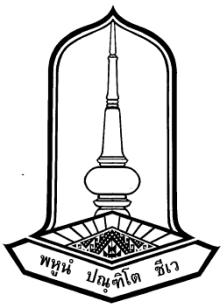

SPECIES DIVERSITY, HOST PLANTS AND DNA BARCODING OFTEPHRITID FRUIT FLIES(DIPTERA: TEPHRITIDAE) IN NORTHEASTERN THAILAND

KOWIT MEEYEN

A thesis submitted in partial fulfillment of the requirements for
the degree of Master of Science in Biology
at Mahasarakham University
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The examining committee has unanimously approved this thesis, submitted by Mr. Kowit Meeyen, as a partial fulfillment of the requirements for the Master of Science degree in Biology at Mahasarakham University.

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ACKNOWLEDGEMENTS

This thesis was financially supported by Mahasarakham University (Grant year 2012).

The thesis would not have been accomplished if without the help from several people. I deeply appreciate Associate Professor Dr. Pairot Pramual, my major advisor, Assistant Professor Dr. Piyamas Nanork Sopaladawan my co-advisor for their guidance, encouraging, invaluable comments and suggestions throughout this study. I would like to thank Assistant Professor Dr. Bangon Kongim, Dr. Pornchai Uttharuk, Department of Biology, Faculty of Science, Mahasarakham University and Assistant Professor Dr. Ubon Tangkawanit, Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University for their comments and suggestions.

I would like to thank Miss Sutthira Khumkratok, Walai Rukhavaj Botanical Research Institute, Mahasarakham University and staffs of the Department of Biology, Faculty of Science, Mahasarakham University for their support in many ways on my study.

I would like to thank all my friends for provided samples of fruit flies, especially Miss Paradee Sriphirom and Miss Kusumart Simwisat, who participated in my study for their friendship and encouragement.

I would like to thank Mr. Sorasak Saiyahuta, Director of Nong Prue Phitthayakhom School, and teachers of Nong Prue Phitthayakhom School for chance of my study.

Finally, I wish express very special thanks to my family for their understanding, encouraging and support in every way on my study.

Kowit Meeyen

TITLE Species diversity, host plants and DNA barcoding of tephritid fruit flies (Diptera: Tephritidae) in northeastern Thailand
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UNIVERSITY Mahasarakham University **YEAR** 2013

ABSTRACT

Fruit flies are important pest of many economically significant agricultural crops. However, little attention has been paid on biodiversity of these insects in northeastern Thailand. The objectives of this study are to examine species diversity, host plants and development of DNA barcoding marker of fruit flies in northeastern Thailand. Nine fruit fly species including five members of *Bactrocera dorsalis* complex (*B. caryeae*, *B. occipitalis*, *B. philippinensis*, *B. dorsalis*, *B. invadens*), *B. correcta*, *B. cucurbitae*, *B. latifrons*, and *B. tau* complex were found. These fruit flies infested 19 host plant species in 10 families. *Bactrocera cucurbitae* and *B. tau* complex was significantly associated with plants family Cucurbitaceae and *B. latifrons* was significantly associated with plants family Solanaceae. Other species used diverse host plants. A total of 140 mitochondrial cytochrome oxidase I barcoding sequences were obtained from nine fruit fly species. Seventy seven mt DNA haplotypes were identified. Haplotype diversity range from 0.834 in *B. latifrons* to 1.000 in *B. cucurbitae*, *B. dorsalis*, *B. invadens* and *B. philippinensis*. Nucleotide diversity in each species ranged from 0.004 in *B. cucurbitae* to 0.018 in *B. invadens*. The results indicated high genetic diversity of tephritid fruit flies in northeastern Thailand. DNA barcode revealed 100% correct identification of *B. correcta*, *B. cucurbitae*, *B. latifrons* and *B. tau*. However, DNA barcode could not differentiate five members of *B. dorsalis* complex. Despite this, DNA barcode is useful to differentiate *B. dorsalis* complex from other species. Therefore, DNA barcode is useful for species identification of fruit fly. Genetic variation and genetic structure were investigated in more details for *B. latifrons*. Higher level of genetic variation and genetic structure of Thai populations compare to other

geographic regions were revealed consistent with the hypothesis that *B. latifrons* was a native species of tropical Asian area. Both an ongoing process (i.e. ongoing gene flow) and historical factor (i.e. Pleistocene climatic change) played roles in determining the genetic structure and diversity of this species. Information gathering from this study will provide significant data for ongoing effort to management and control these economically important insects. It is also strengthen our knowledge of biodiversity in Thailand.

