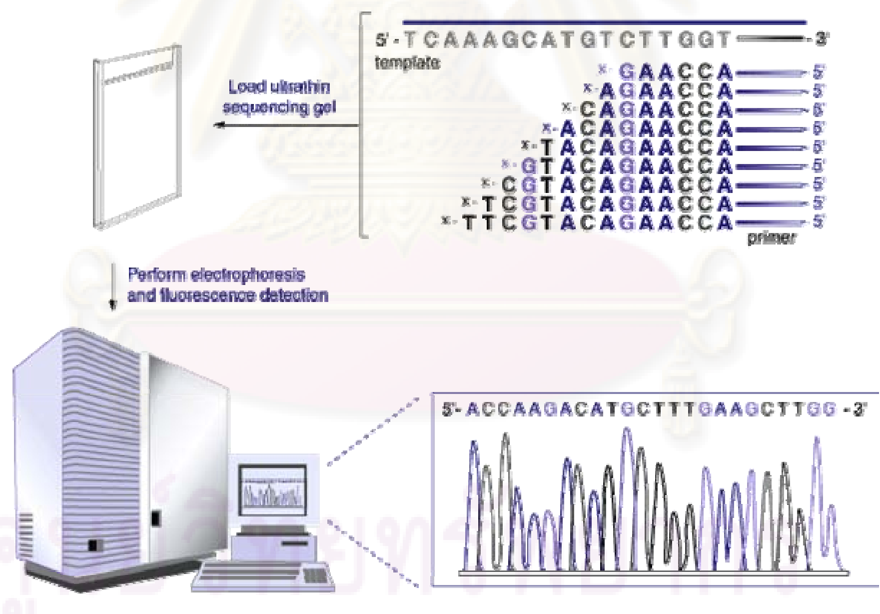

Figure1.5. Automated DNA sequencing.

Source:http://www.biochem.arizona.edu/classes/bioc471/pages/Lecture21/AMG9 .1b.gif

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1.4 Objective of the thesis

Inter-simple sequence repeat (ISSR) markers and the sequencing method for partial mitochondrial DNA control region and the mitochondrial DNA cytochrome *b* gene were used to investigate the genetic diversity, population genetic structure and phylogeographic relationships of *R. brachysoma* in Thai waters.

CHAPTER II

GENETIC DIVERSITY OF *R. brachysoma* IN THAILAND REVEALED BY INTER-SIMPLE SEQUENCE REPEAT (ISSR)

2.1 Introduction

Inter-simple sequence repeat (ISSR) markers are involved the use of polymerase chain reaction (PCR) to amplify the regions between adjacent, inversely oriented microsatellites using a single primer that composed of a short microsatellite sequence (typically, 18-20 base pairs) with one to four degenerate nucleotides anchored at the 5' or 3' end (Zietkiewicz *et al*., 1994). ISSR markers produce multi-locus patterns which are reproducible, abundant and polymorphic in genomes (Zietkiewicz *et al*., 1994). The amplification and data scoring methods used for ISSR markers are similar to RAPD markers, but ISSR markers have advantages in overcoming limitations of RAPD markers that the annealing temperature for amplification is usually higher, resulting in a higher degree of stringency for amplified fragments (Wolfe and Liston, 1998).

ISSR markers have been widely used for population and conservation genetics (Culley and Wolfe, 2001), and investigations in natural populations (Crawford *et al*., 2001). It has also demonstrated a hypervariable nature of the markers and its potential power for examined of genetic relationships within and among species and population studies in recent years (Culley and Wolfe, 2001). Moreover, based on the published, unpublished and in-progress studies that have been recommended using ISSR markers, it is clear that ISSR markers have great potential for studies of natural populations (Wolfe *et al*. 1998).

ISSR markers were introduced and used in the genetic study on cultivated plants (Zietkiewicz *et al*., 1994), fungi (Kerrigan *et al*., 2003), and animals (Chatterjee and Mohandas, 2003). Initially, ISSR markers were used in the population genetic study on plants such as *Nelumbo nucifera* (Chen *et al*., 2008), Croomia (Li *et al*., 2008), and *Camellia sinensis* L. (Thomas *et al*., 2006). Later, the technique has been

used in the population genetic studies on other organisms. For marine species, many studies were reported, such as *Cynoglossus semilaevis* (Liu *et al*., 2008), *Mactra veneriformis* (Hou *et al*., 2006), *Paralichthys olivaceus* (Liu *et al*., 2006), *Apostichopus japonicus* (Bing *et al*., 2007) and Mediterranean cyprinodontiform fish (Maltagliati *et al*., 2006). However, the population genetic study on scombridae species using ISSR markers has not been reported.

In this chapter, the investigation on the genetic diversity and population genetic structure of *R. brachysoma* in Thai water, using ISSR marker was discussed.

2.2 Materials and Methods

2.2.1 Tissue Sampling

Short mackerel *Rastrelliger brachysoma* samples were collected from local fisheries to make sure that the fish are from the eight areas in the Gulf of Thailand and Andaman Sea (Figure2.1). Two hundred and seventy-six individuals were collected (see Table2.1). All tissue samples were immediately placed into absolute ethanol and were stored at -20°C until required.

Table2.1. Details of examined *R. brachysoma* populations consisting of locations, population codes, latitudes and longitudes, sample sizes and collection dates

Locations	Population codes	Latitudes and longitudes	Sample sizes	Collection dates
Songkhla	SOK	7°12’N 100°35’E	38	November, 2006
Surat Thani	SRT	9°13’N 99°30’E	34	October, 2006
Prachuap Khiri Khan	PKK	11°48’N 99°47’E	36	October, 2006
Samut Songkhram	SSK	13°23’N 99°51’E	38	September, 2006
Rayong	RAY	12°39’N101°16’E	38	October, 2006
Chanthaburi	CTB	12°28’N102°04’E	26	October, 2006
Satun	SAT	6°50’N 99° 47’E	34	March, 2007
Krabi	KRB	8°03’N 98° 55’E	32	February, 2007

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Figure2.1. Sampling sites of *R. brachysoma* in the Gulf of Thailand and Andaman Sea. Six populations of the Gulf of Thailand (♦) consist of SOK (Songkhla), SRT (Surat Thani), PKK (Prachuap Khiri Khan), SSK (Samut Songkhram), RAY (Rayong) and CTB (Chanthaburi). Two populations of Andaman Sea (♦) consist of SAT (Satun) and KRB (Krabi).

2.2.2 DNA Extraction

Genomic DNA was extracted from body muscle tissue of each *R. brachysoma* individual using a modified salting out procedure (Miller *et al*., 1988). tissue about 10 mg after removing from a –20°C were transferred into a 1.5 ml microcentrifuge tube containing 485 µl of TNE + 1% SDS buffer (50 mM Tris-base; pH 8.0, 100mM NaCl, 5 mM EDTA; pH8.0, 1% SDS (w/v)). Total proteins were digested by addition of 15 µl of 10 mg/ml proteinase-K solution. The resulting mixture was incubated at 55°C for 3 hours. To remove digested proteins, 250 µl of 6 M Sodium Chloride was added. The sample mixture was then centrifuged at 10,000 rpm for 8 minutes. The aqueous phase was transferred to a new 1.5 ml micorcentrifuge tube. DNA was precipitated by addition of two volume of absolute ethanol and kept at –20°C overnight to ensure complete precipitation. DNA was recovered by centrifugation at 14,000 rpm for 15 minutes before removing ethanol. DNA pellet was air-dried at room temperature and redissolved with appropriate amount with 20 µl TE buffer (10 mM Tris, 0.1 mM EDTA) and kept at –20°C for further analysis.

2.2.3 Agarose Gel Electrophoresis

2.2.3.1 Genomic DNA Analysis

To determine the quality and quantity of the extracted DNA using agarose gel electrophoresis, the loading sample consist of 1 µl of extracted DNA, 2 µl of loading dye (standard stain orange G, 40% (v/v) glycerol) and 6 µl of distilled water was mixed thoroughly. A 0.8 % agarose gels was prepared by weighting 0.4 g of GenePure LE Agarose (Research Organic, Inc) and mixing with 50 ml of 0.5X TBE buffer (0.89 M Tris-base, 0.89 M boric acid and 0.02 M EDTA) in 200 ml flask. The agarose suspension was heated in a microwave about 1 minute (two times) for completely dispersed. Next, the melted agarose was cooled down at room temperature about 10 minutes, 4 µl of 0.4% (w/v) ethidium bromide solution was added and mixed. The mixed solution was poured into the sealed gel tray and the appropriate combs were inserted, the air bubble was removed, and the gel was set at room temperature about 1 hour. The two combs were removed ensuring the gel completely set, the gel was placed in the horizontal electrophoretic chamber containing 0.5X TBE buffer in both wells and extra TBE was added to cover the gel approximately 0.5 cm from the surface of gel. The samples were loaded into each well by using an

automatic micropipette. Concentrations of extracted DNA were estimated by comparison with know quantities of λDNA/Hind III marker (Fermentas). The gel chamber was connected to a power supply and electrophoresis was run at 80 volts for approximately 30 minutes. The DNA bands were visualized as fluorescent bands on a UV transilluminator and photographed using the gel document system (Bio-Rad). The extracted DNA was adjusted to approximately 20-30 ng/μl for use in PCR amplification.

2.2.3.2 ISSR PCR Product Analysis

To determine the quality and quantity of ISSR PCR products using agarose gel electrophoresis, the loading sample consist of 25 μl of PCR product and 5 μl of loading dye (standard stain orange G, 40% (v/v) glycerol) was mixed thoroughly. A 2.0% agarose gels was prepared by weighting 5 g of GenePure LE Agarose (Research Organic, Inc) and mixing with 250 ml of 0.5X TBE buffer (0.89 M Tris-base, 0.89 M boric acid and 0.02 M EDTA) in 500 ml flask. The agarose suspension was heated in a microwave about 3 minutes for completely dispersed. Next, the melted agarose was cooled down at room temperature about 15 minutes, 10 μl of 0.4% (w/v) ethidium bromide solution was added and mixed. The mixed solution was poured into the sealed gel tray and the appropriate combs were inserted, the air bubble was removed, and the gel was set at room temperature about 2 hour. The two combs were removed ensuring the gel completely set, the gel was placed in the horizontal electrophoretic chamber containing 0.5X TBE buffer in both wells and extra TBE was added to cover the gel approximately 0.5 cm from the surface of gel. The samples were loaded into each well by using an automatic micropipette. The size of ISSR PCR products were estimated by comparison with 100 bp ladder DNA marker (Fermentas). The gel chamber was connected to a power supply and electrophoresis was run at 100 volts for approximately 4 hours. The DNA bands were visualized as fluorescent bands on a UV transilluminator and photographed using the gel document system (Bio-Rad).

2.2.4 Primer Screening and Optimization

ISSR primer sequences were repeated dinucleotide and trinucleotide primers. The primers in this study were commercially synthesized (Bio Basic Inc., GeneWorks Pty Ltd., and 1st Base Pty Ltd). Forty-nine primers were screened with *R. brachysoma*

DNA (Table2.2). Initial PCR condition for screening primers was performed in a 25 μl reaction volume containing; 1 μl template DNA (approximately 20-30 ng/μl), 1X reaction buffer with 2.0 mM $MgCl_2$ (Real Biotech Corp.), 0.25 mM dNTPs (Promega), 1 μM primer (Bio Basic Inc., GeneWorks Pty Ltd., and 1st Base Pty Ltd.), and 1.0 U *Taq* DNA polymerase (Real Biotech Corp.). The amplification was followed by 94°C for 5 minutes and 45 cycles of denaturation at 94°C for 45 seconds, annealing at 45°C for 45 seconds, and extension at 72°C for 2 minutes. The amplification products were detected using 2.0% agarose gel. Primers providing reproducible, stable and polymorphic ISSR profiles were selected for the next optimization step. The selected primers were optimized by adjusting $MgCl_2$ concentration, primer concentration, annealing temperature and number of amplification cycles to improve the clarification of ISSR profile.

Table2.2. Forty-nine ISSR primers used to screen for amplification of *R. brachysoma*

No.	Primer	Sequence (5'-3')	No.	Primer	Sequence (5'-3')
1	UBC809	$(AG)_8G$	26	T8711	$(CA)_7YG$
2	UBC811	$(GA)_8C$	27	T8712	$(GA)_8AT$
3	UBC827	$(AC)_8G$	28	T8713	$(CT)_8G$
4	SAS1	$(GTG)_4C$	29	T8714	$(GT)_6RG(CT)_8T$
5	SAS3	$(GAG)_4C$	30	T8715	$(GA)_6C$
6	UBC814	$(CT)_8TG$	31	T8716	$(CA)_6C$
7	844A	$(CT)_8AC$	32	T8717	$(CA)_6T$
8	844B	$(CT)_8GC$	33	T8718	$(GA)_6T$
9	17898A	$(CA)_6AC$	34	UBC813	$(CT)_8T$
10	17898B	$(CA)_6GT$	35	UBC814	$(CT)_8A$
11	17899A	$(CA)_6AG$	36	UBC824	$(CT)_8G$
12	HB12	$(AC)_3GC$	37	UBC845	$(CT)_8RG$
13	HB13	$(GAG)_3GC$	38	UBC840	$(GA)_8YT$
14	HB14	$(CTC)_3GC$	39	UBC848	$(CA)_8RG$
15	HB15	$(GTG)_3GC$	40	TL01	$(CAG)_5$
16	T8701	$(CT)_8RA$	41	TL02	$(CAA)_5$
17	T8702	$(AG)_7YC$	42	TL03	$(GACA)_4$
18	T8703	$(GT)_6YR$	43	TL04	$(GATA)_4$
19	T8704	$(GT)_6AY$	44	UBC812	$(GA)_8A$
20	T8705	$CAA(AG)_5$	45	UBC826	$(AC)_8C$
21	T8706	$GGGC(GA)_8$	46	UBC841	$(GA)_8YC$
22	T8707	$(GAG)_4RC$	47	UBC857	$(AC)_8YC$
23	T8708	$(GA)_7RG$	48	UBC818	$(CA)_8G$
24	T8709	$(GT)_7YG$	49	UBC868	$(GAA)_6$
25	T8710	$(CA)_7YC$			

Mixed bases nomenclature: R=A/G and Y=C/T

2.2.5 ISSR PCR Amplification

ISSR PCR amplification were performed in a 25 µl reaction volume containing 1 µl template DNA (approximately 20-30 ng/µl), 1X reaction buffer with 2.0 mM $MgCl_2$ (Real Biotech Corp.), 0.25 mM dNTPs (Promega), 1 µM primer (Bio Basic Inc., GeneWorks Pty Ltd., and 1st Base Pte Ltd.), and 1.0 U *Taq* DNA polymerase (Real Biotech Corp.). The amplification was followed by 94°C for 5 minutes and 45 cycles of denaturation at 94°C for 45 seconds, annealing at the proper temperature for 45 seconds, and extension at 72°C for 2 minutes, and a final extension at 72°C for 10 minutes. Amplification products were detected using 2.0% agarose gel electrophoresis.

2.2.6 Data Analysis

Assuming two alleles per locus, ISSR profiles were manually scored for each individual in binary character matrix based on the presence as 1 or absence as 0 of amplified bands. Only reproducible DNA bands or loci were selected for data analysis. Three comparable estimators, including the percentage of polymorphic band (PPB), Nei's (1973) genetic diversity (*H*), and Shannon indices of diversity (*I*) (Lewontin, 1960) were used to investigate genetic diversity for each population by using POPGEN 1.32 software (Yeh *et al*., 1999). Assuming Hardy-Weinberg equilibrium, Nei's unbiased genetic distances (Nei, 1978) were determined using POPGEN 1.32 software. AMOVA (Excoffier *et al*., 1992) was used to estimate parameter F-statistic for describe genetic differentiation of intra-population and inter-population was also performed using ARLERQUIN program. To construct Unweighted Pair-group Method Using Arithmetic Average (UPGMA) dendrogram based on Nei's unbiased genetic distances by PHYLIP version 3.67 (Felsenstein, 2007), and draw dendrogram using Treeview (Win32) 1.6.6 program. Mantel test was determined whether the matrix of Nei's unbiased genetic distances between *R. brachysoma* populations correlated with the matrix of geographic distances between locations by 10,000 random permutations, using ARLERQUIN program.

2.3 Results

2.3.1 DNA Extraction

Genomic DNA was extracted from muscle of *R. brachysoma* using salting out method (Miller *et al.*, 1988). The quality and quantity of extracted genomic DNA were determined by using 0.8% agarose gel electrophoresis and comparing with λDNA/Hind III marker. The concentration of extracted genomic at approximately 45-200 ng/μl and the high molecular weight DNA at approximately 23.1 kb were obtained (see Figure2.2). The extracted DNA was adjusted to approximately 20-30 ng/μl for use in PCR amplification.

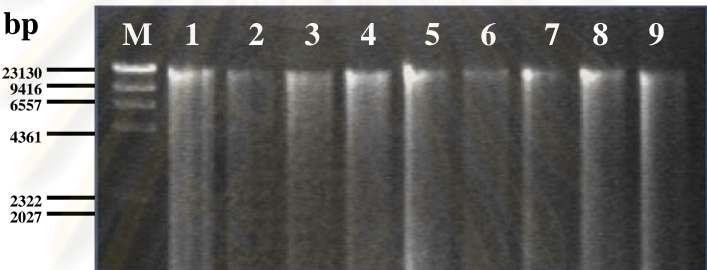

Figure2.2. 0.8% ethidium bromide stained agarose gel showing the quality of total DNA extracted from the muscle of each *R. brachysoma* individual (lanes 1-9). Lane M is λDNA/Hind III marker.

2.3.2 Primer Screening and Optimization

In total, forty-nine commercially synthesized primers were screened for amplification of *R. brachysoma* DNA. After screening, five primers (HB13, HB15, UBC811, UBC840 and UBC841) of forty-nine primers were able to amplify the reproducible and stable bands, and their polymorphic bands were more in contrast. Five primers were selected for further analysis of genetic diversity of *R. brachysoma* populations.

The optimum ISSR amplification parameter consist of all primer were di-repeat nucleotides, the $MgCl_2$ concentrations of all selected primer were 2.0 mM and the annealing temperature at the proper temperature of each primers and the number of amplification cycle in PCR reaction was 45 cycles. The details of ISSR primers and optimized parameters of PCR reaction of each primer were showed (Table2.3).

Table2.3. List of ISSR primers, their sequence and optimized parameters used for amplification consist of $MgCl_2$ concentration and annealing temperature

ISSR primer	Sequence (5’-3’)	$MgCl_2$ concentration (mM)	Annealing temperature (°C)
HB13	$(GAG)_3GC$	2.0	45
HB15	$(GTG)_3GC$	2.0	45
UBC811	$(GA)_8C$	2.0	50
UBC840	$(GA)_8YT$	2.0	45
UBC841	$(GA)_8YC$	2.0	45

Mixed bases nomenclature: Y= C/T

2.3.3 ISSR PCR Amplification

Forty-nine ISSR primers were screened and five of them were able to amplify the reproducible, stable and polymorphic ISSR profiles of the primers (see Figure2.3). A total of 276 *R. brachysoma* individuals from eight populations were investigated and 52 bands were amplified, of which 42 bands were polymorphic (80.77%), and the ranging in size of each primer were showed in Table2.4, corresponding to an average of 10 bands per primer.

Table2.4. Summary of ISSR polymorphism of each primer consisting of total DNA bands, number of polymorphic bands, percentage of polymorphism and size range of DNA bands

ISSR primer	Total DNA bands	Number of polymorphic bands	Percentage of polymorphism (%)	Size range of DNA bands (bp)
HB13	10	8	80	400-800
HB15	9	7	77.78	700-1400
UBC811	10	8	80	350-700
UBC840	12	11	91.67	400-800
UBC841	11	8	72.72	500-1400

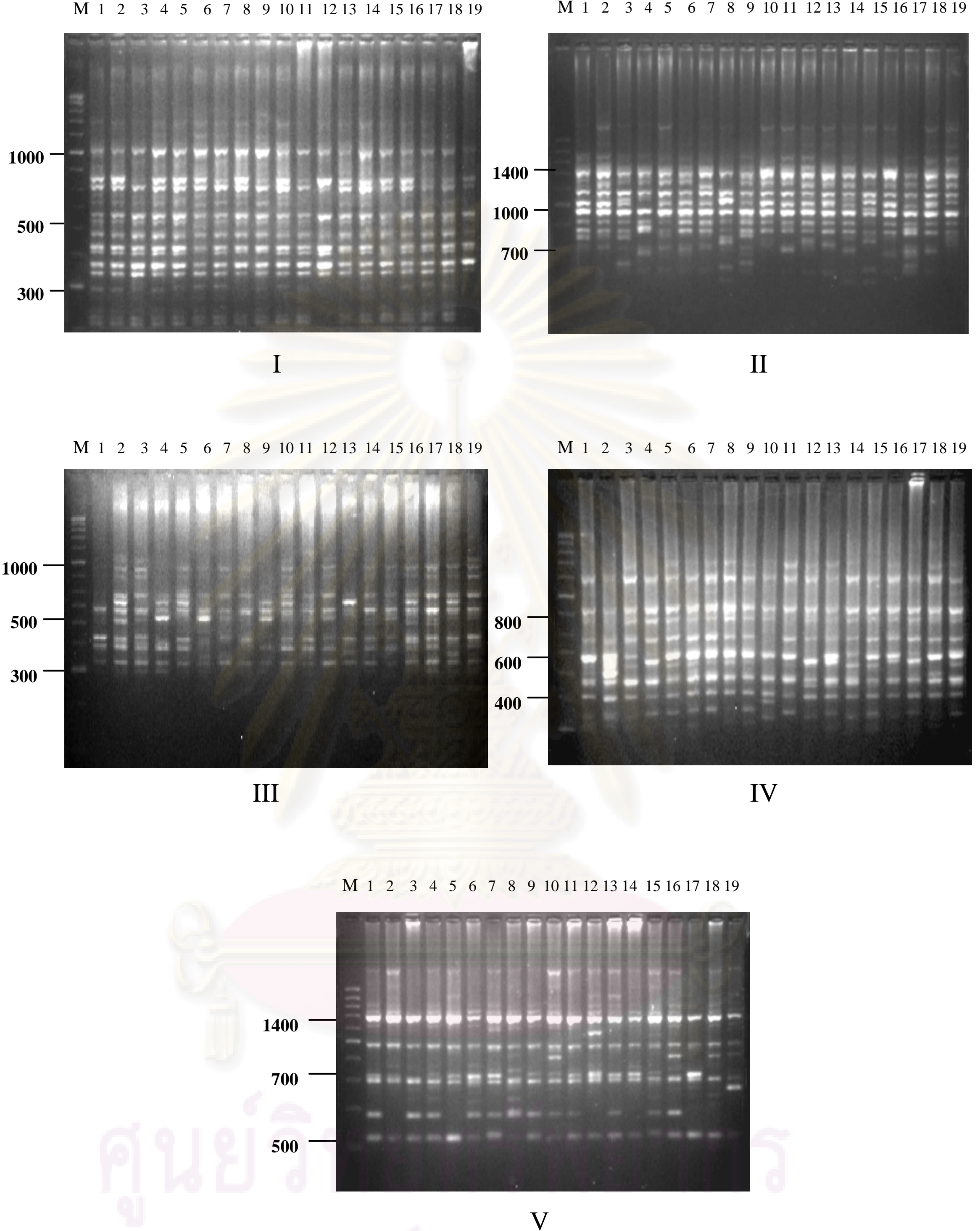

Figure2.3. 2.0% ethidium bromide stained agarose gel showing the ISSR profile of eight *R. brachysoma* populations using five primers. I: primer HB13, II: primer HB15, III: primer UBC811, IV: primer UBC840 and V: primer UBC841. Lanes M showed 100 bp ladder DNA markers. Lanes 1-19 were of *R. brachysoma* samples.

2.3.4 Data Analysis

2.3.4.1 Genetic Diversity of *R. brachysoma*

At species level, the percentage of polymorphic bands was 80.77, the Nei's genetic diversity index (*H*) was 0.1485±0.1801, and the Shannon information index (*I*) was 0.2373±0.2505. At the population level were showed in Table2.5, the percentage of polymorphic bands ranging from 28.85% to 46.15%; the lowest was the SRT population and the highest were the SOK and SAT populations. The Nei's genetic diversity index (*H*) were ranging from 0.0887±0.1591 to 0.1336±0.1791 and the Shannon information index (*I*) were ranging from 0.1356±0.2404 to 0.2064±0.2607; the lowest of *H* and *I* were the PKK and SRT populations respectively, and the highest was the SAT population (see Table2.5).

Table2.5. Summary of genetic diversity of *R. brachysoma* populations, Number of polymorphic bands, Percentage of polymorphic band (PPB), Nei's genetic diversity index (*H*) and Shannon information index (*I*)

Population	Number of polymorphic bands	PPB (%)	*H*	*I*
SOK	24	46.15	0.1151±0.1690	0.1807±0.2465
SRT	15	28.85	0.0887±0.1591	0.1356±0.2404
PKK	20	38.46	0.0893±0.1651	0.1417±0.2236
SSK	21	40.38	0.1176±0.1788	0.1799±0.2587
RAY	22	42.31	0.0915±0.1589	0.1450±0.2302
CTB	17	32.69	0.1057±0.1768	0.1594±0.2563
SAT	24	46.15	0.1336±0.1791	0.2064±0.2607
KRB	21	40.38	0.0908±0.1550	0.1453±0.2254
Species level	42	80.77	0.1485±0.1801	0.2373±0.2505

2.3.4.2 Population Genetic Structure

According to the hierarchical analysis of molecular variance tested by AMOVA, the among population analysis showed highly significant (F_{ST}: 0.2950, *P* <0.001) genetic differences among the 8 populations of *R. brachysoma*, the percentage of variations which 29.50% was attributed to among populations and 70.50% showed differences within populations. The nested analysis, assuming the populations of *R. brachysoma* were divided in two regions: the Gulf of Thailand (SOK, SRT, PKK, SSK, RAY and CTB) and Andaman Sea (SAT and KRB) showed significant genetic differentiation among regions (F_{CT}: 0.1984, *P*=0.0342) which the percentage of variation between two regions were moderately high (19.84%) and indicating to partition of genetic differentiation between the two regions. In addition, the significant genetic differentiation among populations within region (F_{SC}: 0.2223, *P*<0.001) and within populations (F_{ST}: 0.3766, *P* <0.001) were also showed (see Table2.6).

Table2.6. The hierarchical analysis test by Analysis of molecular variance (AMOVA) for 276 individual of *R. brachysoma*

Source of variation	d.f.	Sum of square	Variance component	Percentage of variation	Fixation index	*P*
Among populations	7	289.304	1.12218	29.50	F_{ST} = 0.2950*	<0.001
Within populations	268	718.870	2.68235	70.50		
Among region	1	114.203	0.85375	19.84	F_{CT} =0.1984*	0.0342
Among populations within region	6	175.101	0.76650	17.81	F_{SC} = 0.2223*	<0.001
Within populations	268	718.870	2.68235	62.34	F_{ST} = 0.3766*	<0.001

AMOVA consist of 2 components were among population analysis (among populations and within populations) and nested analysis (Among region, among populations within region and within populations). Significance tests after 10100 random permutations. The (*) represent significant value.

Nei's (1978) unbiased measures of genetic distance between the eight *R. brachysoma* populations ranging from 0.0061 to 0.1226, the highest was between RAY and KRB populations, and the lowest was between SRT and PKK populations (Table2.7).

Table2.7. Nei's unbiased measures of genetic distance (below diagonal) and geographic distance x100 km (above diagonal) among *R. brachysoma* populations

Population	SOK	SRT	PKK	SSK	RAY	CTB	SAT	KRB
SOK	****	2.5499	5.1636	6.8997	6.0907	6.0560	15.3126	17.3549
SRT	0.0595	****	2.9945	4.7849	4.4529	4.7557	17.8655	19.9078
PKK	0.0734	0.0061	****	1.7924	1.8735	2.5815	20.2781	22.3204
SSK	0.0614	0.0350	0.0365	****	1.6056	2.4722	21.8689	23.9112
RAY	0.0706	0.0589	0.0606	0.0344	****	0.8912	20.6839	22.7312
CTB	0.0621	0.0124	0.0144	0.0225	0.0301	****	20.3027	22.3450
SAT	0.0646	0.0440	0.0412	0.0830	0.0963	0.0618	****	2.0423
KRB	0.0878	0.0647	0.0690	0.0959	0.1226	0.0812	0.0155	****

UPGMA dendrogram was constructed using paiwise Nei's unbiased genetic distances (Figure2.4). The dendrogram indicated that the populations of *R. brachysoma* divided into three groups: the upper area of the Gulf of Thailand (SRT, PKK, SSK, RAY and CTB) as groupI, Andaman Sea (SAT and KRB) as groupII and the southern area of the Gulf of Thailand (SOK) as groupIII. Furthermore, within the upper of the Gulf of Thailand (groupI) could be divided into two subgroups: the first consisting of SSK, RAY and CTB, and the second consisting of SRT, PKK.

Nei's unbiased genetic distances and the geographic distances (Table2.7) were used to the correlation study between eight *R. brachysoma* populations by Mantel test method. The significant correlation between genetic distances and geographic distances of *R. brachysoma* populations was showed ($r = 0.6925$, $P<0.0003$) (see Figure2.5).

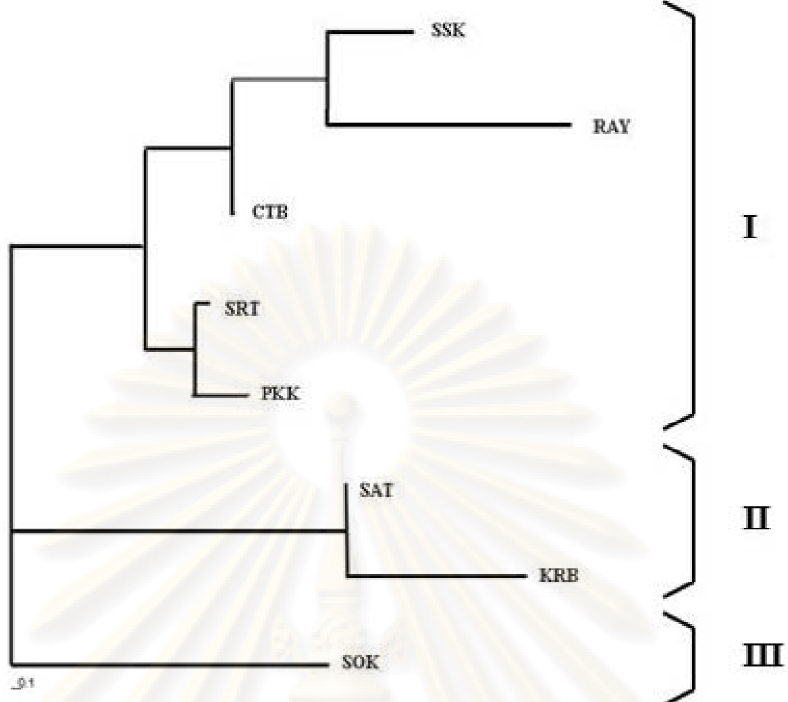

Figure2.4. A UPGMA dendrogram showing genetic relationship of *R. brachysoma* in Thai waters based on Nei's unbiased genetic distance between populations. Dendrogram was divided into three groups: (I) represent to the upper area of Gulf of Thailand, (II) represent to Andaman Sea and (III) represent to the southern area of the Gulf of Thailand.

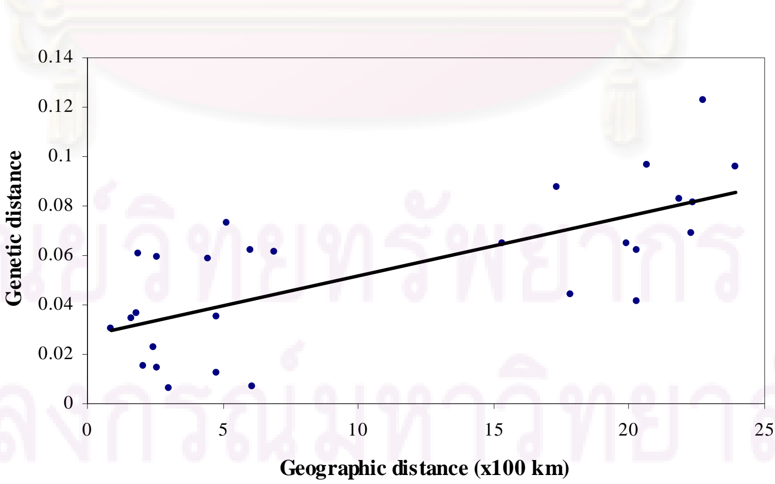

Figure2.5. Correlation between genetic distances and geographic distances of eight *R. brachysoma* populations.

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2.4 Discussion

2.4.1. Genetic Diversity of *R. brachysoma* in Thailand

ISSR is one of the powerful approaches for assessment of genetic variation among populations, especially for species in which no molecular genetic information was previously available like. This study presented that ISSR was highly efficient for investigation of genetic diversity and population genetic structure *R. brachysoma* in Thai waters. Based on ISSR data, the percentage of polymorphic bands of *R. brachysoma* populations was high (80.77%), comparing with ISSR reports of other marine species such as *Cynoglossus semilaevis* (45.26%, Liu *et al*., 2008), *Apostichopus japonicus* (92.2%, Bing *et al*., 2007) and *Mactra veneriformis* (97.9%, Hou *et al*., 2006). The genetic diversity of *R. brachysoma* in the Gulf of Thailand and Andaman Sea was moderately high. The lowest value of genetic diversity was that of Surat Thani population and the highest was Satun and Songkhla populations. Thus, Surat Thani was the most capture fisheries of *R. brachysoma* site in Thailand (Department of Fisheries, 2006) that it could be affected to low genetic diversity value by overexploitation. In contrast, high genetic diversity was found in Satun and Songkhla populations might be caused by the location of the two populations that are situated near the Thai-Malaysia border waters and the open sea (Indian Ocean and South China Sea, respectively) than other populations, it could be affected to transferred fish stocks from neighbors seas and more chance the changing the genetic materials. Comparing genetic diversity of *R. brachysoma* with the previous ISSR studies of other marine animals, genetic diversity of *R. brachysoma* (*H*: 0.1485) was higher than *Cynoglossus semilaevis* (*H*: 0.007, Liu *et al*., 2008) and *Paralichthys olivaceus* (*H*: 0.1086, Liu *et al*., 2006), in contrast, its lower than *Mactra veneriformis* (*H*: 0.3070, Hou *et al*., 2006) and *Apostichopus japonicus* was (*H*: 0.3605, Bing *et al*., 2007).

The high level of genetic variation within population in present study seem similar result in a similar geographical region (the Gulf o f Thailand and Andaman Sea) to that observed in *Penaeus monodon* revealed 16S ribosomal DNA and an intergenic COI-COII RFLP (Kinbunga *et al*., 2001) and three abalone species *Haliotis asinina* and *H. ovina* revealed by RAPD markers (Klinbunga *et al*., 2003).

2.4.2. Population Genetic Structure of *R. brachysoma* in Thailand

R. brachysoma populations can be divided into three groups; the upper and the southern areas of the Gulf of Thailand and Andaman Sea. *R. brachysoma* populations in the Gulf of Thailand and Andaman Sea were genetically divergence that it should be caused by geographical barrier, the Malaysian Peninsular, preventing gene flow between the two populations (Antoro *et al*., 2006). In addition, the gene flow between the Gulf of Thailand and Andaman Sea could also be inhibited due to the north-flowing current in the Strait of Malacca (Great Britain Hydrographic Office, 1958) and the different of temperature range along the Andaman coast was slightly lower than the Gulf of Thailand (Eiamsa-ard and Amornchairojkul 1997). The divergence of the two populations agreed with previous reports in others organisms. For example, Asian moon scallop, *Amusium pleuronectes* revealed by 16S rRNA region sequencing (Mahidol *et al*., 2007), three abalone species, *Haliotis asinina* and *H. ovina* revealed by RAPD markers (Klinbunga *et al*., 2003), banana shrimp, *Penaeus merguiensis* revealed by COI gene sequencing (Hualkasin *et al*., 2003) and giant tiger shrimp, *Penaeus monodon* (Klinbunga *et al*., 2001) revealed by RAPD and mtDNA-RFLP.

In the Gulf of Thailand, *R. brachysoma* populations could be divided into two groups; the upper and the southern areas of the Gulf of Thailand. The two populations were absence of geographical barrier, the surface current circulation pattern should be more considered for the genetic divergence of *R. brachysoma*. In the southwest monsoon period, surface current of South China Sea (including Songkhla area) area move in clockwise directions, while anticlockwise directions rise up near the northern area and the middle of the Gulf of Thailand. In the northeast monsoon, surface current of South China Sea (including Songkhla area) area move in anticlockwise directions, while surface current of the northern area and the middle of the Gulf of Thailand move anticlockwise directions (Neelasri, 1981). Thus, the difference of current circulation patterns of the two areas could be presented as barrier that the fish in these two areas could not be transferred. Moreover, the southern area of the gulf got high-salinity and cold water from the South China Sea enters (Robinson, 1974), while the upper area of the gulf is dominated by the river discharge. The Gulf of Thailand thus functions as a two-layered, shallow estuary with lower-salinity surface water flowing out, while high-salinity, colder water enters from the South China Sea (Naval Hydrographic Department, 1995).

The structure in the upper area of the Gulf of Thailand is not clear agreed with many previously reported such as swimming crab *Portunus pelagicus* (Thamniemdee, 2007) and abalone *Haliotis asinina* and *H. ovina* (Klinbunga *et al*., 2003). Unclear structure might be caused by monsoon winds, tidal currents and the river discharge from four major rivers (the Chao Phraya, the Tha Chin, the Mae Klong and the Bang Pakong) created complex circulation patterns, including localized upwelling and downwelling (Robinson, 1974). The Gene flow between these populations from the upper area of the Gulf of Thailand is possible.

Thereby, the genetic structure of *R. brachysoma* observed in this study agrees with the previous reproductive biology and spawning seasons of *R. brachysoma* studies. The size at first maturity of male and female of these three groups was difference (Maila-iad *et al*., 2006; Sritakon *et al*., 2006; Sutthakorn, 1998). The spawning season of the upper area of the Gulf of Thailand were in period of February to May and August to October while there was higher peak in first period (Maila-iad et al., 2006), the southern area of the Gulf of Thailand were December to February and May to August (Sritakon *et al*., 2006), and Andaman Sea were all year spawning period (Sutthakorn, 1998).

The strong correlation between geographic distances and genetic distances of all geographic locations of *R. brachysoma* indicated that *R. brachysoma* in Thai waters was isolated by distances and was limited by geographical barrier (the Malaysian Peninsular). Generally, geographic distance increasing affect to the high genetic differentiation supported by the previous marine species population studies such as *Chondrus crispus* populations from North Atlantic (r=0.78, *P*=0.002, Wang *et al*., 2008) and *Sargassum muticum* populations around the Shandong peninsula (r=0.9161, P=0.009, Zhao *et al*., 2008). In contrast, some organisms from previously reported showed no significant correlation between geographic distances and genetic distances such as *Castanea mollissima* (r=0.3229, *P*=0.5441, Xiang *et al*., 2007). However, ISSR markers might be provide a clear structure on relatively large geographic distance scale more than 1,000 km (Wang *et al*., 2008).

This study suggested that ISSR clearly offers the ability to investigate the genetic diversity and population genetic structure of *R. brachysoma*. The *R. brachysoma* populations were divided into three groups that there were the upper and

the southern areas of the Gulf of Thailand, and Andaman Sea. However, more studies on life history, tagging, and advanced genetic tool are recommended to gain better understanding of the biology and essential requirements of this species for stock management and conservation in the future.

CHAPTER III

PHYLOGEOGRAPHIC RELATIONSHIPS OF *R. brachysoma* IN THAILAND REVEALED BY MITOCHONDRIAL DNA SEQUENCING

3.1 Introduction

An animal mitochondrial DNA marker is commonly used in many phylogenetic and phylogeographic studies since it evolves faster than nuclear DNA (Brown *et al.* 1985) and different regions of the mitochondrial genome evolve at different rates (Saccone *et al.* 1991) allowing suitable regions to be chosen for study.

The mitochondrial genome of fish is a circular molecule usually about 15-18 kb in length, containing two ribosomal RNA genes (*12S* and *16S*), 13 protein coding genes (*ATPase 6* and *ATPase* 8, *COI-III*, *Cyt b*, *ND1-6* and *4L*), 22 transfer RNA genes and a noncoding control region (Guo *et al*., 2004). The mitochondria DNA control region (or D-loop) of vertebrates is the only large non-coding region in mitochondria and it contains the heavy-strand replication origin and the promoters for both the L- and H-strand transcription (L'Abbe *et al.* 1991). The control region was highest evolutionary rate in the molecule of mtDNA compared with other parts of mtDNA (Brown, 1985). This variability has lead to use control region sequences to examine the population structure and phylogenetic relationship.

The mtDNA control region sequence has made important contributions to the studies on biodiversity and conservation of fishes (Chen, 2004; Tian *et al*., 2004; Cervelli *et al*., 2007), and been used in the population genetic and phylogeographic studies on many scombridae species such as narrow-barred Spanish mackerel *Scomberomorus commerson* (Hoolihan *et al*., 2006), horse mackerel *Trachurus trachurus* (Comesana *et al.*, 2008), Atlantic mackerel *Scomber scombrus* (Nesbo *et al*., 2000), chub mackerel *Scomber japonicus* (Zardoya *et al*., 2004) and albacore *Thunnus alalunga* (Wu *et al.*, 2009). Moreover, the mtDNA control region sequences have also been used for phylogenetic studies in scombridae. For example, Alvarado *et*

al., (1996) sequenced 450 bp control region of the mitochondrial DNA for describe the subgenus Thunnus was not shown to be monophyletic as Neothunnus fell within the Thunnus subgenus.

Cytochrome *b* gene is involved the electron transport in the respiratory chain of mitochondria. The cytochrome *b* gene is the most widely used for phylogenetic and phylogeographic studies. Although it evolves slowly in terms of non-synonymous substitutions, the rate of evolution in silent positions is relatively fast (Irwin *et al.* 1991). Cytochrome *b* is thought to be variable for population and phylogenetic relationship studies. However, cytochrome *b* gene is under strong evolutionary constraints because some parts of the gene are more conserved than others due to functional restrictions (Meyer, 1994). Moore and DeFilipps (1997) suggested that it could be the best way for resolving relatively recent evolutionary history marker.

Cytochrome *b* gene has been the most sources of sequence data for phylogeographic studies on scombridae species such as three horse mackerel species *Trachurus trachurus*, *T.mediterraneus* and *T. picturatus* (Karaiskou *et al*., 2003), Atlantic mackerel *Scomber scombrus* (Nesbo *et al*., 2000) and scad mackerel *Decapterus russelli* (Rohfritsch and Borsa, 2005). Cytochrome *b* gene sequences also used for phylogenetic studies in scombridae species. For example, the 590 bp of cytochrome *b* gene were used for debated in the morphological literature as to the exact relationships among billfishes, tunas, and other scombroids (Carpenter *et al*., 1995; Finnerty and Block, 1995). Finnerty and Block (1995) suggested that their cytochrome *b* gene study indicated that there is strong molecular support for monophyly of Thunnus, but less for the relationships of Euthynnus, Katsuwonus, and Auks to one another and to Thunnus. The study of Chow and Kishino (1995) was the first to include all eight Thunnus species in a molecular phylogeny by using 292 bp fragment of cytochrome *b*.