Keywords Tephritid fruit flies**,** *Bactrocera*, host plants, DNA barcode

ชื่อเรื่อง ความหลากชนิด พืชอาศัย และดีเอ็นเอบาร์โค้ด ของแมลงวันผลไม้ (Diptera: Tephritidae) ในภาคตะวันออกเฉียงเหนือของประเทศไทย
ผู้วิจัย นายโกวิทย์ มีเย็น
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บทคัดย่อ

แมลงวันผลไม้เป็นแมลงศัตรูพืชที่สร้างความเสียหายต่อผลผลิตทางการเกษตร อย่างไรก็ตามข้อมูลเกี่ยวกับความหลากชนิดของแมลงวันผลไม้ในภาคตะวันออกเฉียงเหนือของประเทศไทยยังน้อยมากการศึกษานี้มีวัตถุประสงค์เพื่อศึกษาความหลากชนิด พืชอาศัยรวมถึงเพื่อพัฒนาเครื่องหมายทางพันธุกรรมดีเอ็นเอบาร์โค้ดในการระบุชนิดของแมลงวันผลไม้ในภาคตะวันออกเฉียงเหนือของประเทศไทย จากการศึกษาพบแมลงวันผลไม้จำนวน 9 ชนิด ได้แก่ แมลงวันผลไม้ในกลุ่มสปีชีส์ซับซ้อน *Bactrocer adorsalis* จำนวน 5 ชนิด (*B.caryeae*, *B. occipitalis*, *B. philippinensis*, *B. dorsalis*, *B. invadens*), *B. correcta*, *B. cucurbitae*, *B. latifrons*, และกลุ่มสปีชีส์ซับซ้อน *B. tau* ในพืชอาศัย 19 ชนิด จาก 10 วงศ์ จากการวิเคราะห์ความสัมพันธ์ระหว่างชนิดของแมลงวันผลไม้กับพืชอาศัยพบว่า *B. cucurbitae* และ *B. tau* complex มีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติกับพืชวงศ์ Cucurbitaceae ส่วน *B. latifrons* มีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติกับพืชวงศ์ Solanaceae แมลงวันผลไม้อีก 6 ชนิดที่เหลือไม่พบความสัมพันธ์อย่างมีนัยสำคัญกับพืชอาศัย การวิเคราะห์ลำดับนิวคลีโอไทด์ในตำแหน่งยีน cytochrome oxidase I (COI) ของแมลงวันผลไม้ 140 ตัวอย่าง จากแมลงวันผลไม้ 9 ชนิดพบรูปแบบจำเพาะของลำดับนิวคลีโอไทด์ 77 รูปแบบ ค่าความหลากหลายของแฮพโพลไทป์อยู่ในช่วงระหว่าง 0.834 ใน *B. latifrons* ถึง 1.000 ใน *B. cucurbitae*, *B. dorsalis*, *B. invadens* และ *B. philippinensis* ค่าความหลากหลายของลำดับนิวคลีโอไทด์แต่ละชนิดมีค่าระหว่าง 0.004 ใน *B. cucurbitae* ถึง 0.018 ใน *B. invadens* ผลการศึกษาแสดงให้เห็นว่าแมลงวันผลไม้ในภาคตะวันออกเฉียงเหนือของประเทศไทยมีค่าความแปรผันทางพันธุกรรมสูงการพัฒนาเครื่องหมายพันธุกรรมดีเอ็นเอบาร์โค้ดแสดงให้เห็นว่าเครื่องหมายพันธุกรรมดีเอ็นเอบาร์โค้ดสามารถใช้ในการระบุชนิดของแมลงวันผลไม้ชนิด *B. correcta*, *B. cucurbitae*, *B. latifrons* และ *B. tau* ได้อย่างถูกต้อง 100% แต่ไม่สามารถใช้ระบุชนิดของแมลงวันผลไม้อีก 5 ชนิด ซึ่งอยู่ในกลุ่มสปีชีส์ซับซ้อน *B. dorsalis* ได้ อย่างไรก็ตามการศึกษานี้แสดงให้เห็นว่าเครื่องหมายทางพันธุกรรมดีเอ็นเอบาร์โค้ดมีประโยชน์อย่างยิ่งในการใช้ระบุชนิดของแมลงวันผลไม้ โดยเฉพาะใช้ช่วยในการระบุชนิดของแมลงวันผลไม้ในระยะตัวอ่อน ซึ่งการระบุชนิดโดยใช้ลักษณะทางสัณฐานวิทยาได้ยาก จากการศึกษาความแปรผันทางพันธุกรรมและโครงสร้างทางพันธุกรรมของแมลงวันผลไม้ชนิด *B. latifrons* โดยใช้ลำดับนิวคลีโอไทด์ของยีน COI แสดงให้เห็นว่าแมลงวันผลไม้ชนิด *B. latifrons* ในประเทศไทยมีความแปรผันทางพันธุกรรม และโครงสร้างทางพันธุกรรมที่สูงกว่าภูมิภาคอื่นของโลก ซึ่งสอดคล้องกับสมมติฐานที่ว่าแมลงวันผลไม้ชนิดนี้มีถิ่นกำเนิดอยู่ในเขตร้อนชื้นของทวีปเอเชีย โครงสร้างทางพันธุกรรม และความหลากหลายทาง

พันธุกรรมของแมลงวันผลไม้ชนิดนี้ได้รับอิทธิพลจากเหตุการณ์ปัจจุบันเช่น การเกิดยีนโฟลว รวมถึงประวัติศาสตร์ประชากร และเหตุการณ์ที่เกิดขึ้นในอดีต เช่น การเปลี่ยนแปลงสภาพภูมิอากาศและสภาพแวดล้อมของโลกในยุคไพลสโทซีนข้อมูลที่ได้รับจากการศึกษาครั้งนี้เป็นพื้นฐานสำคัญในการบริหารจัดการและควบคุมแมลงศัตรูพืช และสร้างความเข้าใจความหลากหลายทางชีวภาพของประเทศไทย

คำสำคัญ: แมลงวันผลไม้, *Bactrocera*, พืชอาศัย, เครื่องหมายพันธุกรรมดีเอ็นเอบาร์โค้ด

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List of Abbreviations

A	adenine
AMOVA	analysis of molecular variance
bp	base pairs
º C	degree Celsius
cm	centimeter
COI	cytochrome oxidase subunit I
C	cytosine
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphates
e.g.	example gratia
et al.	etalii
etc.	et cetera
F_{CT}	the correlation of random haplotypes within a group of populations relative to that of random pairs of haplotypes drawn from the whole species.
F_{ST}	the correlation of random haplotypes within populations relative to that of random pairs of haplotypes drawn from the whole species.
Fsc	the correlation of the molecular diversity of random haplotypes within populations relative to that of random pairs of haplotypes drawn from the region.
G	guanine
g	gram
i.e.	idest
ITS	internal transcribed spacer
K2P	Kimura's 2 parameter model
m	meter
min	minute
ml	milliliter
mm	millimeter
mt	mitochondria

PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
rpm	revolution per minute
sec	second
sp.	species
spp.	more than one species
T	Thymine
TBE	Tris-borate
µl	microliter
µM	micromolar
%	percent

Chapter 1

Introduction

1.1 Background

The tephritid fruit flies, which are often called the "true fruit fly," belong to the family Tephritidae. This family composes of more than 4,000 described species in almost 500 genera. Approximately 350 species of tephritid fruit flies in the five genera (*Anastrepha, Bactrocera, Ceratitis, Dacus,* and *Rhagoletis*) are the most economically important. These genera are serious pests of agricultural crops (White and Elson-Harris, 1992; Houdt et al., 2010; Plant Health Australia, 2011). Female fruit flies pierce and lay eggs in unripe or ripe fruits. After that, the larvae feed on the internal tissue for their development. This renders the fruit unsuitable for consumption leading to a loss of economic revenue. The genus *Bactrocera* is the most economically important pests. This genus is one of the largest genera within Tephritidae with about 500 described species arranged in 28 subgenera (Drew, 1989; Drew and Hancock, 2000). Several species (e.g., the Oriental fruit fly, *Bactrocera dorsalis*; the Queensland fruit fly, *B. tryoni*; melon fly, *B. cucurbitae*) capable of attacking a wide variety of commercially produced fruits (White and Elson-Harris, 1992; Allwood *et al*., 1999; Clarke *et al*., 2005). The economic values loss due to the fruit flies were very high. For examples, the horticultural industry was destroyed $4.8 billion in Australia, $300 million in Hawaii, $350 million in California (Armstrong and Jang, 1996; Jang, 2007; Plant Health Australia, 2011).

Basic knowledge on taxonomy, biology, ecology and genetic are crucial for insect pest control. Accurate species identification is critical for understanding all aspects of fruit fly biology and implementing effective control programs (Miller and Rossman, 1995). The traditional taxonomy of fruit flies based mainly on adult morphology. However, morphological identification is complicated by a high level of structural homogeneity and often found misidentification in species complex or closely related species (Drew and Hancock, 1994a; Kapoor, 2005).

Cytological technique was also applied for fruit fly taxonomy. These insects have the giant polytene chromosomes. However, polytene chromosomes of the tephritid fruit flies are inefficiency to use for taxonomy (Baimai, 2010). Variation in the

heterochromatin in mitotic chromosomes can be used to differerntiate members of some species complex (e.g. *Bactrocera tau* complex) (Baimai *et al*., 1999a, b; 2000a, b). However, this limited to some species and are requiring highly experienced staff. Furthermore, specimens used for mitotic chromosome are only workable in larval stage (Bush, 1962; Baimai *et al*., 1999a, b; 2000a, b).