In this chapter, the sequences of partial mitochondrial DNA control region and the mitochondrial DNA cytochrome *b* gene were used for examine the phylogeographic relationships of *R. brachysoma* from the Gulf of Thailand and Andaman Sea.

3.2. Materials and Methods

3.2.1 Tissue Sampling

Forty individuals of *R. brachysoma* were collected from local fisheries to make sure that the fish are from the eight areas in the Gulf of Thailand and Andaman Sea were collected from eight populations (5 individuals for each population). The eight populations were Songkhla (SOK), Surat Thani (SRT), Prachuap Khiri Khan (PKK), Samut Songkhram (SSK), Rayong (RAY) and Chanthaburi (CTB) located in the Gulf of Thailand and Satun (SAT) and Krabi (KRB) located in Andaman Sea (see Table3.1). All tissue samples were preserved in absolute ethanol and kept at -20°C until required.

Table3.1. Details of examined *R. brachysoma* populations consisting of locations, population codes, latitudes and longitudes, sample sizes and collection date

Locations	Population codes	Latitudes and longitudes	Sample sizes	Collection date
Songkhla	SOK	7°12’N 100°35’E	5	November, 2006
Surat Thani	SRT	9°13’N 99°30’E	5	October, 2006
Prachuap Khiri Khan	PKK	11°48’N 99°47’E	5	October, 2006
Samut Songkhram	SSK	13°23’N 99°51’E	5	September, 2006
Rayong	RAY	12°39’N101°16’E	5	October, 2006
Chanthaburi	CTB	12°28’N102°04’E	5	October, 2006
Satun	SAT	6°50’N 99° 47’E	5	March, 2007
Krabi	KRB	8°03’N 98° 55’E	5	February, 2007

3.2.2 DNA Extraction

Genomic DNA was extracted from body muscle tissue of each *R. brachysoma* individual using a modified salting out procedure (Miller *et al.*, 1988). tissue about 10 mg after removing from a –20°C were transferred into a 1.5 ml microcentrifuge tube containing 485 µl of TNE + 1% SDS buffer (50 mM Tris-base; pH 8.0, 100mM NaCl, 5 mM EDTA; pH8.0, 1% SDS (w/v)). Total proteins were digested by addition of 15 µl of 10 mg/ml proteinase-K solution. The resulting mixture was incubated at 55°C

for 3 hours. To remove digested proteins, 250 μl of 6 M Sodium Chloride was added. The sample mixture was then centrifuged at 10,000 rpm for 8 minutes. The aqueous phase was transferred to a new 1.5 ml micorcentrifuge tube. DNA was precipitated by addition of two volume of absolute ethanol and kept at –20°C overnight to ensure complete precipitation. DNA was recovered by centrifugation at 14,000 rpm for 15 minutes before removing ethanol. DNA pellet was air-dried at room temperature and redissolved with appropriate amount with 20 μl TE buffer (10 mM Tris, 0.1 mM EDTA) and kept at –20°C for further analysis.

3.2.3 Agarose Gel Electrophoresis

3.2.3.1 Genomic DNA Analysis

To determine the quality and quantity of the extracted DNA using agarose gel electrophoresis, the loading sample consist of 1 μl of extracted DNA, 2 μl of loading dye (standard stain orange G, 40% (v/v) glycerol) and 6 μl of distilled water was mixed thoroughly. A 0.8 % agarose gels was prepared by weighting 0.4 g of GenePure LE Agarose (Research Organic, Inc) and mixing with 50 ml of 0.5X TBE buffer (0.89 M Tris-base, 0.89 M boric acid and 0.02 M EDTA) in 200 ml flask. The agarose suspension was heated in a microwave about 1 minute (two times) for completely dispersed. Next, the melted agarose was cooled down at room temperature about 10 minutes, 4 μl of 0.4% (w/v) ethidium bromide solution was added and mixed. The mixed solution was poured into the sealed gel tray and the appropriate combs were inserted, the air bubble was removed, and the gel was set at room temperature about 1 hour. The two combs were removed ensuring the gel completely set, the gel was placed in the horizontal electrophoretic chamber containing 0.5X TBE buffer in both wells and extra TBE was added to cover the gel approximately 0.5 cm from the surface of gel. The samples were loaded into each well by using an automatic micropipette. Concentrations of extracted DNA were estimated by comparison with know quantities of λDNA/Hind III marker (Fermentas). The gel chamber was connected to a power supply and electrophoresis was run at 80 volts for approximately 30 minutes. The DNA bands were visualized as fluorescent bands on a UV transilluminator and photographed using the gel document system (Bio-Rad). The extracted DNA was adjusted to approximately 20-30 ng/μl for use in PCR amplification.

3.2.3.2 MtDNA PCR Product Analysis

To determine the quality and quantity of mtDNA products using agarose gel electrophoresis, the loading sample consist of 25 µl of PCR product and 5 µl of loading dye (standard stain orange G, 40% (v/v) glycerol) was mixed thoroughly. A 1.0 % agarose gels was prepared by weighting 0.5 g of GenePure LE Agarose (Research Organic, Inc) and mixing with 50 ml of 0.5X TBE buffer (0.89 M Tris-base, 0.89 M boric acid and 0.02 M EDTA) in 200 ml flask. The agarose suspension was heated in a microwave about 1 minute for completely dispersed. Next, the melted agarose was cooled down at room temperature about 10 minutes, 4 µl of 0.4% (w/v) ethidium bromide solution was added and mixed. The mixed solution was poured into the sealed gel tray and the appropriate combs were inserted, the air bubble was removed, and the gel was set at room temperature about 1 hour. The two combs were removed ensuring the gel completely set, the gel was placed in the horizontal electrophoretic chamber containing 0.5X TBE buffer in both wells and extra TBE was added to cover the gel approximately 0.5 cm from the surface of gel. The samples were loaded into each well by using an automatic micropipette. The size of mtDNA PCR products were estimated by comparison with 100 bp ladder DNA marker (Fermentas). The gel chamber was connected to a power supply and electrophoresis was run at 100 volts for approximately 40 minutes. The DNA bands were visualized as fluorescent bands on a UV transilluminator and photographed using the gel document system (Bio-Rad).

3.2.4 PCR Amplification

PCR primers for partial mtDNA control region amplification of *R. brachysoma* were designed. The forward primer 5’ TCTCACCACTAGCTACCA AAGC 3’ (1st Base Pte Ltd.) was a universal primer for many vertebrates. This forward primer located inside the tRNA-Pro gene which flanks the 5’ end of the control region. The reverse primer 5’ TGCTCATGATATCCTTATATGTG 3’ was designed based on the conserved regions of 5 scombridae species available at the GenBank database: *Scomber scombrus* (AB466272), *Thunnus alalunga* (AY055002), *Thunnus orientalis* (AB185022), *Thunnus thynnus* (AY699946) and Auxis rochei (AB103468). This reverse primer was designed in order to avoid the repeats and it was partly overlapping located inside the control region of which flanks the 3’ end.

PCR primers amplified a section of cytochrome *b* gene, the forward primer 5' CTCCCAGCCCCATCCAACATCTCAGCATGATGAAACTTCG 3' and the reverse primer 5'GGC AAA TAG GAA GTA TCA TTC TG 3' were developed by Kosuch (2001).

Similar PCR amplification reagents were used for both primer pairs. They were performed in a 25 µl reaction volume containing 1 µl template DNA (approximately 20-30 ng/µl), 1X reaction buffer with KCI (Fermentas), 2.0 mM $MgCl_2$ (Fermentas), 0.25 mM dNTPs (Promega), 1 µM primer each primer (forward and reverse primer) (1st Base Pte Ltd.), and 1.0 U *Taq* DNA polymerase (Fermentas).

For partial mtDNA control region amplification was followed by 95°C for 5 minutes and 35 cycles of denaturation at 95°C for 45 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 90 seconds, and a final extension at 72°C for 10 minutes. For cytochrome *b* gene amplification was performed by the following condition, 95°C for 3 minutes and 35 cycles of denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute, and a final extension at 72°C for 10 minutes. A negative control was used in all PCR amplifications. Amplification products were detected using 1.0% agarose gel.

3.2.5 PCR Product Purification

All PCR products were purified by using a MACHEREY-NAGEL PCR clean up Gel extraction kit. The target DNA fragment from an agarose gel was excised using sterile scalpel and the weight of the sliced gel was determined. It was then transferred to a clean 1.5 microcentrifuge tube. The sliced gel was lysed by adding 200 µl buffer NT per each 100 mg of agarose gel, and then sample were incubated at 50°C until the sliced gel was completely dissolved. Third, A NucleoSpin® Extract II Column was placed into a 2 ml collecting tube, the sample was loaded and centrifuged for 1 minute at 11,000 rpm, the DNA was bind with silica membrane. After discarding the flow though, the NucleoSpin® Extract II Column with bound DNA was washed by adding 600 µl buffer NT3 by centrifugation for 1 minute at 11,000 rpm and a further 2 minutes at 11,000 rpm to removed the buffer NT3. Finally, DNA was eluted from NucleoSpin® Extract II Column by adding elution buffer NE 20 µl, and it was then incubated at 50°C for 2 minutes and centrifuged for 1 minute at 11,000 rpm.

3.2.6 Sequencing and Data analysis

The purified PCR products of all samples from previous step were carried out in the sequencing service, Laboratory of Ramathibodi Hospital, Bangkok, Thailand. All nucleotide sequences were manually checked and aligned by using the multiple sequence alignment program CLUSTAL X (Thomson *et al*., 1997).

The obtained sequences of partial mtDNA control region and cytochrome *b* gene of *R. brachysoma* was used to search for any similar sequence in the GenBank database using BLAST program in http://www.ncbi.nlm.nih.gov.

The comparison of haplotype frequency and haplotype distribution of *R. brachysoma* of eight locations from the Gulf of Thailand and Andaman Sea were analyzed by using the sequence data of partial mtDNA control region and cytochrome *b* gene. Phylogeographic relationships among the all individuals from these eight locations used neighbour-joining (NJ) and maximum parsimony (MP) methods. Phylogenetic trees were constructed using PAUP*4ß10 (Swafford, 2000). The NJ (Saitou and Nei, 1987) and MP were analyzed via heuristic searches, 100 random stepwise additions, TBR branch-swapping algorithm (Felsenstein, 1981). ModelTest version 3.7(Posada and Crandall, 1998) was used to determine the best sequence evolution model for the distance (Akaike, 1974). In the part of partial mtDNA control region analysis, the distance estimation and MP were performed using unweighted least squares as the optimality criterion (HKY85+I). Cytochrome *b* gene analysis, the distance estimation and MP were carried out using unweighted least squares as the optimality criterion (HKY85+G). Finally, the combined sequences data of partial mtDNA control region and cytochrome *b* gene analysis, the distance estimation and MP were performed using unweighted least squares as the optimality criterion (TIM+I). Non-parametric bootstrap supports (Felsenstein, 1985) were analyzed using 1000 replicates for NJ and MP, via heuristic searches with starting tree obtained by 100 random stepwise additions, and TBR branch-swapping algorithm (Felsenstein, 1981). In addition, *Rastrelliger kanakurta* was used for an outgroup.

3.3 Results

3.3.1 DNA Extraction

Genomic DNA was extracted from muscle of *R. brachysoma* using salting out method (Miller *et al*., 1988). The quality and quantity of extracted genomic DNA were determined by using 0.8% agarose gel electrophoresis and comparing with λDNA/Hind III marker. The concentration of extracted genomic at approximately 45-200 ng/μl and the high molecular weight DNA at approximately 23.1 kb were obtained (see Figure3.1). The extracted DNA was adjusted to approximately 20-30 ng/μl for use in PCR amplification.

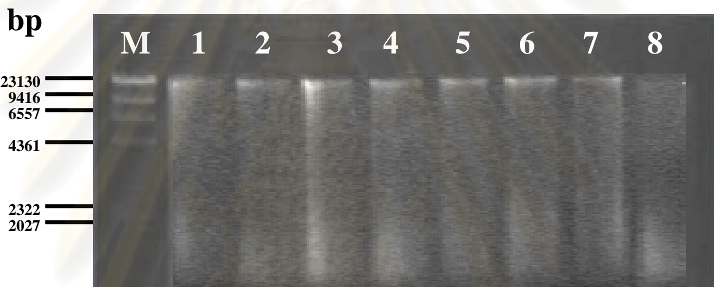


Figure3.1. A 0.8% ethidium bromide stained agarose gel showing the quality of total DNA extracted from the muscle of each *R. brachysoma* individual (lanes 1-7). Lane 8 is *R. kanakurta* sample. Lane M is λDNA/Hind III marker.

3.3.2 PCR Amplification

PCR amplification of specific sequence by synthesed primers extended simultaneously using the complementary strand of DNA template. The partial mtDNA control region and cytochrome *b* gene were successfully amplified with expected product size of about 750 (Figure3.2I) and 800 bp (Figure3.3II), respectively.

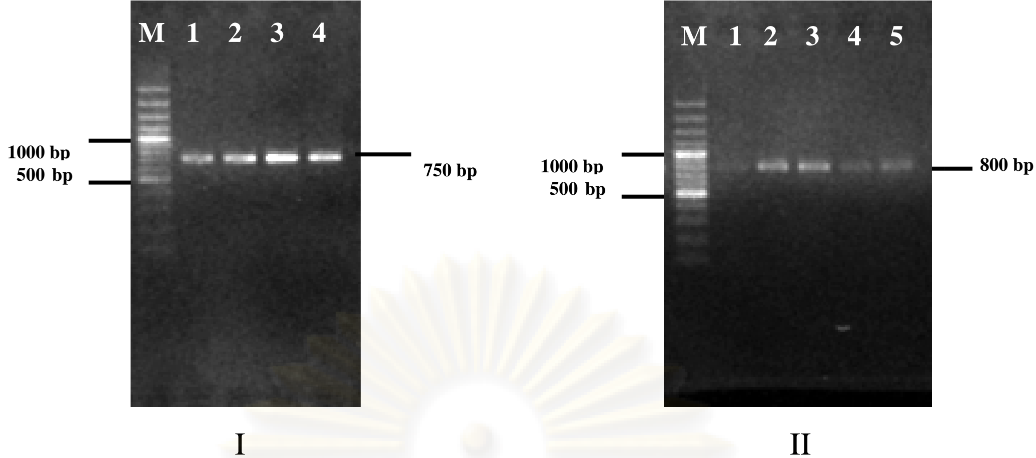


Figure3.2. 1.0% ethidium bromide stained agarose gels showing the PCR products. I represent PCR products of partial mtDNA control region (including partial tRNA-Pro), lane M is 100 bp ladder DNA marker (Fermentas), and lane 1-4 represent the PCR products of each *R. brachysoma* individual. II represent PCR products of cytochrome *b* gene, lane M is 100 bp ladder DNA marker (Fermentas), and lane 1-5 represent the PCR products of each *R. brachysoma* individual.

3.3.3 Data Analysis

After the PCR products of partial mtDNA control region and cytochrome *b* gene of *R. brachysoma* were purified, they were sequenced in both the forward and reverse direction. These resulted sequences were checked the quality by using Chromas version 1.45 (Zajec, 1986) which show bases for each interval (Figure3.3). The chromatograms were manually checked to correct miscalls, noises and secondary smaller peaks. Mostly, in partial mtDNA control region and cytochrome *b* gene the forward and reverse sequences were consistent.

The partial mtDNA control region and cytochrome *b* gene sequencing of PCR product was obtained for *R. brachysoma* (549 and 627 bp, respectively). The partial mtDNA control region sequences from present study were similar with mtDNA control region sequences of other scombridae species such as *Thunnus thynnus* (AB106300), *Scomber colias* (AB361519) and *Scomber japonicus* (EF508464). The present cytochrome *b* gene sequences were similar with cytochrome *b* gene sequences

of other fishes in genus *Rastrelliger* such as *R. faughni* (DQ497846) and *R. kanakurta* (DQ497858).

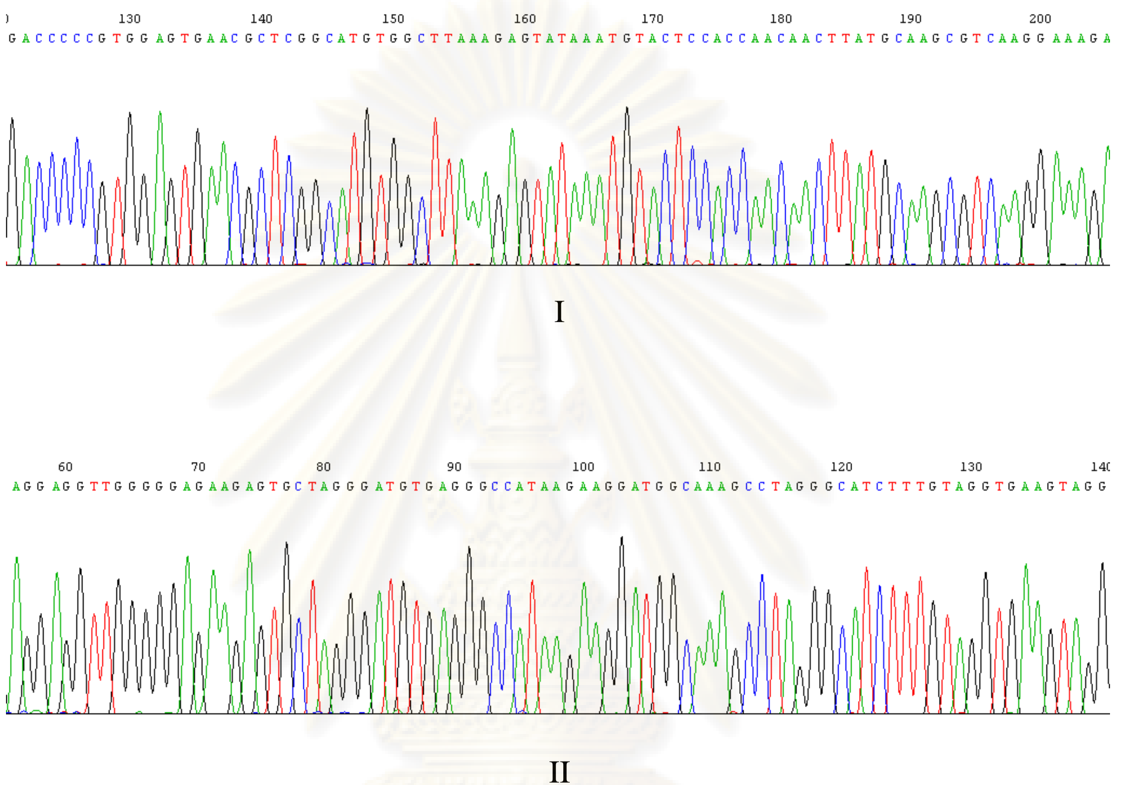


Figure3.3. Chromatograms of DNA sequencing of *R. brachysoma*. Green, blue, black and red show Adenine (A), Cytosine (C), Guanine (G) and Thymine (T), respectively. I: the chromatograms of partial mtDNA control region fragment from RAY1. II: the chromatograms of cytochrome *b* gene fragment from CTB1.

3.3.3.1 The Partial mtDNA Control Region of *R. brachysoma*

In total, 40 *R. brachysoma* individuals from eight locations (5 individuals for each location), including CTB, RAY, SSK, PKK, SRT, SOK, KRB and SAT populations were analyzed. The 549 bp of partial mtDNA control region sequences of *R. brachysoma* (AppendixA.1) showed 7 variable sites were observed and 10 haplotypes were identified. The detail of variable nucleotide position defining the

partial mtDNA control region haplotype (Table3.2) and the haplotype frequencies (Table3.3) of partial mtDNA control region sequences of 40 *R. brachysoma* samples were showed.

Haplotype9 was most abundant (Figure3.4), occurring in 20 samples, which was also found at the highest frequency in all populations of Andaman Sea (KRB and SAT) and the Gulf of Thailand (CTB, RAY, SSK, PKK, SRT and SOK). In addition, all six populations of the Gulf of Thailand found all ten haplotypes, in contrast, two populations of Andaman Sea found only three haplotypes (Haplotype7, Haplotype8 and Haplotype9). However, comparing the haplotypes of the Gulf of Thailand and Andaman Sea, three haplotypes (Haplotype7, Haplotype8 and Haplotype9) were common in both populations (Figure3.4). This result showed no distinct haplotype between the Gulf of Thailand and Andaman Sea samples.

The pairwise genetic distances of 40 *R. brachysoma* individuals evaluate across all pair of sequences were transformed into a distance and ranged from 0 to 0.0018 (see AppendixB.1). The variation between all 40 individuals from the Gulf of Thailand and Andaman Sea populations were very low, with the highest being seen between SRT4 and other 39 individuals. Moreover, the pairwise difference between the Gulf of Thailand individuals and Andaman Sea individuals were low.

The sequences of the partial mtDNA control region of 40 *R. brachysoma* individuals from the Gulf of Thailand and Andaman Sea, including 1 *Rastrelliger kanakurta* individual were used to construct phylogenetic trees. In this report, The NJ and MP tree showed only one cluster, moreover they were showed do not clear structure of *R. brachysoma* in Thailand which may be caused by the low variation of base sequences effected to much lower pairwise genetic distances. Therefore, the phylogeographic study of *R. brachysoma* populations in Thailand may be should select another region of mtDNA for resolve this problem.

Table3.2. Variable nucleotide position defining the partial mtDNA control region haplotype from 40 *R. brachysoma* samples

	0011344 5923809 3759072
Haplotype1	CCACCTA
Haplotype2	T.G....
Haplotype3	..G..G.
Haplotype4	..GT...
Haplotype5	..GT..G
Haplotype6	..GG...
Haplotype7	..G...G
Haplotype8	..G....
Haplotype9	..G....
Haplotype10	.TG.T..

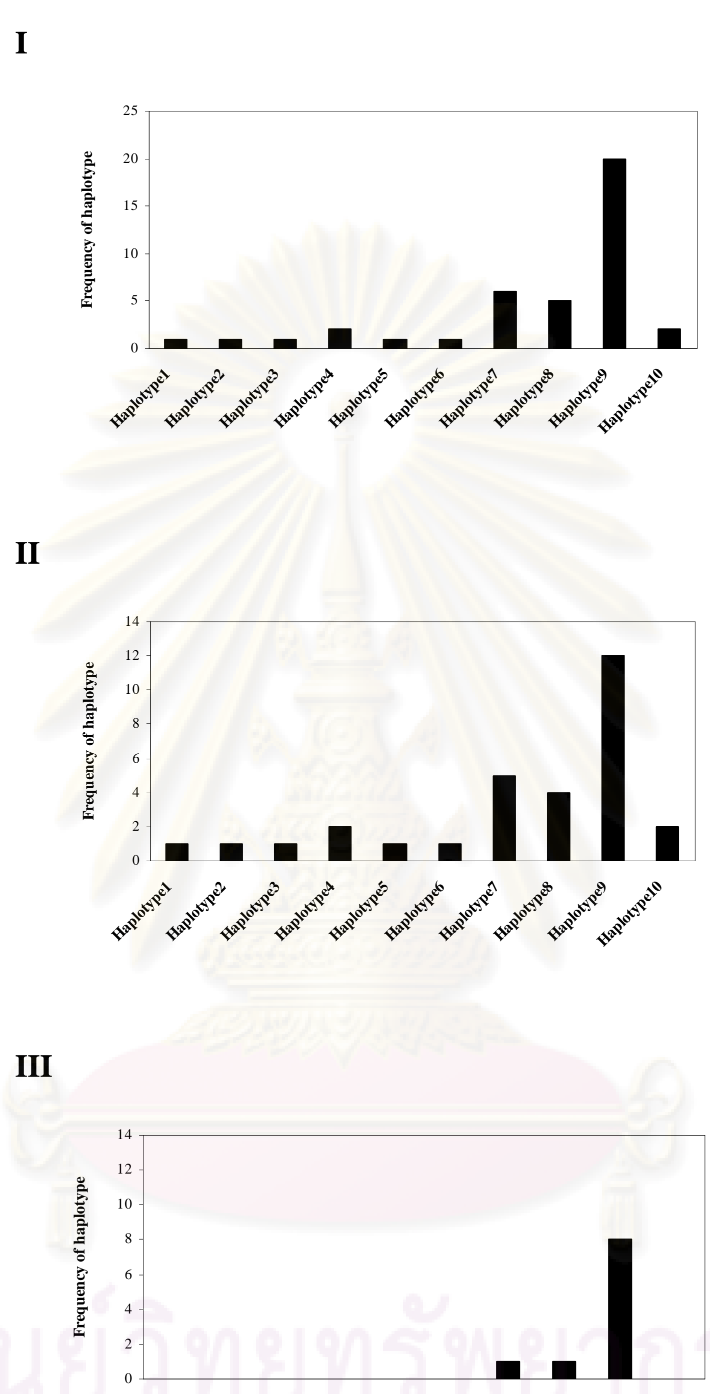
Numbers above nucleotides indicate nucleotide position. Sequence identity to reference sequences in top row (Haplotype1).

Table3.3. Haplotype frequencies of partial mtDNA control region of 40 *R. brachysoma* from eight locations consisting of haplotypes, haplotype frequencies and percentage of haplotype

Haplotypes	**Haplotype frequencies**								**Percentage of haplotype (%)**
	The Gulf of Thailand						**Andaman Sea**		
	CTB	**RAY**	**SSK**	**PKK**	**SRT**	**SOK**	**KRB**	**SAT**	
Haplotype1	-	1	-	-	-	-	-	-	2.5
Haplotype2	-	-	-	1	-	-	-	-	2.5
Haplotype3	-	-	-	-	1	-	-	-	2.5
Haplotype4	1	-	-	-	-	1	-	-	5.0
Haplotype5	-	-	-	1	-	-	-	-	2.5
Haplotype6	1	-	-	-	-	-	-	-	2.5
Haplotype7	1	1	1	-	1	1	1	-	15.0
Haplotype8	-	-	3	-	1	-	-	1	12.5
Haplotype9	2	3	1	1	2	3	4	4	50.0
Haplotype10	-	-	-	2	-	-	-	-	5.0

The Gulf of Thailand populations consist of Chanthaburi (CTB), Rayong (RAY), Samut Songkhram (SSK), Prachuap Khiri Khan (PKK), Surat Thani (SRT) and Songkhla (SOK). Andaman Sea populations consist of Krabi (KRB) and Satun (SAT).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



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จุฬาลงกรณ์มหาวิทยาลัย

Figure3.4. Histrogram showing the distribution of haplotype obtained from partial mtDNA control region sequences of 40 *R. brachysoma* individuals in Thailand. I: the eight populations from the Gulf of Thailand and Andaman Sea, II: the Gulf of Thailand population (CTB, RAY, SSK, PKK, SRT and SOK), and III: Andaman Sea population (KRB and SAT).

3.3.3.2 The Cytochrome *b* Gene of *R. brachysoma*

The 40 *R. brachysoma* individuals from eight locations (5 individuals for each location), including CTB, RAY, SSK, PKK, SRT, SOK, KRB and SAT populations were analyzed. The 627 bp of cytochrome *b* gene sequences (AppendixA.2) showed 17 variable sites and 6 haplotypes were identified. The detail of variable nucleotide position defining the partial mtDNA control region haplotype (Table3.4) and the haplotype frequencies (Table3.5) of cytochrome *b* gene sequences of 40 *R. brachysoma* samples were showed.

The Haplotype6 was also found at the highest frequency in all populations of the Gulf of Thailand (CTB, RAY, SSK, PKK and SRT) except SOK population was found only Haplotype5. In contrast, Haplotype1 and Haplotype2 were restricted to Andaman Sea populations (KRB was found only Haplotype1 but SAT was found both haplotypes). Comparing the haplotypes found in Thai water, the Gulf of Thailand haplotypes (Haplotype3, Haplotype4, Haplotype5 and Haplotype6) were slightly nucleotide difference from each other that 1-3 bp differences among haplotypes. The same with Andaman Sea haplotypes (Haplotype1 and Haplotype2) were slightly nucleotide difference only 2 bp. However, comparing nucleotide difference between the Gulf of Thailand and Andaman Sea haplotypes were as high as 15 to 17 bp. From all results, the cytochrome *b* gene haplotypes could be able to devided between *R. brachysoma* of the Gulf of Thailand and Andaman Sea populations (Figure3.5).

The pairwise genetic distances of 40 *R. brachysoma* individuals evaluate across all pair of sequences were transformed into a distance and ranged from 0 to 0.0262, with the highest between SRT1 and SAT5. The variation between all individuals from the Gulf of Thailand populations was low at 0 to 0.0048, with the highest between SRT1 and all five SOK individuals. Variation between all individuals from Andaman Sea populations was also low at 0 to 0.0032, with the highest being seen between SAT5 and all other individuals. In addition, the pairwise difference between individuals from the Gulf of Thailand and Andaman Sea were much higher differences ranging from 0.0195 to 0.0262, with the highest between SRT1 and SAT5 (AppendixB.2).

Table3.4. Variable nucleotide position defining cytochrome *b* gene haplotype from 40 *R. brachysoma* samples

```
              00112233344455556
              25160714425602562
              17484682800518247

Haplotype1    TCTACATGGCTATCTGT
Haplotype2    .....G..A........
Haplotype3    CTCGT.CA.TCGCTC.C
Haplotype4    CTCGT.CA.TCGC.C.C
Haplotype5    .TCGT.CA.TCGC.C.C
Haplotype6    .TCGT.CA.TCGC.CAC
```

Numbers above nucleotides indicate nucleotide position. Sequence identity to reference sequences in top row (Haplotype1).

Table3.5. Haplotype frequencies of cytochrome *b* gene of 40 *R. brachysoma* from eight locations consisting of haplotypes, haplotype frequencies and percentage of haplotype

Haplotypes	**Haplotype frequencies**								**Percentage of haplotype (%)**
	The Gulf of Thailand						**Andaman Sea**		
	CTB	**RAY**	**SSK**	**PKK**	**SRT**	**SOK**	**KRB**	**SAT**	
Haplotype1	-	-	-	-	-	-	5	4	22.5
Haplotype2	-	-	-	-	-	-	-	1	2.5
Haplotype3	-	-	-	-	1	-	-	-	2.5
Haplotype4	-	-	-	-	1	-	-	-	2.5
Haplotype5	-	-	-	-	-	5	-	-	12.5
Haplotype6	5	5	5	5	3	-	-	-	57.5

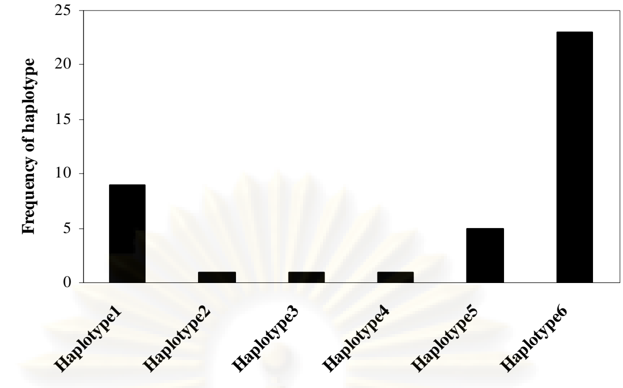
The Gulf of Thailand populations consist of Chanthaburi (CTB), Rayong (RAY), Samut Songkhram (SSK), Prachuap Khiri Khan (PKK), Surat Thani (SRT) and Songkhla (SOK). Andaman Sea populations consist of Krabi (KRB) and Satun (SAT).

In the part of phylogenetic relationship, the cytochrome *b* gene sequences data of 40 *R. brachysoma* individuals from the Gulf of Thailand and Andaman Sea, including a single of *R. kanakurta* were analyzed. Phylogenetic trees were constructed using NJ and MP methods, and non-parametric bootstrap supports were assessed in both methods. According to the NJ tree (Figure3.6), *R. brachysoma* in Thailand were separated in two major groups. The first major group comprised of Andaman Sea (all 5 individuals of each KRB and SAT), with 73% bootstrap support. In contrast, the second major group comprised of the Gulf of Thailand (all five individuals of each CTB, RAY, SSK, PKK, SRT and SOK), with high bootstrap support at 100%. Moreover, the Gulf of Thailand group could be able to divide into two subgroups, the first subgroup (subgroupI) was consisted of all the Gulf of Thailand individuals except all 5 SOK individuals, and the second subgroup (subgroupII) was composed of all 5 individuals from SOK.

According to the MP analysis, six trees were retained, however only one was showed (Figure3.7). From MP tree, the separation of 40 *R. brachysoma* individuals were in two groups same with NJ trees. The first group comprised of Andaman Sea (all Andaman Sea individuals), with the high bootstrap support at 99%, which this group could not be able to divide into subgroup. In contrast, the second group comprised of the Gulf of Thailand with high bootstrap support at 100%, which this group could be able to divide into three subgroups. The first subgroup (subgroupI) consisted of all 5 individuals each CTB, RAY, SSK and PKK, and three individuals of SRT (SRT2, SRT3 and SRT5). In addition, the second subgroup (subgroupII) consisted of SRT1 and SRT4, which they were slightly divergence. The last subgroup (subgroupIII) consisted of all 5 individuals from SOK.

From both NJ and MP analysis, cytochrome *b* gene sequences data were constructed similar tree topology, with slightly differences. However, both phylogenetic trees were obtained the same separation of the two groups (Andaman Sea and the Gulf of Thailand), but differ only in the relationship of each subgroup to the other between the two analytical methods. However, these two methods were confirmed similar result of phylogeographic relationships of *R. brachysoma* in Thailand were separated in two regions.

I



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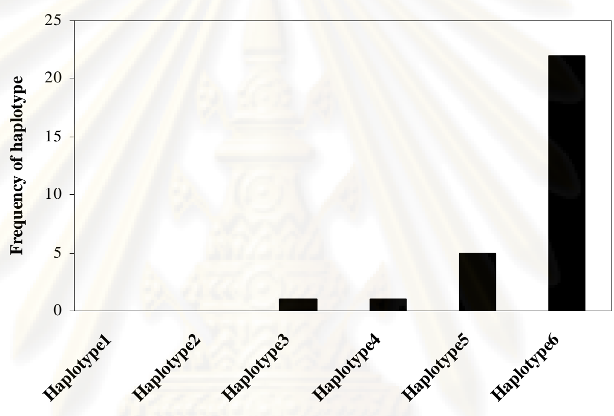

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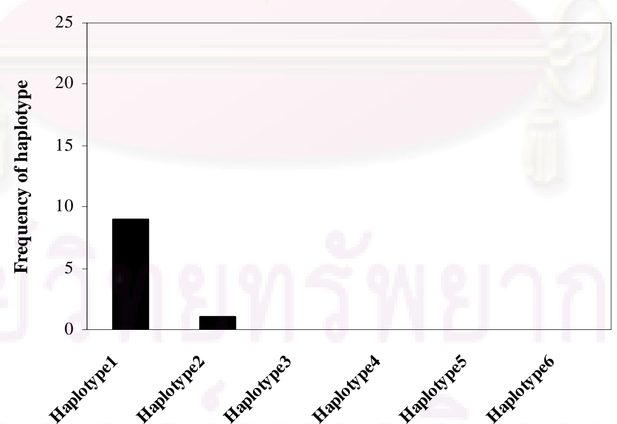


Figure3.5. Histrogram showing the distribution of haplotype obtained from cytochrome *b* gene sequences of 40 *R. brachysoma* individuals in Thailand. I: the eight populations from the Gulf of Thailand and Andaman Sea, II: the Gulf of Thailand population (CTB, RAY, SSK, PKK, SRT and SOK), and III: Andaman Sea population (KRB and SAT).

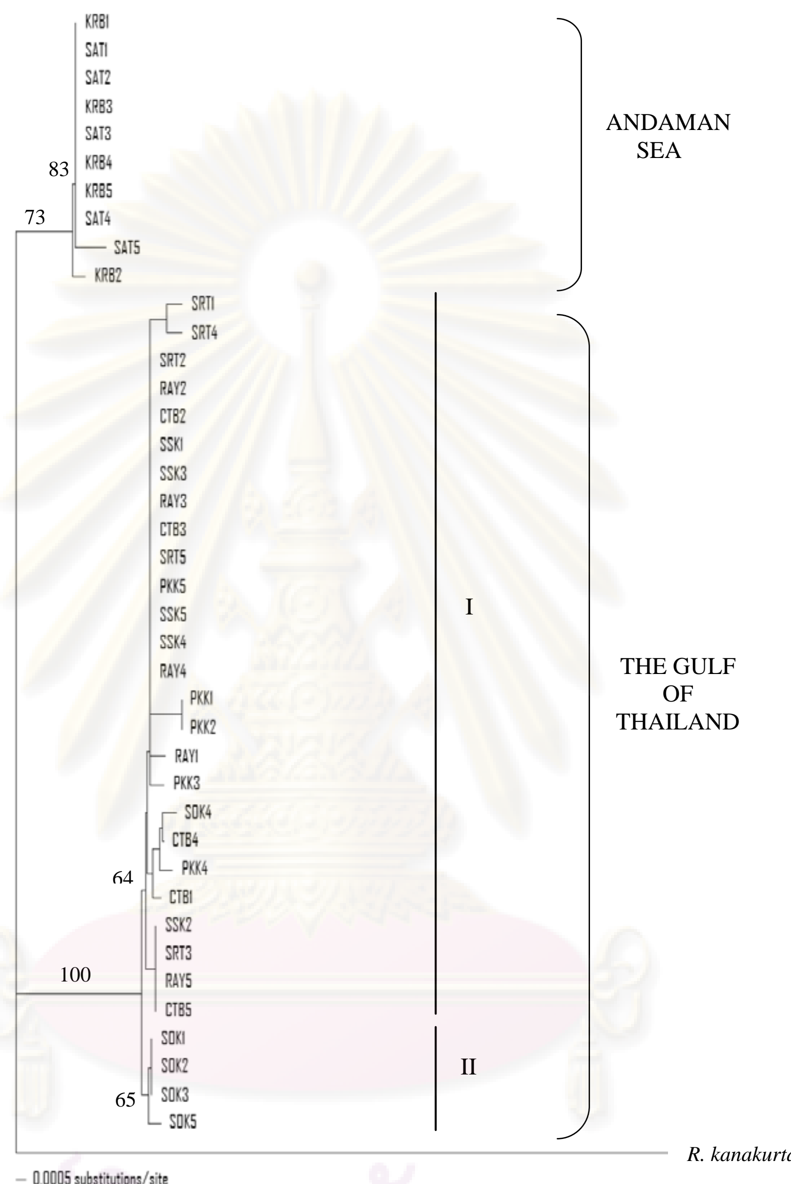


Figure3.6. Neighbor-joining tree derived from genetic distance estimated from HKY85+G model of cytochrome *b* gene sequences of 40 *R. brachysoma* samples and *R. kanakurta* as the outgroup. Numbers indicate bootstrap supports (%) on the branches of the phylogenetic only values > 50% are shown. I and II represent subgroupI and subgroupII of the Gulf of Thailand group, respectively.

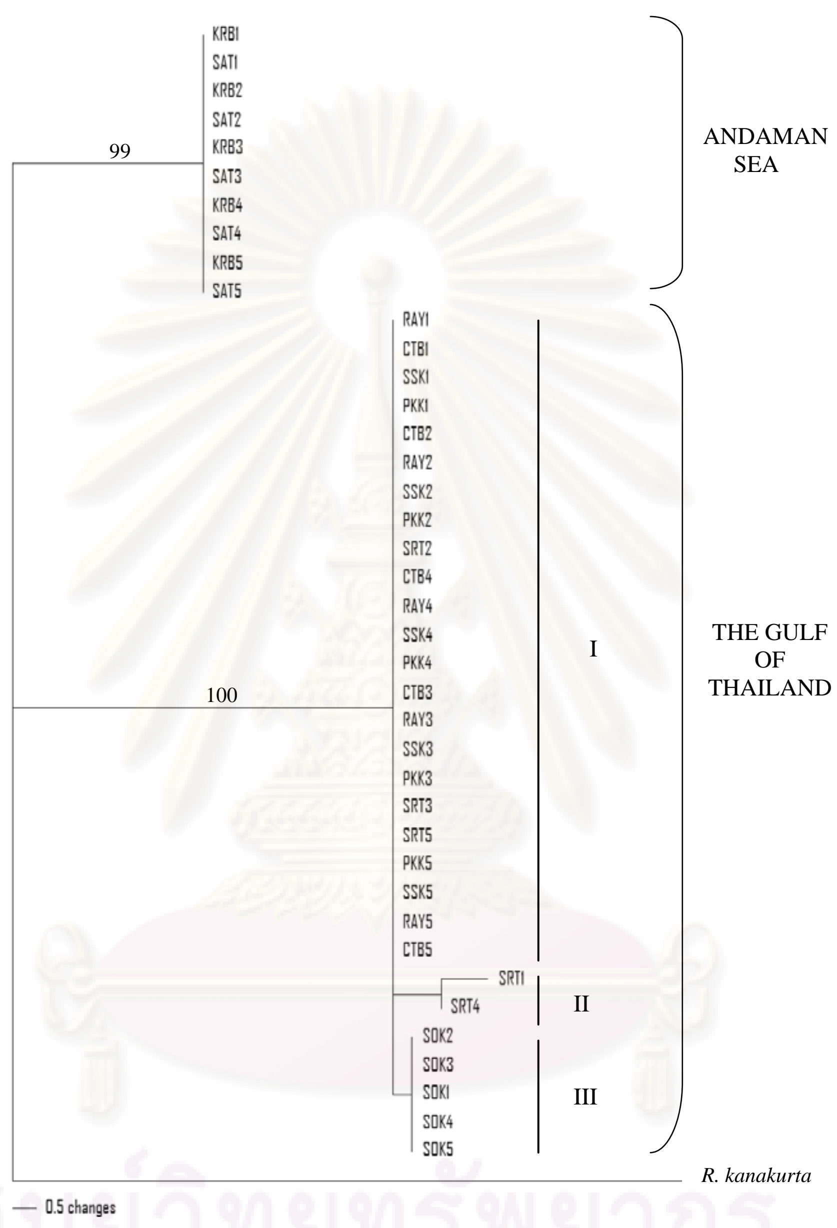


Figure3.7. Maximum parsimony tree derived from the cytochrome *b* gene sequences of 40 *R. brachysoma* samples and *R. kanakurta* as the outgroup. Numbers indicate bootstrap supports (%) on the branches of the phylogenetic only values > 50% are shown. I, II and III represent subgroupI, subgroupII and subgroupIII of the Gulf of Thailand group, respectively.

3.3.3.3 The Combined Sequences of Partial mtDNA Control Region and Cytochrome *b* Gene

In total, 40 *R. brachysoma* individuals from eight locations (5 individuals for each location), including CTB, RAY, SSK, PKK, SRT, SOK, KRB and SAT populations were analyzed. The 1176 bp combined sequences of partial mtDNA control region and cytochrome *b* gene showed 24 variable sites were observed and 16 haplotypes were identified. The detail of variable nucleotide position defining the composited haplotype (Table3.6) and the haplotype frequencies (Table3.7) of combined sequences of partial mtDNA control region and cytochrome *b* gene of 40 *R. brachysoma* samples were showed.