Molecular techniques have been used effectively complementarity to morphological identification (Ball *et al*., 2005; Timm *et al*., 2007; 2008). Recently, DNA barcoding was proposed as one of the solution of the limitation of traditional taxonomy (Hebert *et al*., 2003a). Several reports revealed the successful of DNA barcode in taxonomic study in many organisms. For examples, springtails, mayflies, spiders, fish, mosquitos, birds, blackflies. (Hogg and Hebert, 2004; Ball *et al*., 2005; Barrett and Hebert, 2005; Ward *et al*., 2005; Cywinska *et al*., 2006; Kerr *et al*., 2007; Rivera and Currie, 2009; Pramual *et al*. 2011b).

DNA barcoding of the Tephritidae have been developed as an international project (i.e. Tephritidae Barcoding Initiative, TBI) in 2006. Several trials have started to develop DNA barcodes for three main pest genera occurring in Africa including *Bactrocera*, *Ceratitis* and *Dacus* (Houdt *et al*., 2010). Recent studies revealed that DNA barcode was successful to differentiate fruit flies species (Armstrong and Ball, 2005; Liu *et al*., 2011; Blacket *et al*., 2012). DNA barcoding could also reveal cryptic biodiversity of the fruit flies, especially the genus *Bactrocera* which has several species as complex species (Hancock *et al*., 2000; Armstorng and Ball, 2005; Clarke *et al*., 2005; Liu *et al*., 2011).

In Thailand and bordering countries (i.e. Cambodia, Laos, Vietnam, Malaysia, and Tenasserim, Lower Burma) 211 species of fruit flies have been reported. These species are arranged in 4 subfamilies, 13 tribes, 69 genera, and 7 subgenera (Hardy, 1973). Of these, 182 species are belong to genus *Bactrocera* (Drew and Hancock, 2001a). Several aspects of fruit flies in Thailand have been reported such as biological, behavioral and control method (e.g. Poramarcom *et al*., 1994; Poramarcom and Baimai, 1996; Kitthawee, 2000; Aemprapa, 2007; Jaturat, 2007; Orankanok *et al*., 2006; 2007; Rattanapun *et al*., 2009; Aketarawong *et al*., 2011). However, less attention have been paid to the population genetic and evolution (e.g. Bush, 1962; Hunwattanakul and Baimai, 1994; Baimai *et al*., 1995; 1996d; 1999a,b; 2000a,b;

Jamnongluk *et al*., 2003a,b; Aketarawong *et al*., 2006; Saelee *et al*., 2006). There is only one report on DNA barcoding of the fruit flies in Thailand (Nopparat *et al*., 2011).

The host plant relationship is another important aspect of the fruit fly biology. There are some reports on the associations between fruit fly species and host plants in Thailand (Clarke *et al*., 2001; Baimai *et al*., 2002). It has been hypothesize that different host usage could play a significant role in evolution of the fruit fly in Thailand (Jamnongluk *et al*., 2003a,b).

In this study, species diversity, genetic variation, host plant associations and DNA barcoding of the fruit fly genus *Bactrocera* Macquart (1835) in northeastern Thailand were determined. In addition, intraspecific phylogeography based on COI barcoding sequences were used to infer the population genetic structure of Solanum fruit fly *B. latifrons* (Hendel) in Thailand. This basic knowledge will be useful for effective management of the fruit fly. Information gathering from this study could also strengthening our knowledge of biodiversity in Thailand.

1.2 Objectives of the research

The objectives of the present study are:

1) to examine species diversity of the fruit fly in northeastern Thailand.

2) to examine the association between fruit fly and host plant species.

3) to assessment level of genetic variation of fruit flies in northeastern Thailand.

4) to develop DNA barcoding marker for fruit flies in northeastern Thailand.

5) to infer population genetic structure and population history of *Bactrocera latifrons*in Thailand using COI barcoding sequences.

1.3 Scope of the research

In this study both adults and larvae of tephritid fruit fly of the genus *Bactrocera* were collected from various host plants throughout northeastern Thailand. The larvae were reared into adult in the laboratory. Adults were identified based on morphology. DNA was extracted from adult flies. The cytochrome oxidase subunit I (COI) gene barcoding region was amplified using polymerase chain reaction (PCR). PCR products were checked, purified and sequencing. Intraspecific and interspecific genetic divergences were calculated based on COI sequences. The present or absent of the fruit fly species in each host plant was recorded for further analysis of the relationship between the fruit fly species and their hosts. In addition, COI sequences of *B. latifrons* throughout northern and northeastern Thailand were used to investigate genetic variation, population genetic structure and population history of this species.

Chapter 2

Literature Review

2.1 Classification of the genus *Bactrocera*

Phylum: Arthropoda
Class: Insecta
Order: Diptera
Suborder: Brachycera
Infraorder: Muscomorpha
Superfamily: Tephritoidae
Family: Tephritidae
Subfamily: Dacinae
Tribe: Dacini
(Macquart, 1835)

Common name: true fruit fly

True fruit flies (Tephritid fruit flies) are a group of insects forming the family Tephritidae of the order Diptera. The following higher classification revealed that there are 6 subfamilies including Tachiniscinae, Blepharoneurinae, Phytalmiinae, Dacinae, Trypetinae, and Tephritinae, which compose of 27 tribes, in the family Tephritidae (Korneyev, 1999; Norrbom *et al*., 1999). There are more than 4,000 described species arranged in 500 genera within Tephritidae. This making it one of the largest families within Diptera (White and Elson-Harris, 1992; Norrbom *et al*., 1998; Houdt *et al*., 2010; Plant Health Australia, 2011).

Dacini is one of three tribes in the subfamily Dacinae. This tribe contains approximately 770 described species in 4 genera (i.e. two small genera, *Ichneumonopsis* Hardy (one sp.) and *Monacrostichus* Bezzi (two spp.), and two very large genera, *Dacus* Fabricius (245 spp.) and *Bactrocera* Macquart (528 spp.) (Drew 1989; Drew and Hancock 2000). These genera were divided based on adult morphology (Table 2.1) (Drew and Hand cock, 2001a).The tribe Dacini is very important because it contains

many genera that are the serious pests of agricultural crops such as *Bactrocera,* and *Dacus* (White and Elson-Harris, 1992).

The genus *Bactrocera* Macquart (Diptera: Tephritidae: Dacini) is the largest and most important genus of the family Tephritidae. This genus contains 528 described species arranged in 28 subgenera. Members of the Tephritidae cause serious reductions in yields and quality of fruits and vegetables in many countries (Hardy, 1973; White and Elson-Harris, 1992; Allwood *et al*., 1999; Clarke *et al*., 2005; Plant Health Australia, 2011). Moreover, the subgenera of the genus *Bactrocera* were divided into four groups based on morphological characters (i.e. length of lateral surstylus and shape of abdominal tergum V of male) such as *Bactrocera*, *Melanodacus*, *Queenslandacus*, and *Zeugodacus* (Drew, 1989) (Table 2.2).The genus *Bactrocera* has the most geographically widespread. They can be found in the tropical and subtropical rain forests in West Africa, coastal East Africa, Madagascar and the Mascarene Islands, southwest India, Southeast Asia from Nepal to southern China in the north to the Indonesian islands in the south, Papua New Guinea, northeastern Australia, and some South Pacific islands (Drew and Hand cock, 2001a). In Southeast Asia and Australia the *Bactrocera* spp. are dominate group (Drew 1989; Drew and Hancock 2000; Drew, 2004). In Thailand and bordering countries 211 species of fruit flies have been reported. These species are arranged in 4 subfamilies, 13 tribes, 69 genera, and 7 subgenera (Hardy, 1973).

Table 2.1 Character states in the genera of Dacini.

Character	***Ichneumonopsis***	***Monacrostichus***	***Dacus***	***Bactrocera***
Abdomen shape	Elongate oval	Elongate oval	Elongate oval or oval	Generally oval
Abdomen shape	Terga not fused	Terga not fused	Elongate-oval or oval	Terga not fused
Abdomen fusion	Absent	Absent	Terga fused **	Usually present *
Petcen on male tergum III	Absent	Absent	Present * or absent	Usually present *
Ceromata on tergum V	Short but distinct	Short but distinct	Present *	Vestigial, disassociated *
Female tergite VI	Very long	Short	Vestigial, disassociated *	Short or long
Oviscape	Rounded	Convoluted *^	Short or long	Convoluted *^
Spermathecae	Long	Short	Convoluted *^	Long or short
Posterior lobe of lateral surstylus	With moderate concavity	With moderate concavity	Long or short	With shallow to moderate or deep concavity
Posterior margin of male sternite V	Short	Elongate and broad **	With shallow to moderate concavity	Short
Wing cell sc	With a transverse sclerotized line **	Without sclerotized line	Short	Without sclerotized line
Wing cell r_1	Narrow and not broadened	Narrow, basally broadened *	Without sclerotized line	Broad *
Wing cell bm	Broad	Basally narrow ***	Broad *	Broad
Wing cell dm	Setose	Bare *^	Broad	Bare *^
Vein R4+5	Ends at wing margin	Ends well before wing margin **	Bare *^	Ends at wing margin
Vein A1+Cu2	Present	Present	Ends at wing margin	Absent
Ventroapical spines on forefemur	Short and broad	Short and broad	Present or absent	Subtriangular
Scutellum shape	Absent	Absent	Usually short and broad	Usually present ***
Prescutellar acrostichal bristles	Vestigial **	Present	Absent	Present
Inner postalar bristle	Present	Present	Present	Present or absent
Supra-alar bristle	One pair (apical)	One pair (apical)	Present or absent	One or two pairs

Table 2.1 (Continued).