Table3.6. Variable nucleotide position defining the combined sequences of partial mtDNA control region and cytochrome *b* gene haplotype from 40 *R. brachysoma* samples

	000000000000000000000111 001122333444555566777001 251607144256025628256031 174846828005182470462749
Haplotype1	TTCGTACAGTCGCCCGCCCGCCTA
Haplotype2	..................T..T..
Haplotype3	C............T..........
Haplotype4	C.....................G.
Haplotype5	...................A....
Haplotype6	.................T......
Haplotype7	....................T..G
Haplotype8	....................T...
Haplotype9	....................G...
Haplotype10	...............A........
Haplotype11	...............A....T...
Haplotype12	...............A.......G
Haplotype13	.......................G
Haplotype14	.CTAC.TG.CTAT.T.T......G
Haplotype15	.CTACGTGACTAT.T.T.......
Haplotype16	.CTAC.TG.CTAT.T.T.......

Numbers above nucleotides indicate nucleotide position. Sequence identity to reference sequences in top row (Haplotype1).

Table3.7. Haplotype frequencies of the combined sequences of partial mtDNA control region and cytochrome *b* gene of 40 *R. brachysoma* from eight locations consisting of haplotype, haplotype frequencies and percentage of haplotype

Haplotypes	**Haplotype frequencies**								**Percentage of haplotype (%)**
	The Gulf of Thailand						**Andaman Sea**		
	CTB	**RAY**	**SSK**	**PKK**	**SRT**	**SOK**	**KRB**	**SAT**	
Haplotype1	2	3	4	1	2	-	-	-	30.0
Haplotype2	-	-	-	2	-	-	-	-	5.0
Haplotype3	-	-	-	-	1	-	-	-	2.5
Haplotype4	-	-	-	-	1	-	-	-	2.5
Haplotype5	-	1	-	-	-	-	-	-	2.5
Haplotype6	-	-	-	1	-	-	-	-	2.5
Haplotype7	-	-	-	1	-	-	-	-	2.5
Haplotype8	1	-	-	-	-	-	-	-	2.5
Haplotype9	1	-	-	-	-	-	-	-	2.5
Haplotype10	-	-	-	-	-	3	-	-	7.5
Haplotype11	-	-	-	-	-	1	-	-	2.5
Haplotype12	-	-	-	-	-	1	-	-	2.5
Haplotype13	1	1	1	-	1	-	-	-	10.0
Haplotype14	-	-	-	-	-	-	1	-	2.5
Haplotype15	-	-	-	-	-	-	-	1	2.5
Haplotype16	-	-	-	-	-	-	4	4	20.0

The Gulf of Thailand populations consist of Chanthaburi (CTB), Rayong (RAY), Samut Songkhram (SSK), Prachuap Khiri Khan (PKK), Surat Thani (SRT) and Songkhla (SOK). Andaman Sea populations consist of Krabi (KRB) and Satun (SAT).

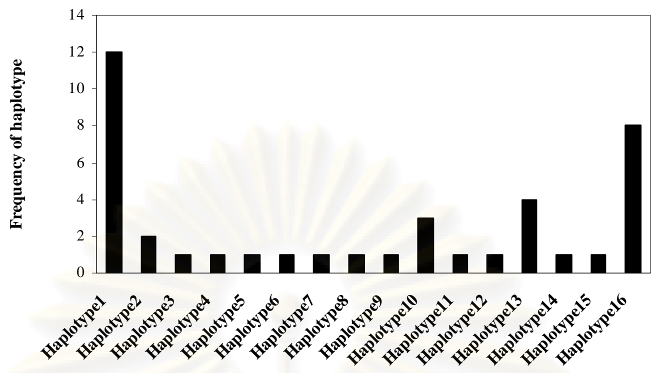
The Haplotype1 was also found at the highest frequency in all populations of the Gulf of Thailand (CTB, RAY, SSK, PKK and SRT) except SOK population. In contrast, the SOK population was restricted three haplotype (Haplotype10, Haplotype11 and Haplotype12). Moreover, Haplotype14, Haplotype15 and Haplotype16 were restricted in Andaman Sea populations (KRB was found Haplotype14 and Haplotype16 but SAT was found Haplotype15 and Haplotype16), with the Haplotype16 was abundant. Comparing the haplotypes found in Thailand, the Gulf of Thailand haplotypes (Haplotype1 to Haplotype13) were nucleotide difference from each other that 1-11 bp differences among haplotypes. The same with Andaman Sea haplotypes (Haplotype1 and Haplotype2) were nucleotide difference at 1-3 bp. However, comparing nucleotide difference between the Gulf of Thailand and

Andaman Sea haplotypes were as high as 12 to 24 bp. For the combined sequences of partial mtDNA control region and cytochrome *b* gene haplotypes could be able to divide between *R. brachysoma* of the Gulf of Thailand and Andaman Sea populations (Figure3.8).

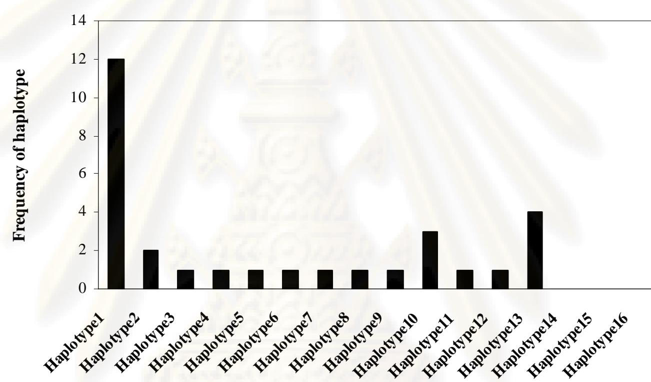
The pairwise genetic distances of 40 *R. brachysoma* individuals evaluate across all pair of sequences were transformed into a distance and ranged from 0 to 0.0138. The variation between all individuals from the Gulf of Thailand population was low at 0 to 0.0034. In addition, variation between all individuals from Andaman Sea populations was also low at 0 to 0.0026, with the highest being seen between SAT5 and KRB2. The pairwise difference between individuals from the Gulf of Thailand and Andaman Sea were much higher differences ranging from 0.0103 to 0.0138, with the highest between SAT5 and six individuals as SRT1, PKK1, PKK2, PKK4, SOK4 and SOK5 (AppendixB.3).

In the part of phylogenetic relationship, the combined sequences of patial mtDNA control region and cytochrome *b* gene of 40 *R. brachysoma* individuals from the Gulf of Thailand and Andaman Sea, including a single of *R. kanakurta* were analyzed. Phylogenetic trees were constructed using NJ and MP methods, and non-parametric bootstrap supports were assessed in both method. According to the NJ tree (Figure3.9), *R. brachysoma* in Thailand were separated in two major groups similar with the cytochrome *b* analysis. The first major group comprised of Andaman Sea, with 74% bootstrap support. The second major group comprised of the Gulf of Thailand, with high bootstrap support at 99%. The Gulf of Thailand group could be able to divide into two subgroups, the first subgroup (subgroupI) composed of all individuals from CTB, RAY, SSK, PKK and SRT, and SOK4, and the second subgroup (subgroupII) also composed of four individuals from SOK (SOK1, SOK2, SOK3 and SOK5), as slight convergence of individual. However, within subgroupI composed of many sister clades as slightly diverged in many individuals.

I



II



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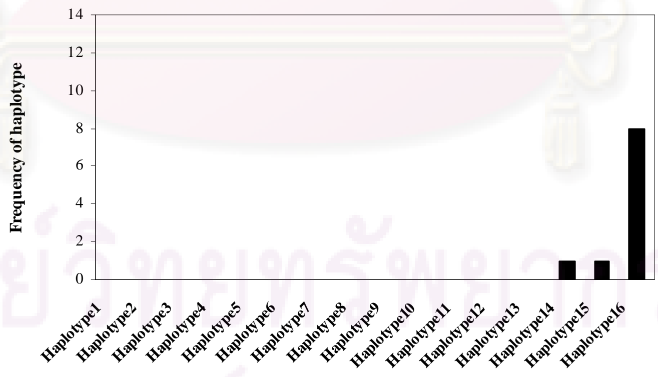

Figure3.8. Histrogram showing the distribution of haplotype obtained from the combined sequences of partial mtDNA control region and cytochrome *b* gene of 40 *R. brachysoma* individuals in Thailand. I: the eight populations from the Gulf of Thailand and Andaman Sea, II: the Gulf of Thailand population (CTB, RAY, SSK, PKK, SRT and SOK), and III: Andaman Sea population (KRB and SAT).

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According to the MP analysis, one-hundred trees were retained, however; only one was showed (Figure3.10). From MP tree, the separation of 40 *R. brachysoma* individuals were in two groups same with NJ trees. The first group comprised of Andaman Sea, with bootstrap support at 82%, and the second group comprised of the Gulf of Thailand with high bootstrap support at 99%. However, within the Gulf of Thailand group consisted of many subgroups.

From both NJ and MP analysis, the combined sequences of partial mtDNA control region and cytochrome *b* gene data were constructed the similar tree topology showed with slightly differences. However, the both phylogenetic trees were obtained the same separation of the two groups (Andaman Sea and the Gulf of Thailand). However, these both trees obtained the complexity of subgroups from many individuals of each group.

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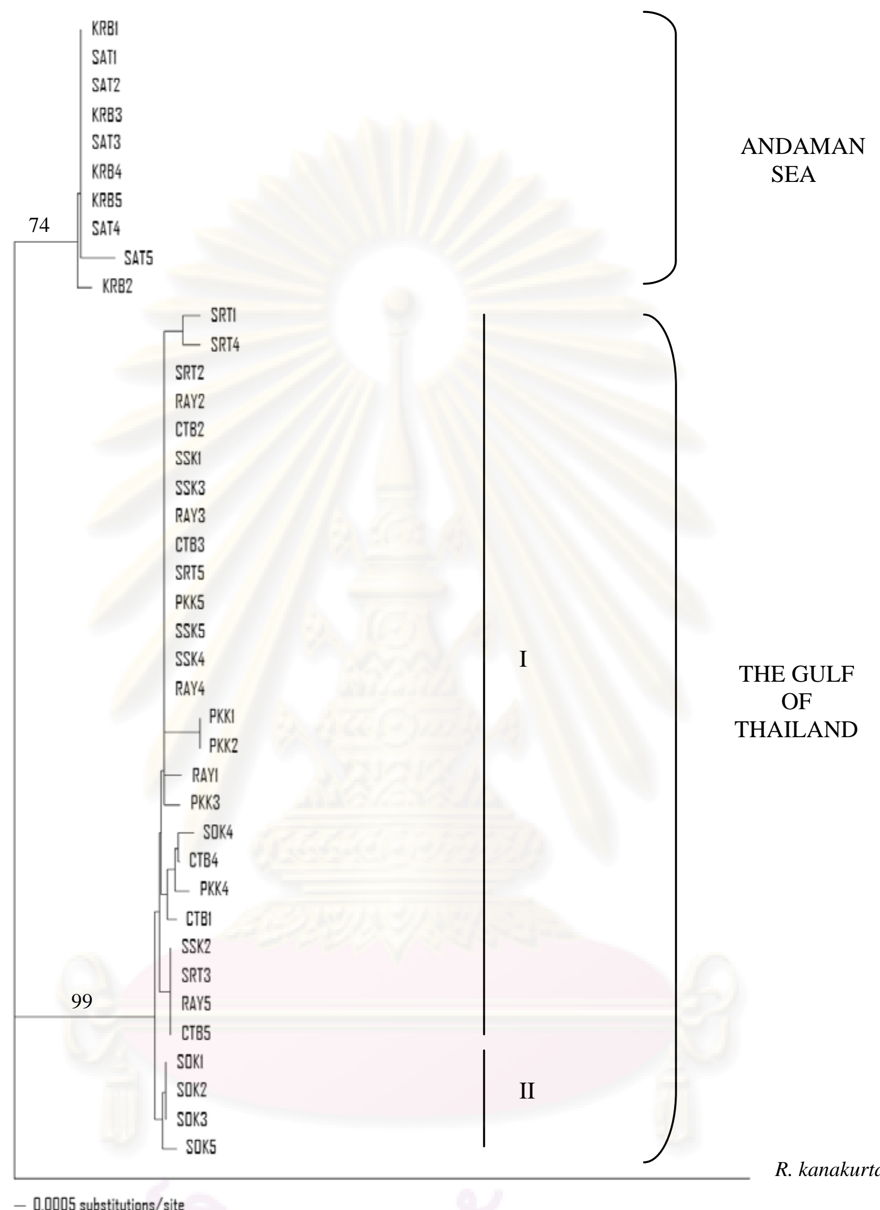

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Figure3.9. Neighbor-joining tree derived from genetic distance estimated from TIM+I model of the combined sequences of partial mtDNA control region and cytochrome *b* gene of 40 *R. brachysoma* samples and *R. kanakurta* as the outgroup. Numbers indicate bootstrap supports (%) on the branches of the phylogenetic only values > 50% are shown. I and II represent subgroupI and subgroupII of the Gulf of Thailand group, respectively.

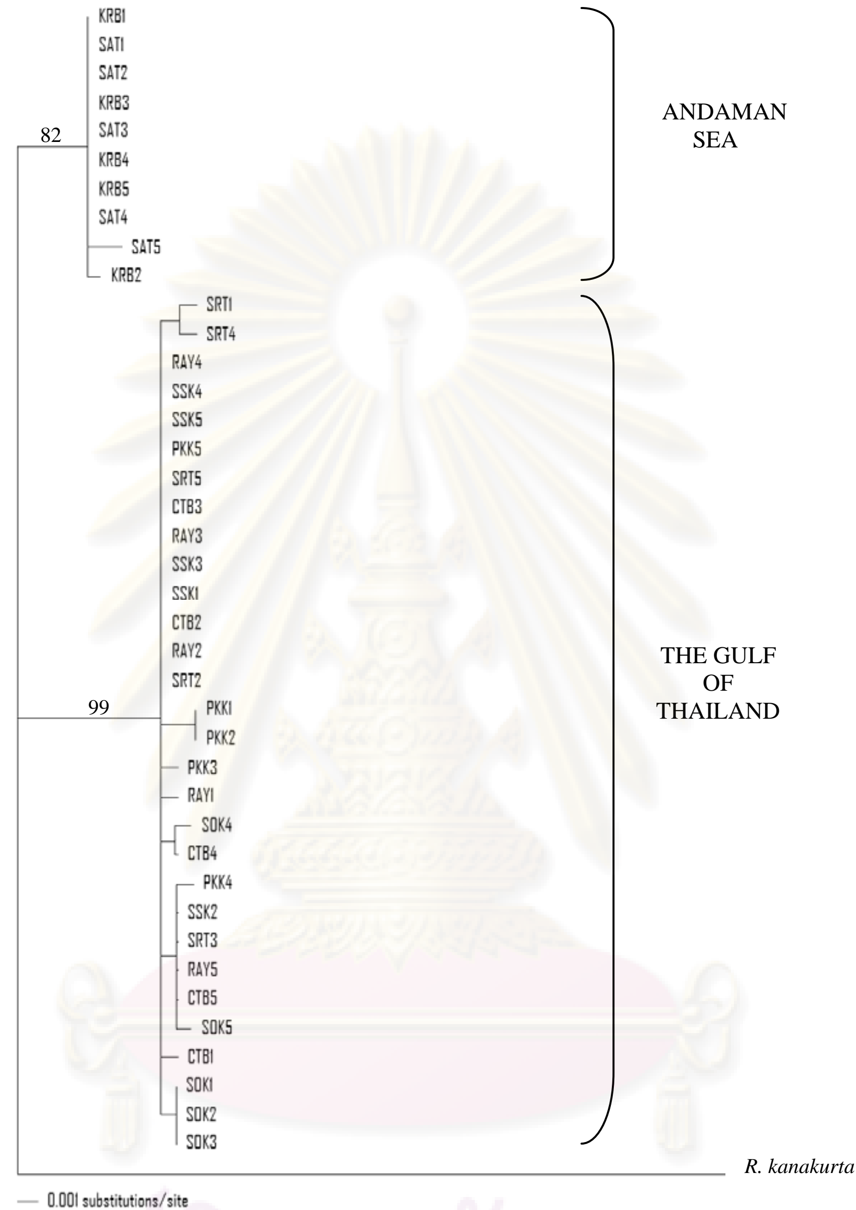

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Figure3.10. Maximum parsimony tree derived from the combined sequences of partial mtDNA control region and cytochrome *b* gene from 40 *R. brachysoma* samples and *R. kanakurta* as the outgroup. Numbers indicate bootstrap supports (%) on the branches of the phylogenetic only values > 50% are shown.

3.4 Discussion

In this chapter, the DNA sequencing of partial mtDNA control region and cytochrome *b* gene is a direct approach for phylogeographic relationships study of *R. brachysoma* in Thailand. The partial mtDNA control region and cytochrome *b* gene are suitable for population studies due to maternal inheritance and rapid evolution (Ferris and Berg, 1987). Considering animal mtDNA is a haploid and non-recombinant molecule reflecting only one type mtDNA in an organism. Therefore, mtDNA is generally useful for examine the genetic relationship among populations (Brown *et al*., 1985).

3.4.1 Genetic Diversity of *R. brachysoma* in Thailand

The 40 samples from eight geographic locations in the Gulf of Thailand and Andaman Sea were used for partial mtDNA control region and cytochrome *b* gene sequencing analysis. The degree of genetic variation in both analyzed mtDNA segments is not similar. The sequences of cytochrome *b* gene showed much higher variable sites of sequences than partial mtDNA control region sequences. The combined sequences of the two regions showed 24 variable sites from 1176 bp in total. cytochrome *b* gene sequences of *R. brachysoma* obtained in this study showed 17 variable sites from 627 bp. Similar result of cytochrome *b* gene sequences were also found in other migratory scombridae fishes. For instance, 485 samples of scad mackerel, *decapterus russelli* showed 20 variable sites from 307 bp fragment (Rohfritsch, 2005). In contrast, 205 samples of Atlantic mackerel, *Scomber scombrus* showed higher degree of variation, 27 variable sites from 197 bp fragment (Nesbo *et al*., 2000). Partial mtDNA control region sequences of *R. brachysoma* obtained in this study showed 7 variable sites from 549 bp fragment, which should a very low degree of variability. On the other hand, there is higher degree of variation in the region of other scombridae species and migratory fishes. For example, 21 samples of bigeye tuna, *Thunnus obesus* showed 75 variable sites in 347 bp fragment (Alvarado-Bremer *et al*., 1998) and 205 samples of Atlantic mackerel, *Scomber scombrus* showed 106 variable sites in 272 bp fragment (Nesbo *et al*., 2000).

3.4.2. Phylogeographic Relationships of *R. brachysoma* in Thailand

According to the phylogenetic analysis by NJ and MP method, the result of cytochrome *b* and the combined sequences of partial mtDNA control region and cytochrome *b* gene showed that there were genetically separation between the Gulf of Thailand and Andaman Sea by all samples segregating completely between the Gulf of Thailand (all individuals of CTB, RAY, SSK, PKK, SRT and SOK) and Andaman Sea (all individuals of KRB and SAT), with high bootstrap support. Interestingly, Songkhla population is likely to be separated from other individuals of the Gulf of Thailand group from NJ and MP trees.

The results of haplotype distribution, pairwise genetic distance and phylogenetic analysis of cytochrome *b* and the combined sequences of the two regions indicated that *R. brachysoma* populations from the Gulf of Thailand and Andaman Sea were genetically different. The genetic difference between populations of *R. brachysoma* from the two regions might be caused by geographical barrier, the Malaysian Peninsular preventing gene flow between the two populations (Antoro *et al*., 2006). In addition, the gene flow between the Gulf of Thailand and Andaman Sea is inhibited due to the north-flowing current in the Strait of Malacca (Great Britain Hydrographic Office, 1958) and the different of temperature range along the Andaman coast was slightly lower than the Gulf of Thailand (Eiamsa-ard and Amornchairojkul 1997). These genetic difference of the two regions agreed with previous reports in others organisms. For example, Asian moon scallop, *Amusium pleuronectes* revealed by 16S rRNA region sequencing (Mahidol *et al*., 2007), abalone, *Haliotis asinina* and *H. ovina* revealed by RAPD markers (Klinbunga *et al*., 2003), banana shrimp, *Penaeus merguiensis* (Hualkasin *et al*., 2003) revealed by *COI* gene sequencing and giant tiger shrimp, *Penaeus monodon* (Klinbunga *et al*., 2001) revealed by RAPD and mtDNA-RFLP.

Moreover, the results indicated that Songkhla population is separated from other populations of the Gulf of Thailand (Surat Thani, Prachuap Khiri Khan, Samut Songkhram, Rayong and Chanthaburi). Songkhla is represented to the southern area of the Gulf of Thailand, while the other populations of the Gulf of Thailand are represented the upper area of the Gulf of Thailand. The divergence might be caused by the different surface current circulation pattern of these two areas. In the southwest

monsoon period, surface current of South China Sea (including Songkhla area) area move in clockwise directions, while anticlockwise directions rise up near the northern area and the middle of the Gulf of Thailand. In the northeast monsoon, surface current of South China Sea (including Songkhla area) area move in anticlockwise directions, while surface current of the northern area and the middle of the Gulf of Thailand move anticlockwise directions (Neelasri, 1981). Thus, the difference of current circulation patterns of the two areas could be presented as barrier that the fish in these two areas could not be transferred. In addition, the southern area of the gulf got high-salinity and cold water from the South China Sea enters (Robinson 1974), while the upper area of the gulf is dominated by the river discharge. The Gulf of Thailand thus functions as a two-layered, shallow estuary with lower-salinity surface water flowing out, while high-salinity, colder water enters from the South China Sea (Naval Hydrographic Department 1995).

The structure of the upper area of the Gulf of Thailand populations were not clear agreeing with many previous reports in many organisms such as swimming crab, *Portunus pelagicus* (Thamniemdee, 2007) and abalone, *Haliotis asinina* and *H. ovina* (Klinbunga *et al*., 2003). Unclear genetic structure might be caused by monsoon winds, tidal currents and the river discharge from four major rivers (the Chao Phraya, the Tha Chin, the Mae Klong and the Bang Pakong) created complex circulation patterns, including localized upwelling and downwelling (Robinson, 1974). The Gene flow between these populations in the upper area of the Gulf of Thailand is possible.

The genetic structure of *R. brachysoma* observed in this study agrees with the previous reproductive biology and spawning seasons of *R. brachysoma* studies. The size at first maturity of male and female of these three groups was difference (Maila-iad *et al*., 2006; Sritakon *et al*., 2006; Sutthakorn, 1998). The spawning season of the upper area of the Gulf of Thailand were in period of February to May and August to October while there was higher peak in first period (Maila-iad et al., 2006), the southern area of the Gulf of Thailand were December to February and May to August (Sritakon *et al*., 2006), and Andaman Sea were all year spawning period (Sutthakorn, 1998).

However, more comprehensive study on population genetic structure of *R. brachysoma* in Thailand is required. The geographic location ranges of the Gulf of Thailand and Andaman Sea, and more samples per population should be added for the analysis. This should provide clearer structure of *R. brachysoma* populations in Thai waters.

CHAPTER IV

GENERAL DISCUSSION

The thesis can be divided into two main parts. The first part is the development of Inter-simple sequence repeats (ISSR) markers used for investigation on genetic diversity and population genetic structure of *R. brachysoma* in Thailand (Chapter II). The second part is the study on phylogeographic relationships of *R. brachysoma* in Thailand using sequencing method of partial mtDNA control region and cytochrome *b* gene (Chapter III). In this Chapter, the results obtained from the two previous chapters will be discussed.

4.1 Genetic diversity of *R. brachysoma* in Thailand

Genetic diversity of *R. brachysoma* was moderately high (Genetic diversity: 0.1485), comparing with previous studies on other marine species in Thai waters. For example, genetic diversity of *Haliotis asinine* was revealed by 16s rRNA and 18s rRNA RFLP (haplotype diversity: 0.6762 and nucleotide diversity: 0.3716) (Klinbunga *et al*., 2003) and in *Penaeus monodon*, genetic diversity was revealed by 16S ribosomal DNA and an intergenic COI-COII RFLP (haplotype diversity: 0.855 and nucleotide diversity: 3.328%) (Klinbunga *et al*., 2001). In contrast, difference results were found in *Amusium pleuronectes*. The result showed low genetic diversity by using 16s rRNA sequencing analysis (haplotype diversity: 0.0237 and nucleotide diversity: 0.0006) (Mahidol *et al*., 2007). With the same technique (ISSR), genetic diversity of *R. brachysoma* in Thai waters was lower than *Mactra veneriformis* in the Chinese coast (Hou *et al*., 2006) and *Apostichopus japonicus* in the Shandong Peninsula (Bing *et al*., 2007), but higher than *Cynoglossus semilaevis* in Laizhou Bay (Liu *et al*., 2008).

The number of haplotype sequences of 40 *R. brachysoma* samples was low. This might be caused by a small numbers of samples used in this study. The number of haplotypes of Andaman Sea samples was lower than the Gulf of Thailand samples.

By comparing the haplotype and variable site obtained from both cytochrome *b* and partial mtDNA control region sequencesin this study, it showed much lower variation than other scombridae species such as *Trachurus trachurus* (Comesana *et al*., 2008), *Scomber scombrus* (Nesbo *et al*., 2000) and *Thunnus obesus* (Alvarado-Bremer *et al*., 1998). In contrast, similar result was found on *decapterus russelli* (Rohfritsch, 2005).

The high level of genetic variation within population of *R. brachysoma* was observed. In general, marine fish tend to show a higher genetic variation than freshwater and anadromous fishes (Ward *et al*, 1994; DeWoody and Avise, 2000) because many marine fishes have been attributed to larger population sizes than freshwater fishes and geographical barriers (for gene flow) among freshwater localities, which isolate populations (Ward *et al*, 1994). The high level of genetic variation within population in present study similar result in a similar geographical region (the Gulf of Thailand and Andaman Sea) to that observed in *Penaeus monodon* revealed 16S ribosomal DNA and an intergenic COI-COII RFLP (Kinbunga *et al*., 2001) and three abalone species *Haliotis asinina* and *H. ovina* revealed by RAPD markers (Klinbunga *et al*., 2003).

In conclusion, the high degree of genetic diversity found in present study might be accordant with the concept that widely distributed marine animal species must adapt to a broad range of environmental conditions to maintain their large geographic distributions. Consequently, many widespread species have high genetic diversity and evolved into a series of ecological races (Turesson, 1922). However, it should be noted that *R. brachysoma* populations used in this study were sampled from narrow geographical area of distribution.

4.2 Population Genetic Structure of *R. brachysoma* in Thailand

From the results of ISSR and mtDNA sequencing analysis showed that *R. brachysoma* populations could be divided into three groups; the upper and the southern areas of the Gulf of Thailand and Andaman Sea. The genetic divergence between populations might be cause by geographical barrier. Between the Gulf of Thailand and Andaman Sea, the divergence might be caused by the Malaysian Peninsular preventing gene flow between the two populations (Antoro *et al*., 2006). A

clear genetic differentiation between the populations of the Gulf of Thailand and Andaman Sea could occur since the Pleistocene isolation of marine basins during the connection of the Asian landmass and Sunda Shelf (south of the South China Sea, the Gulf of Thailand and Java Sea) with lowered sea levels (McManus, 1958). In addition, the temperature range along the Andaman coast slightly lower than the Gulf of Thailand (Eiamsa-ard and Amornchairojkul, 1997) that could be made genetic differentiated of the two areas. The similar result of the genetic differentiated of the two areas were reported in *Amusium pleuronectes* (Mahidol *et al*., 2007), *Haliotis asinina* and *H. ovina* (Klinbunga *et al*., 2003), *Penaeus merguiensis* (Hualkasin *et al*., 2003) and. *Penaeus monodon* (Klinbunga *et al*., 2001).

In the Gulf of Thailand, genetic divergence of *R. brachysoma* between the upper and southern areas might be caused by the different surface current circulation patterns of the two areas, which is likely to be a major factor inhibiting gene flow between the areas. In the southwest monsoon period, surface current of South China Sea (including Songkhla area) area move in clockwise directions, while anticlockwise directions rise up near the northern area and the middle of the Gulf of Thailand. In the northeast monsoon, surface current of South China Sea (including Songkhla area) area move in anticlockwise directions, while surface current of the northern area and the middle of the Gulf of Thailand move anticlockwise directions (Neelasri, 1981). Thus, the difference of current circulation patterns of the two areas could be presented as barrier that the fish in these two areas could not be transferred. Moreover, the divergence might be caused by physical barrier from the difference in salinity and temperature of water between the areas. The southern area of the Gulf got high salinity and cold water from the South China Sea enters (Robinson 1974), while the upper area of the gulf is dominated by the river discharge. The Gulf of Thailand thus functions as a two-layered, shallow estuary with lower-salinity surface water flowing out, while high-salinity, colder water enters from the South China Sea (Suvapepun, 1991; Naval Hydrographic Department, 1995).

However, the genetic structure of populations within the upper area of the Gulf of Thailand was not clear. This indicates that value of gene flow of *R. brachysoma* populations in the upper of the Gulf of Thailand was high. The unclear structure might be caused by monsoon winds, tidal currents and the river discharge

from four major rivers (the Chao Phraya, the Tha Chin, the Mae Klong and the Bang Pakong) create complex circulation patterns, including localized upwelling and downwelling (Robinson, 1974), which effect to the well mixture between populations. The previous reported for unclear population genetic structure of the upper area of the Gulf of Thailand similar with this study were founded in many marine organisms such as *Portunus pelagicus* (Thamniemdee, 2007) and *Haliotis asinina* and *H. ovina* (Klinbunga *et al*., 2003).

The genetic structure of *R. brachysoma* observed in this study agrees with the previous reproductive biology and spawning seasons of *R. brachysoma* studies. The size at first maturity of male and female of these three groups was difference (Maila-iad *et al*., 2006; Sritakon *et al*., 2006; Sutthakorn, 1998). The spawning season of the upper area of the Gulf of Thailand were in period of February to May and August to October while there was higher peak in first period (Maila-iad et al., 2006), the southern area of the Gulf of Thailand were December to February and May to August (Sritakon *et al*., 2006), and Andaman Sea were all year spawning period (Sutthakorn, 1998).

In general, *R. brachysoma* dispersed in coastal habitats that the first migration of the fish might be presented in pelagic larval stage. Although pelagic larvae of many marine organisms could be dispersed across hundreds of kilometers, oceanographic or behavioral mechanisms can force dispersal for unclear of genetic differentiation (Taylor and Hellberg, 2003). *R. brachysoma* has a high retention period of pelagic larvae that can potentially connect between populations through dispersal on the Gulf of Thailand currents. The study shows that populations of *R. brachysoma* of the upper and the southern areas of the Gulf of Thailand are differentiated, while the absence geographical barriers. The result suggested that gene flow among the populations have been restricted, it might be caused by larvae behavior (Burton and Feldman, 1982; Bousfield, 1995) or current circulation pattern (Benzie and Stoddart, 1992; Bertness and Gaines, 1992). In monsoon season, the current circulation pattern in the upper and the southern areas of the Gulf of Thailand were different; as a consequence, the larvae in these two areas could not be transferred (Wanna *et al*., 2004). Moreover, the absence of population structure for species with board larval dispersal potential or high larval retention like *R. brachysoma* were presented such as blue head wrasse,

Thalassoma bifasciatum (Swearer *et al*., 1999). The pelagic larvae of *R. brachysoma* among populations in the upper area of the Gulf of Thailand could be mixed by the complex circulation patterns of monsoon wind, tidal currents and the river discharge from four major rivers (the Chao Phraya, the Tha Chin, the Mae Klong and the Bang Pakong), the result showed unclear structure of *R. brachysoma* in the upper area of the Gulf of Thailand. The result indicated that gene flow among populations of the upper area of the Gulf was high.

4.3 Implication on Conservation and Stock Management

The estimation and partition of the level of genetic variation between populations in any species is fundamental for management of natural resources (Avise, 1994). In recent years, DNA analysis has been commonly used to determine the level of genetic variation and population genetic structure of many species (Benzie, 2000).

In present study, *R. brachysoma* populations in Thailand could be divided into three stocks (the upper of the Gulf of Thailand, the southern area of the Gulf of Thailand and Andaman Sea). The result agrees with the reports on spawning ground and season of *R. brachysoma* studies in Thai waters, which were different in the three areas (see section 1.2.4 and 1.2.5). Therefore, fishing season of *R. brachysoma* in Thai waters should be different in the three areas.

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 *R. brachysoma* in Thailand and prevention the genetic contamination among the three stocks when stock rehabilitation is needed. The strategies for stock rehabilitation were control the yield at a reduced level, direct reduction in the fishing effort, and increase the size at first capture (Sanders and Beinssen, 1998). In the future, stock rehabilitation of *R. brachysoma* might be established to maintain high yields and to conserve in Thai waters.

However, the conservation and management of *R. brachysoma* are required information in many disciplines such as biology (reproductive biology, spawning

characteristics and spawning season), fisheries science, population dynamic (migration, growth and recruitment, genetic structure) and social science to obtain efficient and sustainable use of *R. brachysoma* in Thailand.

CHAPTER V

CONCLUSION

In the previous chapter, Inter-simple sequence repeat (ISSR) method was used to investigate genetic diversity and population genetic structure of *R. brachysoma*. The partial mitochondrial DNA control region and the cytochrome *b* gene sequences were used for phylogeographic study of *R. brachysoma* from the Gulf of Thailand and Andaman Sea. The following studies, the results obtained from previous chapters will be concluded.

1. Genetic diversity of *R. brachysoma* in Thai water was moderately high by comparing with another marine species such as *Amusium pleuronectes*, *Cynoglossus semilaevis*, *Penaeus monodon* and *Haliotis asinine*. Genetic diversity of the Gulf of Thailand and Andaman Sea populations were relatively similar. The high degree of genetic diversity found in present study might be accordant with the concept that widely distributed marine animal species must adapt to a broad range of environmental conditions to maintain their large geographic distributions.

2. The result obtained from ISSR markers and mtDNA sequencing analysis showed that the populations of *R. brachysoma* in Thai waters were divided into three groups that there were the upper area of the Gulf of Thailand (Chanthaburi, Rayong, Samut Songkhram, Prachuap Khiri Khan and Surat Thani), the southern area of the Gulf of Thailand (Songkhla) and Andaman Sea (Satun and Krabi). However, the mtDNA sequencing analysis, partial mtDNA control region sequences do not showed population genetic structure. The partition of *R. brachysoma* between the Gulf of Thailand and Andaman Sea might be caused by the geographical barrier (the Malaysian Peninsular) and the different water temperature. The partition of the upper and the southern areas of the Gulf of Thailand might be caused by the different surface current circulation patterns of the two areas, which is likely to be a major factor inhibiting gene flow between the areas. Moreover, the divergence might be

caused by physical barrier from the difference in salinity and temperature of water between the upper and the southern areas of the Gulf of Thailand.

3. This study suggested that ISSR markers and mtDNA sequencing analysis showed clearly offers the ability to investigate the genetic diversity, population genetic structure and phylogeographic relationships of *R. brachysoma* in Thai waters.

4. The result obtained from this study will be useful information for stock management and conservation plans of *R. brachysoma* in Thai waters. We should set a precedent for stock management of *R. brachysoma* in Thai waters by separate the fishes into three stocks as the upper and the southern areas of the Gulf of Thailand and Andaman Sea for conservation and fisheries.

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APPENDICES

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Appendix A

AppendixA.1. A character matrix of 40 *R.brachysoma* samples and 1 *R. kanakurta* sample based on partial mtDNA control region sequences. Asterisks(*) represent conserved nucleotides across all samples.

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                      10        20        30        40        50        60
              ....|....|....|....|....|....|....|....|....|....|....|....|
PKK1          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
PKK2          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
SOK4          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
CTB4          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
PKK4          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
CTB1          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
KRB2          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
SSK2          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
SRT3          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
SOK5          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
RAY5          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
CTB5          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
SRT4          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
SAT2          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
SOK2          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
SAT1          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
SRT1          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
RAY2          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
KRB3          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
SSK4          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
RAY4          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
KRB5          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
SAT5          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
SRT5          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
PKK5          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
SSK5          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
SSK3          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
SAT4          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
KRB4          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
CTB3          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
RAY3          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
SOK3          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
SAT3          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
CTB2          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
SSK1          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
SRT2          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
SOK1          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
KRB1          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
PKK3          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTTTATGTAT
RAY1          TTAACACCATATATTTATGTCGAACATTTATTATCAATGCTTTAAAGATATTCTATGTAT
R.kanakurta   TTAACACCATATATTTATGTCGAACATTTATTATCAATGTTTTAAAGACATTTTATGTAT
ClustalConsens*************************************** ******** *** *******
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AppendixA.1. (continued)

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                       70        80        90       100       110       120
              ....|....|....|....|....|....|....|....|....|....|....|....|
PKK1          TATCACCATTTATAGTAATAGAACATTCACATGTCATCATTTCATACTAAGGGGTACATA
PKK2          TATCACCATTTATAGTAATAGAACATTCACATGTCATCATTTCATACTAAGGGGTACATA
SOK4          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
CTB4          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
PKK4          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
CTB1          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
KRB2          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
SSK2          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
SRT3          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
SOK5          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
RAY5          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
CTB5          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
SRT4          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
SAT2          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
SOK2          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
SAT1          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
SRT1          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
RAY2          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
KRB3          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
SSK4          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
RAY4          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
KRB5          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
SAT5          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
SRT5          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
PKK5          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
SSK5          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
SSK3          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
SAT4          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
KRB4          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
CTB3          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
RAY3          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
SOK3          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
SAT3          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
CTB2          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
SSK1          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
SRT2          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
SOK1          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
KRB1          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
PKK3          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
RAY1          TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
R.kanakurta   TATCACCATTTATAGTAATAGAACATTCACATGTCACCATTTCATACTAAGGGGTACATA
ClustalConsens************************************ ***********************
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AppendixA.1. (continued)

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                           130       140       150       160       170       180
                   ....|....|....|....|....|....|....|....|....|....|....|....|
PKK1               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
PKK2               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
SOK4               AACCATTAGGTCCATATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
CTB4               AACCATTAGGTCCATATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
PKK4               AACCATTAGGTCCATATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
CTB1               AACCATTAGGTCCAGATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
KRB2               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
SSK2               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
SRT3               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
SOK5               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
RAY5               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
CTB5               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
SRT4               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
SAT2               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
SOK2               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
SAT1               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
SRT1               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
RAY2               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
KRB3               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
SSK4               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
RAY4               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
KRB5               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
SAT5               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
SRT5               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
PKK5               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
SSK5               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
SSK3               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
SAT4               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
KRB4               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
CTB3               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
RAY3               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
SOK3               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
SAT3               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
CTB2               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
SSK1               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
SRT2               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
SOK1               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
KRB1               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
PKK3               AACCATTAGGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
RAY1               AACCATTAAGTCCACATATTACATATACTTCATTCAAGGACTGGCGATGGAGGGAACCCT
R.kanakurta        AACCATTAAGTCCTCATATTACATACATTTCACTCAAGGACTGGCGATGGAGGGAACCCC
ClustalConsens     ******** ****  ********** * **** ************************** 
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AppendixA.1. (continued)

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                        190       200       210       220       230       240
                ....|....|....|....|....|....|....|....|....|....|....|....|
PKK1            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
PKK2            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
SOK4            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
CTB4            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
PKK4            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
CTB1            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
KRB2            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
SSK2            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
SRT3            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
SOK5            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
RAY5            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
CTB5            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
SRT4            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
SAT2            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
SOK2            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
SAT1            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
SRT1            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
RAY2            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
KRB3            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
SSK4            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
RAY4            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
KRB5            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
SAT5            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
SRT5            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
PKK5            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
SSK5            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
SSK3            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
SAT4            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
KRB4            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
CTB3            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
RAY3            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
SOK3            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
SAT3            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
CTB2            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
SSK1            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
SRT2            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
SOK1            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
KRB1            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
PKK3            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
RAY1            GTACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
R.kanakurta     GCACCCTGAATCTCTCGTGTCTCGATATACCAAGTATCAACATCTCTACAACCGAGGATA
ClustalConsens  * **********************************************************
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ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

AppendixA.1. (continued)

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                  250       260       270       280       290       300
             ....|....|....|....|....|....|....|....|....|....|....|....|
PKK1         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
PKK2         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
SOK4         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
CTB4         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
PKK4         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
CTB1         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
KRB2         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
SSK2         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
SRT3         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
SOK5         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
RAY5         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
CTB5         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
SRT4         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
SAT2         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
SOK2         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
SAT1         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
SRT1         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
RAY2         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
KRB3         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
SSK4         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
RAY4         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
KRB5         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
SAT5         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
SRT5         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
PKK5         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
SSK5         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
SSK3         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
SAT4         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
KRB4         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
CTB3         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
RAY3         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
SOK3         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
SAT3         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
CTB2         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
SSK1         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
SRT2         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
SOK1         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
KRB1         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
PKK3         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
RAY1         CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAATGCATACTCTTATTGAAGG
R.kanakurta  CTCATACGCAGTAAGAGCCCACCAACAAGCTCATAACTTAAAGCATACTCTTATTGAAGG
ClustalConsens *****************************************  ******************
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จุฬาลงกรณ์มหาวิทยาลัย

AppendixA.1. (continued)

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                      310       320       330       340       350       360
              ....|....|....|....|....|....|....|....|....|....|....|....|
PKK1          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
PKK2          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
SOK4          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
CTB4          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
PKK4          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
CTB1          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
KRB2          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
SSK2          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
SRT3          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
SOK5          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
RAY5          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
CTB5          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
SRT4          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
SAT2          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
SOK2          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
SAT1          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
SRT1          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
RAY2          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
KRB3          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
SSK4          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
RAY4          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
KRB5          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
SAT5          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
SRT5          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
PKK5          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
SSK5          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
SSK3          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
SAT4          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
KRB4          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
CTB3          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
RAY3          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
SOK3          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
SAT3          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
CTB2          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
SSK1          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
SRT2          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
SOK1          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
KRB1          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
PKK3          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
RAY1          TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
R.kanakurta   TGAGGGACAAAAATTGTGGGGGTTTCACTTAGTGAATTATTCCTGGCATTTGGTTCCTAT
ClustalConsens ************************************************************
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AppendixA B.1. (continued)

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                              370       380       390       400       410       420
                     ....|....|....|....|....|....|....|....|....|....|....|....|
PKK1                 TTCAGGGCCATTACTTGATTTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
PKK2                 TTCAGGGCCATTACTTGATTTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
SOK4                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
CTB4                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
PKK4                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
CTB1                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
KRB2                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
SSK2                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
SRT3                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
SOK5                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
RAY5                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
CTB5                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
SRT4                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTGGCATAAGTTGTTG
SAT2                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
SOK2                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
SAT1                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
SRT1                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
RAY2                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
KRB3                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
SSK4                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
RAY4                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
KRB5                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
SAT5                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
SRT5                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
PKK5                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
SSK5                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
SSK3                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
SAT4                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
KRB4                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
CTB3                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
RAY3                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
SOK3                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
SAT3                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
CTB2                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
SSK1                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
SRT2                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
SOK1                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
KRB1                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
PKK3                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
RAY1                 TTCAGGGCCATTACTTGATCTACTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
R.kanakurta          TTCAGGGCCATTACTTGATTTGTTTCCCCATTCTTTCCTTGACGCTTGCATAAGTTGTTG
ClustalConsens       ******************* *  *********************** *************
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AppendixA.1. (continued)