Character	***Ichneumonopsis***	***Monacrostichus***	***Dacus***	***Bactrocera***
Scutellar bristles	Absent	Present **	One pair (apical)	Absent
Facial and scutal furrows	Much reduced *^	Much reduced *^	Absent	Much reduced *^
Chaetotaxy of head and thorax	Absent or with 1 vestigial pair *	Absent *	Much reduced *^	Usually 2 pairs present
Frontal bristles	Plumose	Bare *^	Usually 2 pairs present	Bare *^
Arista	SE Asia	SE Asia	Bare *^	Primarily SE Asia–Pacific
Distribution	Unknown	Rutaceae (*Citrus* spp.)	Primarily Africa and SE Asia	Primarily tropical and subtropical rain forest fruits
Host plants			Primarily Asclepiadaceae, Passifloraceae, and Cucurbitaceae	

* = Shared apomorphy (^ = character state occurs outside tribe but presumed convergent); ** = apomorphy unique to genus;
*** = possible apomorphy for genus within Dacini (character state also occurs outside tribe).

(From: Drew and Hand cock, 2001a)

Table 2.2 Four groups and their subgenera of *Bactrocera*.

***Bactrocera* group**	***Zeugodacus* group**
'Afrodacus' *Apodacus* *Bactrocera* *Bulladacus* *'Gymnodacus'* *Notodacus* *Semicallantra* *Tetradacus* *Trypetidacus*	*Asiadacus* *Austrodacus* *Diplodacus* *Hemigymnodacus* *Heminotodacus* *Hemiparatridacus* *Javadacus* *Nesodacus* *Niuginidacus* *Papuodacus* *Paradacus* *Paratridacus* *Parazeugodacus* *Sinodacus* *Zeugodacus*
***Melanodacus* group** *Hemisurstylus* *Hemizeugodacus* *Melanodacus*	
***Queenslandacus* group** *Queenslandacus*	

(Modified from: Drew, 1989)

2.2 Biology of tephritid fruit fly

The life cycle of the tephritid fruit fly (Fig. 2.1) composed of four basic stages, egg, larva, pupa and adult. These stages may be divided into three parts, host fruits, soil and aerial. The female fruit flies lay eggs into host fruits, and these eggs hatch to larvae. The larvae that hatch initially are small and delicate first instar larvae. They molt into slightly more robust second instar larvae, and these in turn molt into quite stout and tough third instar larvae. Every stages of larva feed on the internal tissue of the fruits for their development. When the third instars have finished feeding they leave the fruits, fall to the ground, and crawl away to a sheltered spot (usually in the soil) where they pupate. The larval skin becomes barrel-shaped, tanned brown and hard, and is known as the puparium. The true pupa is formed inside this puparium "shell". The pupa turns into an adult fly, which escapes from the puparium by splitting open the anterior end and

squeezing out (Christenson and Foote, 1960; Ferrar, 2010). Many reports indicated that the several species of fruit fly capable of attacking a wide variety of host fruits (e.g., the Oriental fruit fly, *Bactrocera dorsalis*; the Queensland fruit fly, *B. tryoni*; melon fly, *B. cucurbitae*) (White and Elson-Harris, 1992; Allwood *et al*., 1999; Clarke *et al*., 2005).

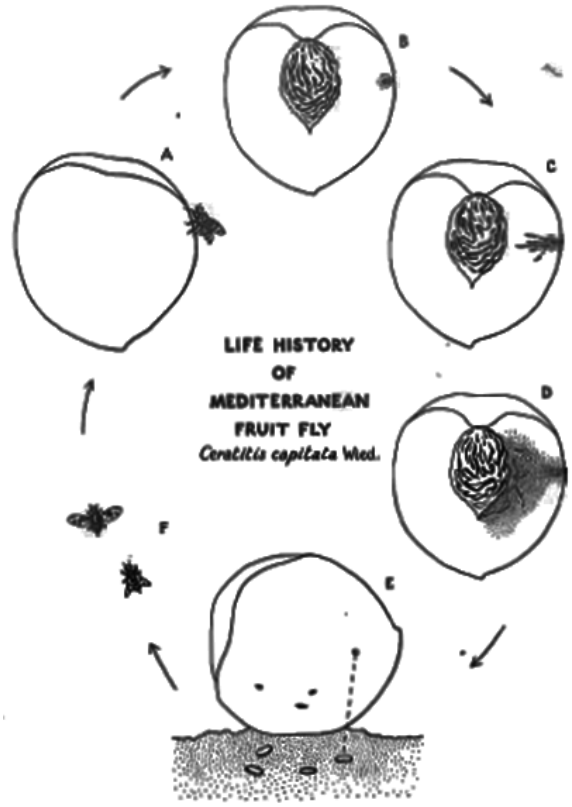


Figure 2.1 Life cycle of the fruit fly (from: Swan, 1949)

Although tephritid fruit flies have numerous species but the life cycle of them follows a closely similar pattern.