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                   430       440       450       460       470       480
               ....|....|....|....|....|....|....|....|....|....|....|....|
PKK1           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
PKK2           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
SOK4           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
CTB4           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
PKK4           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
CTB1           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
KRB2           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
SSK2           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
SRT3           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
SOK5           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
RAY5           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
CTB5           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
SRT4           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
SAT2           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
SOK2           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
SAT1           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
SRT1           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
RAY2           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
KRB3           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
SSK4           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
RAY4           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
KRB5           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
SAT5           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
SRT5           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
PKK5           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
SSK5           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
SSK3           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
SAT4           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
KRB4           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
CTB3           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
RAY3           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
SOK3           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
SAT3           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
CTB2           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
SSK1           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
SRT2           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
SOK1           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
KRB1           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
PKK3           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
RAY1           GTGGAGTACATTTATACTCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
R.kanakurta    GTGGAGTTCATAT-TACCCTTTAAGCCACATGCCGAGCGTTCACTCCACGGGGGTCAGGT
ClustalConsens ******* *** * *** ******************************************
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AppendixA.1. (continued)

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                     490       500       510       520       530       540
                 ....|....|....|....|....|....|....|....|....|....|....|....|
PKK1             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
PKK2             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
SOK4             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
CTB4             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
PKK4             TATTTTTTTCTGTTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
CTB1             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
KRB2             TATTTTTTTCTGTTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
SSK2             TATTTTTTTCTGTTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
SRT3             TATTTTTTTCTGTTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
SOK5             TATTTTTTTCTGTTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
RAY5             TATTTTTTTCTGTTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
CTB5             TATTTTTTTCTGTTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
SRT4             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
SAT2             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
SOK2             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
SAT1             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
SRT1             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
RAY2             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
KRB3             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
SSK4             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
RAY4             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
KRB5             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
SAT5             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
SRT5             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
PKK5             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
SSK5             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
SSK3             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
SAT4             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
KRB4             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
CTB3             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
RAY3             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
SOK3             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
SAT3             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
CTB2             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
SSK1             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
SRT2             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
SOK1             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
KRB1             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
PKK3             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
RAY1             TATTTTTTTCTATTTCCTTTCATTTGACCCTTCAGAGTGAACACCGATAATGACGTTCAA
R.kanakurta      TATTTTTTTCTATTTCCTTTCACTTGACATTTCAGAGTGAGCACCGGCAATGAGGTTCAA
ClustalConsens   *********** ********** *****  ********** *****  ***** ******
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AppendixA.1. (continued)

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              ....|....
PKK1          GGTTGAACA
PKK2          GGTTGAACA
SOK4          GGTTGAACA
CTB4          GGTTGAACA
PKK4          GGTTGAACA
CTB1          GGTTGAACA
KRB2          GGTTGAACA
SSK2          GGTTGAACA
SRT3          GGTTGAACA
SOK5          GGTTGAACA
RAY5          GGTTGAACA
CTB5          GGTTGAACA
SRT4          GGTTGAACA
SAT2          GGTTGAACA
SOK2          GGTTGAACA
SAT1          GGTTGAACA
SRT1          GGTTGAACA
RAY2          GGTTGAACA
KRB3          GGTTGAACA
SSK4          GGTTGAACA
RAY4          GGTTGAACA
KRB5          GGTTGAACA
SAT5          GGTTGAACA
SRT5          GGTTGAACA
PKK5          GGTTGAACA
SSK5          GGTTGAACA
SSK3          GGTTGAACA
SAT4          GGTTGAACA
KRB4          GGTTGAACA
CTB3          GGTTGAACA
RAY3          GGTTGAACA
SOK3          GGTTGAACA
SAT3          GGTTGAACA
CTB2          GGTTGAACA
SSK1          GGTTGAACA
SRT2          GGTTGAACA
SOK1          GGTTGAACA
KRB1          GGTTGAACA
PKK3          GGTTGAACA
RAY1          GGTTGAACA
R.kanakurta   GGTTGAACA
ClustalConsens *********
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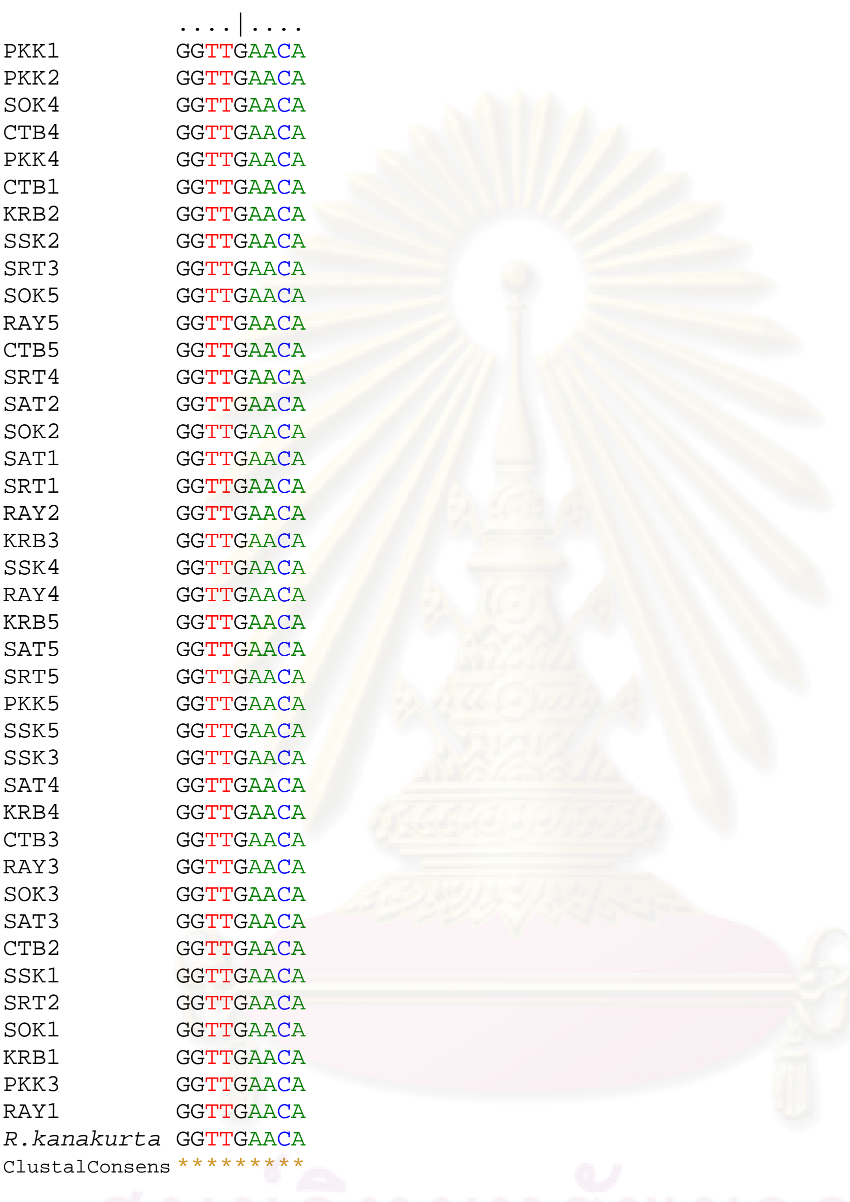

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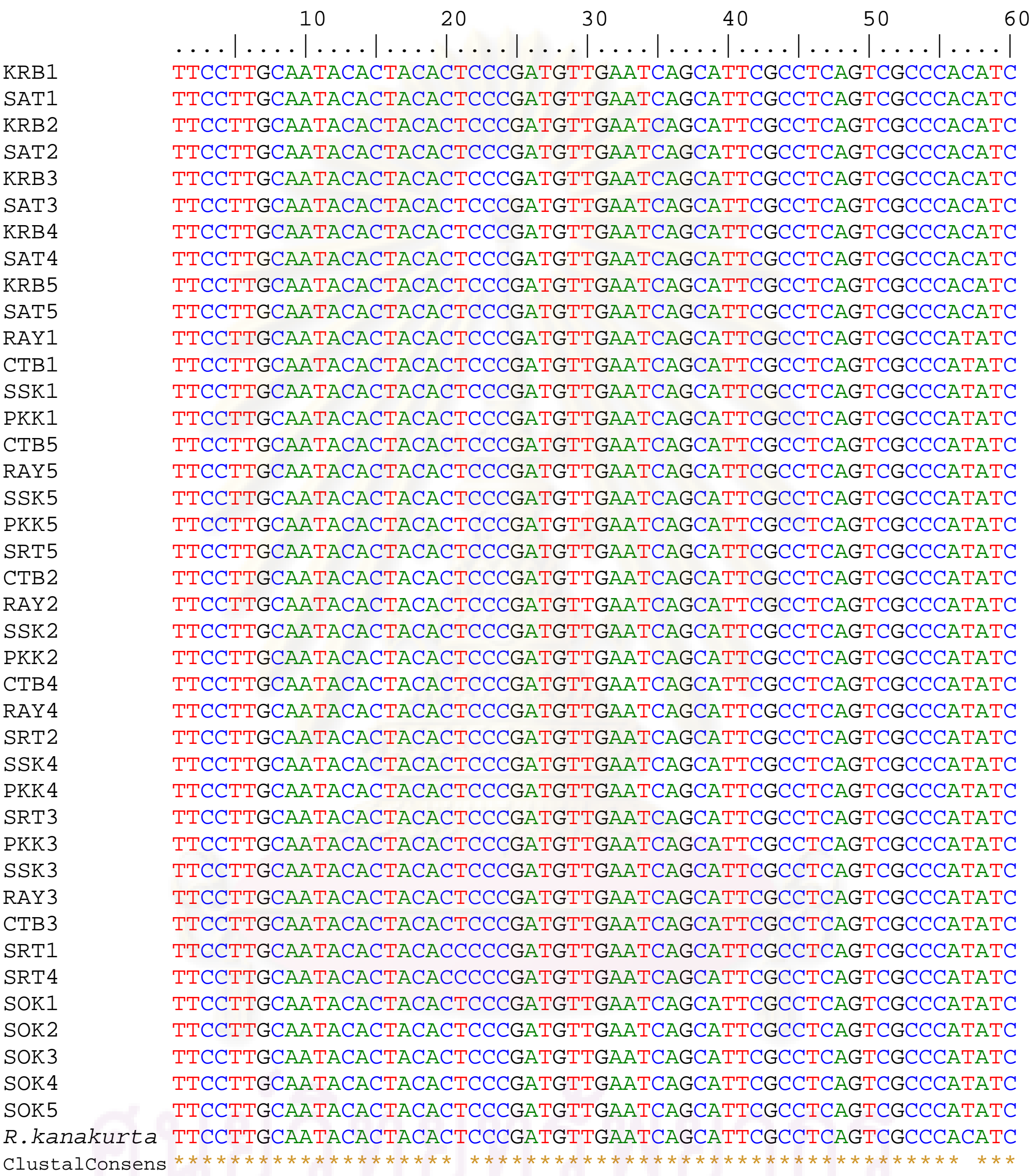
AppendixA.2. A character matrix of 40 *R.brachysoma* samples and 1 *R. kanakurta* sample based on cytochrome *b* gene sequences. Asterisks(*) represent conserved nucleotides across all samples.



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                      10        20        30        40        50        60
              ....|....|....|....|....|....|....|....|....|....|....|....|
KRB1          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCACATC
SAT1          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCACATC
KRB2          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCACATC
SAT2          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCACATC
KRB3          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCACATC
SAT3          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCACATC
KRB4          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCACATC
SAT4          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCACATC
KRB5          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCACATC
SAT5          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCACATC
RAY1          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
CTB1          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
SSK1          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
PKK1          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
CTB5          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
RAY5          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
SSK5          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
PKK5          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
SRT5          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
CTB2          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
RAY2          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
SSK2          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
PKK2          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
CTB4          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
RAY4          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
SRT2          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
SSK4          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
PKK4          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
SRT3          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
PKK3          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
SSK3          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
RAY3          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
CTB3          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
SRT1          TTCCTTGCAATACACTACACCCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
SRT4          TTCCTTGCAATACACTACACCCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
SOK1          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
SOK2          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
SOK3          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
SOK4          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
SOK5          TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCATATC
R.kanakurta   TTCCTTGCAATACACTACACTCCCGATGTTGAATCAGCATTCGCCTCAGTCGCCCACATC
ClustalConsens ******************** *********************************** ***
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ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

AppendixA.2. (continued)

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                        70        80        90        100       110       120
               ....|....|....|....|....|....|....|....|....|....|....|....|
KRB1           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCTTCTTTC
SAT1           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCTTCTTTC
KRB2           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCTTCTTTC
SAT2           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCTTCTTTC
KRB3           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCTTCTTTC
SAT3           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCTTCTTTC
KRB4           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCTTCTTTC
SAT4           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCTTCTTTC
KRB5           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCTTCTTTC
SAT5           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCTTCTTTC
RAY1           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
CTB1           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
SSK1           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
PKK1           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
CTB5           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
RAY5           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
SSK5           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
PKK5           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
SRT5           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
CTB2           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
RAY2           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
SSK2           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
PKK2           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
CTB4           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
RAY4           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
SRT2           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
SSK4           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
PKK4           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
SRT3           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
PKK3           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
SSK3           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
RAY3           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
CTB3           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
SRT1           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
SRT4           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
SOK1           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
SOK2           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
SOK3           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
SOK4           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
SOK5           TGCCGAGACGTAAACTTCGGCTGACTCATCCGCAACCTCCACGCAAATGGCGCCTCTTTC
R.kanakurta    TGCCGAGACGTAAACTTCGGCTGACTCATCCGTAACCTCCACGCAAATGGCGCTTCTTTC
ClustalConsens ******************************** ******************** ******
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AppendixA.2. (continued)

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                        130       140       150       160       170       180
                ....|....|....|....|....|....|....|....|....|....|....|....|
KRB1            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGATCCTACCTCTAC
SAT1            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGATCCTACCTCTAC
KRB2            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGATCCTACCTCTAC
SAT2            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGATCCTACCTCTAC
KRB3            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGATCCTACCTCTAC
SAT3            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGATCCTACCTCTAC
KRB4            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGATCCTACCTCTAC
SAT4            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGATCCTACCTCTAC
KRB5            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGATCCTACCTCTAC
SAT5            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGATCCTACCTCTAC
RAY1            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
CTB1            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
SSK1            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
PKK1            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
CTB5            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
RAY5            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
SSK5            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
PKK5            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
SRT5            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
CTB2            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
RAY2            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
SSK2            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
PKK2            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
CTB4            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
RAY4            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
SRT2            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
SSK4            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
PKK4            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
SRT3            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
PKK3            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
SSK3            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
RAY3            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
CTB3            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
SRT1            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
SRT4            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
SOK1            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
SOK2            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
SOK3            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
SOK4            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
SOK5            TTCTTTATTTGCATCTACATGCACATTGGACGAGGCCTCTACTACGGGTCCTACCTCTAC
R.kanakurta     TTCTTTATTTGCATCTACATGCACATCGGACGAGGCCTTTACTACGGATCCTACCTCTAT
ClustalConsens  ************************** *********** ******* ************ 
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AppendixA.2. (continued)

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                        190       200       210       220       230       240
               ....|....|....|....|....|....|....|....|....|....|....|....|
KRB1           ATAGAAACATGAAACATCGGAGTCGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
SAT1           ATAGAAACATGAAACATCGGAGTCGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
KRB2           ATAGAAACATGAAACATCGGAGTCGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
SAT2           ATAGAAACATGAAACATCGGAGTCGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
KRB3           ATAGAAACATGAAACATCGGAGTCGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
SAT3           ATAGAAACATGAAACATCGGAGTCGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
KRB4           ATAGAAACATGAAACATCGGAGTCGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
SAT4           ATAGAAACATGAAACATCGGAGTCGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
KRB5           ATAGAAACATGAAACATCGGAGTCGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
SAT5           ATAGAAACATGAAACATCGGAGTCGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
RAY1           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
CTB1           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
SSK1           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
PKK1           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
CTB5           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
RAY5           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
SSK5           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
PKK5           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
SRT5           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
CTB2           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
RAY2           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
SSK2           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
PKK2           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
CTB4           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
RAY4           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
SRT2           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
SSK4           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
PKK4           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
SRT3           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
PKK3           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
SSK3           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
RAY3           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
CTB3           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
SRT1           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
SRT4           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
SOK1           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
SOK2           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
SOK3           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
SOK4           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
SOK5           ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
R.kanakurta    ATAGAAACATGAAACATCGGAGTTGTTCTTCTCCTCTTAGTAATGATAACCGCTTTCGTT
ClustalConsens *********************** ************************************
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AppendixA.2. (continued)

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                        250       260       270       280       290       300
                ....|....|....|....|....|....|....|....|....|....|....|....|
KRB1            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
SAT1            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
KRB2            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
SAT2            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
KRB3            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
SAT3            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
KRB4            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
SAT4            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
KRB5            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
SAT5            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGGGGTGCAACTGTCATTACTAATCTC
RAY1            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
CTB1            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
SSK1            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
PKK1            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
CTB5            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
RAY5            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
SSK5            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
PKK5            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
SRT5            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
CTB2            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
RAY2            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
SSK2            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
PKK2            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
CTB4            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
RAY4            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
SRT2            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
SSK4            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
PKK4            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
SRT3            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
PKK3            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
SSK3            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
RAY3            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
CTB3            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
SRT1            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
SRT4            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
SOK1            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
SOK2            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
SOK3            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
SOK4            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
SOK5            GGCTACGTCCTTCCCTGAGGACAAATGTCTTTCTGAGGTGCAACTGTCATTACTAATCTC
R.kanakurta     GGCTACGTCCTTCCCTGAGGACAAATGTCCTTCTGGGGTGCAACTGTCATTACTAACCTC
ClustalConsens  ***************************** ***** ******************** ***
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AppendixA.2. (continued)

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                           310       320       330       340       350       360
                  ....|....|....|....|....|....|....|....|....|....|....|....|
KRB1              CTTTCCGCAGTTCCTTATGTAGGCACTACCCTCGTAGAATGGATCTGGGGTGGCTTCTCC
SAT1              CTTTCCGCAGTTCCTTATGTAGGCACTACCCTCGTAGAATGGATCTGGGGTGGCTTCTCC
KRB2              CTTTCCGCAGTTCCTTATGTAGGCACTACCCTCGTAGAATGGATCTGGGGTGGCTTCTCC
SAT2              CTTTCCGCAGTTCCTTATGTAGGCACTACCCTCGTAGAATGGATCTGGGGTGGCTTCTCC
KRB3              CTTTCCGCAGTTCCTTATGTAGGCACTACCCTCGTAGAATGGATCTGGGGTGGCTTCTCC
SAT3              CTTTCCGCAGTTCCTTATGTAGGCACTACCCTCGTAGAATGGATCTGGGGTGGCTTCTCC
KRB4              CTTTCCGCAGTTCCTTATGTAGGCACTACCCTCGTAGAATGGATCTGGGGTGGCTTCTCC
SAT4              CTTTCCGCAGTTCCTTATGTAGGCACTACCCTCGTAGAATGGATCTGGGGTGGCTTCTCC
KRB5              CTTTCCGCAGTTCCTTATGTAGGCACTACCCTCGTAGAATGGATCTGGGGTGGCTTCTCC
SAT5              CTTTCCGCAGTTCCTTATGTAGGCACTACCCTCGTAGAATGGATCTGAGGTGGCTTCTCC
RAY1              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
CTB1              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
SSK1              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
PKK1              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
CTB5              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
RAY5              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
SSK5              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
PKK5              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
SRT5              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
CTB2              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
RAY2              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
SSK2              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
PKK2              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
CTB4              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
RAY4              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
SRT2              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
SSK4              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
PKK4              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
SRT3              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
PKK3              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
SSK3              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
RAY3              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
CTB3              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
SRT1              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
SRT4              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
SOK1              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
SOK2              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
SOK3              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
SOK4              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
SOK5              CTTTCCGCAGTTCCTTACGTAGGCACTACCCTCGTAGAATGAATCTGGGGTGGCTTCTCC
R.kanakurta       CTTTCCGCAGTCCCTTATGTAGGCACTACCCTAGTAGAATGAATCTGAGGTGGCTTCTCC
ClustalConsens    *********** ***** ************** ******** ***** ************
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AppendixA.2. (continued)

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                         370       380       390       400       410       420
                ....|....|....|....|....|....|....|....|....|....|....|....|
KRB1            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATC
SAT1            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATC
KRB2            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATC
SAT2            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATC
KRB3            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATC
SAT3            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATC
KRB4            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATC
SAT4            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATC
KRB5            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATC
SAT5            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATC
RAY1            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
CTB1            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
SSK1            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
PKK1            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
CTB5            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
RAY5            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
SSK5            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
PKK5            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
SRT5            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
CTB2            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
RAY2            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
SSK2            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
PKK2            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
CTB4            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
RAY4            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
SRT2            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
SSK4            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
PKK4            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
SRT3            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
PKK3            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
SSK3            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
RAY3            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
CTB3            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
SRT1            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
SRT4            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
SOK1            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
SOK2            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
SOK3            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
SOK4            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
SOK5            GTCGACAATGCAACCCTCACTCGATTCTTCGCATTCCATTTCCTCTTCCCATTCGTCATT
R.kanakurta     GTCGACAATGCAACCCTCACTCGGTTCTTCGCATTCCATTTCCTTTTCCCATTCGTCATC
ClustalConsens  *********************** ******************** ************** 
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AppendixA.2. (continued)