2.2.1 Egg

Eggs of the fruit fly (Fig. 2.2) are small usually 0.5 – 1.0 mm in length. The egg shape is spindle and color of the egg vary from creamy to white. The female fruit fly selects ripe or ripening fruit to lay the eggs. The ovipositor is used to puncture the fruit for laying (Fig. 2.3). The bacteria around the fruit surface are pushed into the fruit and cause fruit decay, providing a medium in which the larvae feed. A small group of the eggs is laid in an egg pocket just beneath the skin of fruit. Several egg pockets

may be found in a single fruit, and the same or different flies may later lay more eggs into the same pocket (Swan, 1949). Moreover, the clutch size of the fruit flies may be depends on species of fruit fly and number of host crop. Fitt (1990) suggested that generalist species produced smaller clutch sizes and specialists larger clutch sizes and that this may be a result of exploitation of a diverse range of wild fruits by generalist fly species. The egg deposition and diapause depend on type of fruit fly and environmental temperature. However, several reports revealed that their eggs will hatch within one or two days at room temperature (Swan, 1949; Christenson and Foote, 1960). For the egg deposition, the individual of female fruit fly can produce the eggs about 400 to 1,000 eggs (Oriental fruit fly, Mediterranean fruit fly and Melon fruit fly can deposit more than 1,000 eggs 700 eggs and 400 eggs respectively).

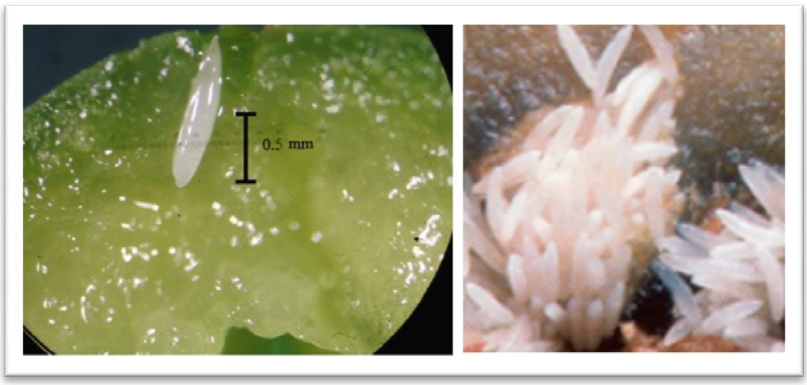


Figure 2.2 Eggs of the fruit fly (from: Jaturat, 2007)

Figure 2.3 Females fruit fly, *Bactrocera*, are punching the fruit using oviporsitor to lays their eggs.

2.2.2 Larva

The larva of various kinds of the fruit flies follows a closely similar pattern. A larva is cylindrical-maggot shape, elongate, anterior end narrowed and curved ventrally, with anterior mouth hooks, ventral fusiform areas and flattened caudal end (Fig. 2.4). Size of the larvae are varying from 7.0 to 11.0 mm and color varies from creamy white to pale yellow depend on the stage of the larva. In general, the larvae have fluid-feeding mouthpart structures and in *Bactrocera* species they inhabit the bacterial soup in the decaying areas of infested fruit. Drew and Lloyd (1987) revealed that the bacteria fed to flies as the only protein source were distributed onto fruit surfaces when the flies were released into a caged netcarine tree. The bacteria were the only microorganisms in oviposition holes, the fruit rot, alimentary tracts of larvae, pupae, and the next generation of adults. Most larval diets contain hydrolyzed protein and some vitamins (in the form of yeast), sucrose as a carbohydrate source and antimicrobial agents (Tsitsipis, 1989). The larvae feed on the internal tissue of fruit and grow in size by molting twice, defining three larval stages. The larva lived in host fruit about 10 – 14 days and when the lava is fully grown, it escapes from the fruit and drops onto the ground below, burrowing into the soil or organic matter (Leblanc *et al*., 2001).

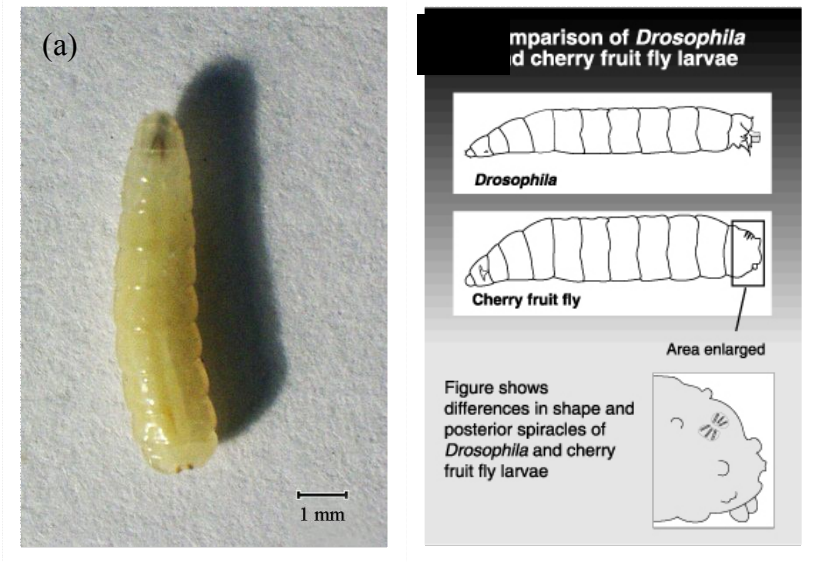


Figure 2.4 The structure of fruit fly larva: (a) tephritid fruit fly larva (b) compare with *Drosophila* larva (from: http://jojo-pestexclusion.blogspot.com)