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                      430       440       450       460       470       480
              ....|....|....|....|....|....|....|....|....|....|....|....|
KRB1          GCAGCAATAACAATCCTGCACCTTCTCTTTCTTCATGAAACTGGATCAAACAACCCAATG
SAT1          GCAGCAATAACAATCCTGCACCTTCTCTTTCTTCATGAAACTGGATCAAACAACCCAATG
KRB2          GCAGCAATAACAATCCTGCACCTTCTCTTTCTTCATGAAACTGGATCAAACAACCCAATG
SAT2          GCAGCAATAACAATCCTGCACCTTCTCTTTCTTCATGAAACTGGATCAAACAACCCAATG
KRB3          GCAGCAATAACAATCCTGCACCTTCTCTTTCTTCATGAAACTGGATCAAACAACCCAATG
SAT3          GCAGCAATAACAATCCTGCACCTTCTCTTTCTTCATGAAACTGGATCAAACAACCCAATG
KRB4          GCAGCAATAACAATCCTGCACCTTCTCTTTCTTCATGAAACTGGATCAAACAACCCAATG
SAT4          GCAGCAATAACAATCCTGCACCTTCTCTTTCTTCATGAAACTGGATCAAACAACCCAATG
KRB5          GCAGCAATAACAATCCTGCACCTTCTCTTTCTTCATGAAACTGGATCAAACAACCCAATG
SAT5          GCAGCAATAACAATCCTGCACCTTCTCTTTCTTCATGAAACTGGATCAAACAACCCAATG
RAY1          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
CTB1          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
SSK1          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
PKK1          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
CTB5          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
RAY5          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
SSK5          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
PKK5          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
SRT5          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
CTB2          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
RAY2          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
SSK2          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
PKK2          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
CTB4          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
RAY4          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
SRT2          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
SSK4          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
PKK4          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
SRT3          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
PKK3          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
SSK3          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
RAY3          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
CTB3          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
SRT1          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
SRT4          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
SOK1          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
SOK2          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
SOK3          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
SOK4          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
SOK5          GCAGCAATAACAATCCTGCACCTTCTCTTCCTTCATGAAACTGGGTCAAACAACCCAATG
R.kanakurta   GCAGCAATAACAATCCTGCACCTTCTCTTCCTACATGAAACTGGATCAAACAACCCAATG
ClustalConsens *****************************  * *********** ***************
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AppendixA.2. (continued)

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                      490       500       510       520       530       540
              ....|....|....|....|....|....|....|....|....|....|....|....|
KRB1          GGCCTAAACTCAAATGCAGATAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
SAT1          GGCCTAAACTCAAATGCAGATAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
KRB2          GGCCTAAACTCAAATGCAGATAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
SAT2          GGCCTAAACTCAAATGCAGATAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
KRB3          GGCCTAAACTCAAATGCAGATAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
SAT3          GGCCTAAACTCAAATGCAGATAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
KRB4          GGCCTAAACTCAAATGCAGATAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
SAT4          GGCCTAAACTCAAATGCAGATAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
KRB5          GGCCTAAACTCAAATGCAGATAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
SAT5          GGCCTAAACTCAAATGCAGATAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
RAY1          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
CTB1          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
SSK1          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
PKK1          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
CTB5          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
RAY5          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
SSK5          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
PKK5          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
SRT5          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
CTB2          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
RAY2          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
SSK2          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
PKK2          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
CTB4          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
RAY4          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
SRT2          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
SSK4          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
PKK4          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
SRT3          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
PKK3          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
SSK3          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
RAY3          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
CTB3          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
SRT1          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACTTACAAAGATGCC
SRT4          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
SOK1          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
SOK2          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
SOK3          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
SOK4          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
SOK5          GGCCTAAACTCAAATGCAGACAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
R.kanakurta   GGCCTAAACTCAAATGCAGATAAAATCTCCTTCCACCCCTACTTCACCTACAAAGATGCC
ClustalConsens *******************  *************************** ************
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AppendixA.2. (continued)

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                      550       560       570       580       590       600
              ....|....|....|....|....|....|....|....|....|....|....|....|
KRB1          CTAGGCTTTGCTATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
SAT1          CTAGGCTTTGCTATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
KRB2          CTAGGCTTTGCTATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
SAT2          CTAGGCTTTGCTATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
KRB3          CTAGGCTTTGCTATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
SAT3          CTAGGCTTTGCTATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
KRB4          CTAGGCTTTGCTATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
SAT4          CTAGGCTTTGCTATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
KRB5          CTAGGCTTTGCTATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
SAT5          CTAGGCTTTGCTATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
RAY1          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
CTB1          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
SSK1          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
PKK1          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
CTB5          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
RAY5          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
SSK5          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
PKK5          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
SRT5          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
CTB2          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
RAY2          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
SSK2          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
PKK2          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
CTB4          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
RAY4          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
SRT2          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
SSK4          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
PKK4          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
SRT3          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
PKK3          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
SSK3          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
RAY3          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
CTB3          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
SRT1          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
SRT4          CTAGGCTTTGCCATCCTTCTTATGGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
SOK1          CTAGGCTTTGCCATCCTTCTTATAGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
SOK2          CTAGGCTTTGCCATCCTTCTTATAGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
SOK3          CTAGGCTTTGCCATCCTTCTTATAGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
SOK4          CTAGGCTTTGCCATCCTTCTTATAGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
SOK5          CTAGGCTTTGCCATCCTTCTTATAGCCCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
R.kanakurta   CTAGGATTTGCCATCCTTCTTATAGCTCTCACATCCCTAGCACTCTTCTCCCCCAACCTC
ClustalConsens***** ***** *********** ** *********************************
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AppendixA.2. (continued)

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                    610       620
               ....|....|....|....|....|..
KRB1           CTCGGCGACCCAGACAACTTCACGCCT
SAT1           CTCGGCGACCCAGACAACTTCACGCCT
KRB2           CTCGGCGACCCAGACAACTTCACGCCT
SAT2           CTCGGCGACCCAGACAACTTCACGCCT
KRB3           CTCGGCGACCCAGACAACTTCACGCCT
SAT3           CTCGGCGACCCAGACAACTTCACGCCT
KRB4           CTCGGCGACCCAGACAACTTCACGCCT
SAT4           CTCGGCGACCCAGACAACTTCACGCCT
KRB5           CTCGGCGACCCAGACAACTTCACGCCT
SAT5           CTCGGCGACCCAGACAACTTCACGCCT
RAY1           CTCGGCGACCCAGACAACTTCACGCCC
CTB1           CTCGGCGACCCAGACAACTTCACGCCC
SSK1           CTCGGCGACCCAGACAACTTCACGCCC
PKK1           CTCGGCGACCCAGACAACTTCACGCCC
CTB5           CTCGGCGACCCAGACAACTTCACGCCC
RAY5           CTCGGCGACCCAGACAACTTCACGCCC
SSK5           CTCGGCGACCCAGACAACTTCACGCCC
PKK5           CTCGGCGACCCAGACAACTTCACGCCC
SRT5           CTCGGCGACCCAGACAACTTCACGCCC
CTB2           CTCGGCGACCCAGACAACTTCACGCCC
RAY2           CTCGGCGACCCAGACAACTTCACGCCC
SSK2           CTCGGCGACCCAGACAACTTCACGCCC
PKK2           CTCGGCGACCCAGACAACTTCACGCCC
CTB4           CTCGGCGACCCAGACAACTTCACGCCC
RAY4           CTCGGCGACCCAGACAACTTCACGCCC
SRT2           CTCGGCGACCCAGACAACTTCACGCCC
SSK4           CTCGGCGACCCAGACAACTTCACGCCC
PKK4           CTCGGCGACCCAGACAACTTCACGCCC
SRT3           CTCGGCGACCCAGACAACTTCACGCCC
PKK3           CTCGGCGACCCAGACAACTTCACGCCC
SSK3           CTCGGCGACCCAGACAACTTCACGCCC
RAY3           CTCGGCGACCCAGACAACTTCACGCCC
CTB3           CTCGGCGACCCAGACAACTTCACGCCC
SRT1           CTCGGCGACCCAGACAACTTCACGCCC
SRT4           CTCGGCGACCCAGACAACTTCACGCCC
SOK1           CTCGGCGACCCAGACAACTTCACGCCC
SOK2           CTCGGCGACCCAGACAACTTCACGCCC
SOK3           CTCGGCGACCCAGACAACTTCACGCCC
SOK4           CTCGGCGACCCAGACAACTTCACGCCC
SOK5           CTCGGCGACCCAGACAACTTCACGCCC
R.kanakurta    CTTGGCGACCCAGACAACTTCACGCCT
ClustalConsens ** *********************** 
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Appendix B

AppendixB.1. The HKY85 distance matrix of partial mtDNA control region sequences from 40 *R. brachysoma* samples and *R. kanakurta* as outgroup.

	PKK1	PKK2	SOK4	CTB4	PKK4	CTB1	KRB2	SSK2	SRT3	SOK5	RAY5	CTB5	SRT4	SAT2	SOK2	SAT1	SRT1	RAY2	KRB3	SSK4
PKK1																				
PKK2	0.0000																			
SOK4	0.0000	0.0000																		
CTB4	0.0000	0.0000	0.0000																	
PKK4	0.0000	0.0000	0.0000	0.0000																
CTB1	0.0000	0.0000	0.0000	0.0000	0.0000															
KRB2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000														
SSK2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000													
SRT3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000												
SOK5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000											
RAY5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000										
CTB5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000									
SRT4	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018								
SAT2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018							
SOK2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000						
SAT1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000					
SRT1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000				
RAY2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000			
KRB3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000		
SSK4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	
RAY4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
KRB5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SAT5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SRT5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
PKK5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SSK5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SSK3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SAT4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
KRB4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
CTB3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
RAY3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SOK3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SAT3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
CTB2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SSK1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SRT2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SOK1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
KRB1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
PKK3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
RAY1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
R. kanakurta	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400	0.0419	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400

AppendixB.1. (continued)

	RAY4	KRB5	SAT5	SRT5	PKK5	SSK5	SSK3	SAT4	KRB4	CTB3	RAY3	SOK3	SAT3	CTB2	SSK1	SRT2	SOK1	KRB1	PKK3	RAY1
PKK1																				
PKK2																				
SOK4																				
CTB4																				
PKK4																				
CTB1																				
KRB2																				
SSK2																				
SRT3																				
SOK5																				
RAY5																				
CTB5																				
SRT4																				
SAT2																				
SOK2																				
SAT1																				
SRT1																				
RAY2																				
KRB3																				
SSK4																				
RAY4																				
KRB5	0.0000																			
SAT5	0.0000	0.0000																		
SRT5	0.0000	0.0000	0.0000																	
PKK5	0.0000	0.0000	0.0000	0.0000																
SSK5	0.0000	0.0000	0.0000	0.0000	0.0000															
SSK3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000														
SAT4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000													
KRB4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000												
CTB3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000											
RAY3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000										
SOK3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000									
SAT3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000								
CTB2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000							
SSK1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000						
SRT2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000					
SOK1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000				
KRB1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000			
PKK3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		
RAY1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	
R. kanakurta	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400	0.0400

AppendixB.2. The HKY85 distance matrix of cytochrome *b* gene from 40 *R. brachysoma* samples and *R. kanakurta* as outgroup.

	KRB1	SAT1	KRB2	SAT2	KRB3	SAT3	KRB4	SAT4	KRB5	SAT5	RAY1	CTB1	SSK1	PKK1	RAY2	CTB2	SSK2	PKK2	SRT2	CTB4
KRB1																				
SAT1	0.0000																			
KRB2	0.0000	0.0000																		
SAT2	0.0000	0.0000	0.0000																	
KRB3	0.0000	0.0000	0.0000	0.0000																
SAT3	0.0000	0.0000	0.0000	0.0000	0.0000															
KRB4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000														
SAT4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000													
KRB5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000												
SAT5	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032											
RAY1	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229										
CTB1	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229	0.0000									
SSK1	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229	0.0000	0.0000								
PKK1	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229	0.0000	0.0000	0.0000							
CTB2	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229	0.0000	0.0000	0.0000	0.0000						
RAY2	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229	0.0000	0.0000	0.0000	0.0000	0.0000					
SSK2	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000				
PKK2	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000			
SRT2	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		
CTB4	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	
RAY4	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SSK4	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
PKK4	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
CTB3	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
RAY3	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SSK3	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
PKK3	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SRT3	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SRT5	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
PKK5	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SSK5	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
RAY5	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
CTB5	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0195	0.0229	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SRT1	0.0229	0.0229	0.0229	0.0229	0.0229	0.0229	0.0229	0.0229	0.0229	0.0262	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032
SRT4	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0246	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016
SOK2	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0246	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016
SOK3	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0246	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016
SOK1	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0246	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016
SOK4	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0246	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016
SOK5	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0246	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016
R.Kanakurta	0.0346	0.0346	0.0346	0.0346	0.0346	0.0346	0.0346	0.0346	0.0346	0.0311	0.0414	0.0414	0.0414	0.0414	0.0414	0.0414	0.0414	0.0414	0.0414	0.0414

AppendixB.2. (continued)

	RAY4	SSK4	PKK4	CTB3	RAY3	SSK3	PKK3	SRT3	SRT5	PKK5	SSK5	RAY5	CTB5	SRT1	SRT4	SOK2	SOK3	SOK1	SOK4	SOK5
KRB1																				
SAT1																				
KRB2																				
SAT2																				
KRB3																				
SAT3																				
KRB4																				
SAT4																				
KRB5																				
SAT5																				
RAY1																				
CTB1																				
SSK1																				
PKK1																				
CTB2																				
RAY2																				
SSK2																				
PKK2																				
SRT2																				
CTB4																				
RAY4																				
SSK4	0.0000																			
PKK4	0.0000	0.0000																		
CTB3	0.0000	0.0000	0.0000																	
RAY3	0.0000	0.0000	0.0000	0.0000																
SSK3	0.0000	0.0000	0.0000	0.0000	0.0000															
PKK3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000														
SRT3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000													
SRT5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000												
PKK5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000											
SSK5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000										
RAY5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000									
CTB5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000								
SRT1	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032							
SRT4	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016						
SOK2	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0048	0.0032					
SOK3	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0048	0.0032	0.0000				
SOK1	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0048	0.0032	0.0000	0.0000			
SOK4	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0048	0.0032	0.0000	0.0000	0.0000		
SOK5	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0048	0.0032	0.0000	0.0000	0.0000	0.0000	
R.Kanakurta	0.0414	0.0414	0.0414	0.0414	0.0414	0.0414	0.0414	0.0414	0.0414	0.0414	0.0414	0.0414	0.0414	0.0449	0.0432	0.0397	0.0397	0.0397	0.0397	0.0397

AppendixB.3. The TIM distance matrix of the combined sequences from 40 *R. brachysoma* samples and *R. kanakurta* as outgroup.

	KPB1	SAT1	SAT2	KPB3	SAT3	KPB4	KPB5	SAT4	SAT5	KPB2	SRT1	SRT4	RAY4	SSK4	SSK5	PKK5	SRT5	CTB3	RAY3	SSK3
KPB1																				
SAT1	0.0000																			
SAT2	0.0000	0.0000																		
KPB3	0.0000	0.0000	0.0000																	
SAT3	0.0000	0.0000	0.0000	0.0000																
KPB4	0.0000	0.0000	0.0000	0.0000	0.0000															
KPB5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000														
SAT4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000													
SAT5	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017												
KPB2	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0026											
SRT1	0.0121	0.0121	0.0121	0.0121	0.0121	0.0121	0.0121	0.0121	0.0138	0.0129										
SRT4	0.0121	0.0121	0.0121	0.0121	0.0121	0.0121	0.0121	0.0121	0.0138	0.0129	0.0017									
RAY4	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0121	0.0112	0.0017	0.0017								
SSK4	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0121	0.0112	0.0017	0.0017	0.0000							
SSK5	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0121	0.0112	0.0017	0.0017	0.0000	0.0000						
PKK5	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0121	0.0112	0.0017	0.0017	0.0000	0.0000	0.0000					
SRT5	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0121	0.0112	0.0017	0.0017	0.0000	0.0000	0.0000	0.0000				
CTB3	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0121	0.0112	0.0017	0.0017	0.0000	0.0000	0.0000	0.0000	0.0000			
RAY3	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0121	0.0112	0.0017	0.0017	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		
SSK3	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0121	0.0112	0.0017	0.0017	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	
SSK1	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0121	0.0112	0.0017	0.0017	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
CTB2	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0121	0.0112	0.0017	0.0017	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
RAY2	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0121	0.0112	0.0017	0.0017	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SRT2	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0103	0.0121	0.0112	0.0017	0.0017	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
PKK1	0.0121	0.0121	0.0121	0.0121	0.0121	0.0121	0.0121	0.0121	0.0138	0.0129	0.0034	0.0034	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017
PKK2	0.0121	0.0121	0.0121	0.0121	0.0121	0.0121	0.0121	0.0121	0.0138	0.0129	0.0034	0.0034	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017
PKK3	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0129	0.0121	0.0026	0.0026	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009
RAY1	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0129	0.0121	0.0026	0.0026	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009
SOK4	0.0121	0.0121	0.0121	0.0121	0.0121	0.0121	0.0121	0.0121	0.0138	0.0129	0.0034	0.0034	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017
CTB4	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0129	0.0121	0.0026	0.0026	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009
PKK4	0.0121	0.0121	0.0121	0.0121	0.0121	0.0121	0.0121	0.0121	0.0138	0.0112	0.0034	0.0034	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017
CTB1	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0129	0.0121	0.0026	0.0026	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009
SSK2	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0129	0.0103	0.0026	0.0026	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009
SRT3	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0129	0.0103	0.0026	0.0026	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009
RAY5	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0129	0.0103	0.0026	0.0026	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009
CTB5	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0129	0.0103	0.0026	0.0026	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009
SOK1	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0129	0.0121	0.0026	0.0026	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009
SOK2	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0129	0.0121	0.0026	0.0026	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009
SOK3	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0112	0.0129	0.0121	0.0026	0.0026	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009
SOK5	0.0121	0.0121	0.0121	0.0121	0.0121	0.0121	0.0121	0.0121	0.0138	0.0112	0.0034	0.0034	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017
R.kanakurta	0.0397	0.0397	0.0397	0.0397	0.0397	0.0397	0.0397	0.0397	0.0379	0.0406	0.0452	0.0452	0.0434	0.0434	0.0434	0.0434	0.0434	0.0434	0.0434	0.0434

AppendixB.3. (continued)

	SSK1	CTB2	RAY2	SRT2	PKK1	PKK2	PKK3	RAY1	SOK4	CTB4	PKK4	CTB1	SSK2	SRT3	RAY5	CTB5	SOK1	SOK2	SOK3	SOK5
KRB1																				
SAT1																				
SAT2																				
KRB3																				
SAT3																				
KRB4																				
KRB5																				
SAT4																				
SAT5																				
KRB2																				
SRT1																				
SRT4																				
RAY4																				
SSK4																				
SSK5																				
PKK5																				
SRT5																				
CTB3																				
RAY3																				
SSK3																				
SSK1																				
CTB2	0000																			
RAY2	0000	0.0000																		
SRT2	0000	0.0000	0.0000																	
PKK1	0017	0.0017	0.0017	0.0017																
PKK2	0017	0.0017	0.0017	0.0017	0.0000															
PKK3	0009	0.0009	0.0009	0.0009	0.0026	0.0026														
RAY1	0009	0.0009	0.0009	0.0009	0.0026	0.0026	0.0017													
SOK4	0017	0.0017	0.0017	0.0017	0.0034	0.0034	0.0026	0.0026												
CTB4	0009	0.0009	0.0009	0.0009	0.0026	0.0026	0.0017	0.0017	0.0009											
PKK4	0017	0.0017	0.0017	0.0017	0.0034	0.0034	0.0026	0.0026	0.0017	0.0009										
CTB1	0009	0.0009	0.0009	0.0009	0.0026	0.0026	0.0017	0.0017	0.0017	0.0009	0.0017									
SSK2	0009	0.0009	0.0009	0.0009	0.0026	0.0026	0.0017	0.0017	0.0026	0.0017	0.0009	0.0017								
SRT3	0009	0.0009	0.0009	0.0009	0.0026	0.0026	0.0017	0.0017	0.0026	0.0017	0.0009	0.0017	0.0000							
RAY5	0009	0.0009	0.0009	0.0009	0.0026	0.0026	0.0017	0.0017	0.0026	0.0017	0.0009	0.0017	0.0000	0.0000						
CTB5	0009	0.0009	0.0009	0.0009	0.0026	0.0026	0.0017	0.0017	0.0026	0.0017	0.0009	0.0017	0.0000	0.0000	0.0000					
SOK1	0009	0.0009	0.0009	0.0009	0.0026	0.0026	0.0017	0.0017	0.0009	0.0017	0.0026	0.0017	0.0017	0.0017	0.0017	0.0017				
SOK2	0009	0.0009	0.0009	0.0009	0.0026	0.0026	0.0017	0.0017	0.0009	0.0017	0.0026	0.0017	0.0017	0.0017	0.0017	0.0017	0.0000			
SOK3	0009	0.0009	0.0009	0.0009	0.0026	0.0026	0.0017	0.0017	0.0009	0.0017	0.0026	0.0017	0.0017	0.0017	0.0017	0.0017	0.0000	0.0000		
SOK5	0017	0.0017	0.0017	0.0017	0.0034	0.0034	0.0026	0.0026	0.0017	0.0026	0.0017	0.0026	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	
R.kanakurta	0434	0.0434	0.0434	0.0434	0.0434	0.0434	0.0424	0.0425	0.0434	0.0443	0.0452	0.0443	0.0443	0.0443	0.0443	0.0443	0.0425	0.0425	0.0425	0.0434

BIOGRAPHY

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