2.2.3 Pupa

After the mature larva burrowed into the soil or the organic matter, except in *Dacus*, especially the Asclepiadaceae feeders, the larvae pupate withinthe fruiting body. This characteristic is rare in the *Bactrocera* with the only known records being *Bactrocera oleae* which can pupate in fruit or soil (Koveos and Tzanakakis, 1993) and *B. melastomatos* Drew and Hancock which pupates in the fruiting body which is the base of the flower of *Melastomatos malabathricum* (Drew and Romig, 2001b), the larval skin thickens and hardens to form a shell called a puparium where thelarva turns into adult (Norrbom *et al*., 1999). The pupa is light brown, shining, seed like structure, about 5 – 8 mm. long. Similar the other insect pupa they does not need the nutrients for development. The pupa is changed to nigger brown coulor. They remain in the soil for varying periods, depends on temperature. Under average summer conditions this stage lasts 12 – 14 days; in the winter it may last 25 – 50 days (Swan, 1949). The pupa turns into an adult fly, which escapes from the puparium by splitting open the anterior end and squeezing out. The general structure of the pupa is shown in Figure 2.5.

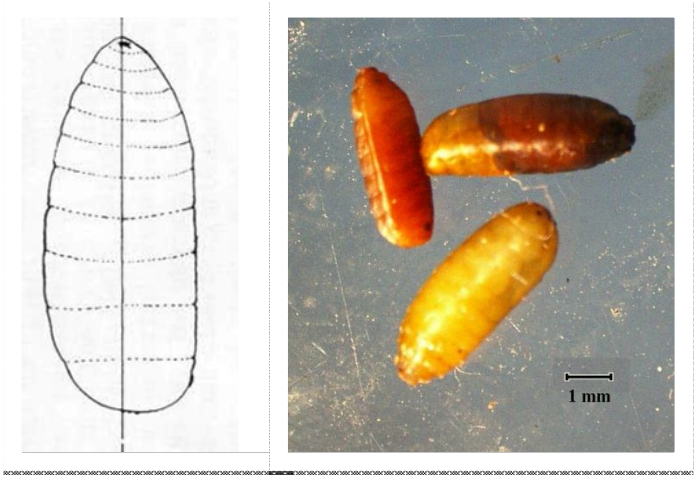


Figure 2.5 The structure of fruit fly pupa

2.2.4 Adults

The adult stage is started when it emerged from the pupa. The adult fruit fly is capable of forcing its way through surprising depths of soil and flies to the aerial (Swan, 1949). The common physical morphological features of Dacini fruit flies are as presented in Figure 2.6. Size of adults about house fly (Allwood and Leblanc, 1997),

the thorax length and width about 2 mm while abdomen width about 3 mm (Arita and Kaneshiro, 1988; http://forecast.doae.go.th/web/mango/218-insect-pests-of-mango/921-oriental-fruit-fly.html; 22 September 2012). The lifespans of the adult fruit flies range between 2 – 300 days depend on various factors e.g. body size, food abundance, mating behavior etc. (Sivinski, 1993; Jaturat, 2007).

Newly emerged fruit flies return to the shelter of vegetation. They are anautogenous (after emerged from puparia as sexually immature adults) thus, many nutrients are required for their survival and reproduction (Raghu, 2003). The basic nutrients which are required by adult fruit flies have long been defined as protein (in the form of free amino acids), minerals, sugars, B-complex vitamins, and water (Hagen, 1953). Moreover, the most fruit flies have a high reproductive rate due to a large number of ovarioles per ovary (20 to 40), short life cycles allowing many generations per year (six to eight in the subtropics to tropics as long as host plants are available) and having multiple host species (about 50% being polyphagous) Thus, the adult female fruit flies require many nutrients to produce their eggs especially protein and lipids (Drew and Romig, 2001b). Because most fruit flies are polyphagous, there are various resources for them e.g. sugar sources include honeydew and other plant exudate, protein from phylloplane bacteria and bird faeces etc. (Bateman, 1972; Drew *et al*., 1983; Courtice and Drew, 1984; Drew and Romig, 2001b). The adult fruit flies feed in this way for 7 – 10 days before the first eggs are laid (Leblanc *et al*., 2001).

In Dacini fruit fly, probably the most important behavioral characteristic that influences both survival of the species and the process of speciation is host plant mating. Zwölfer (1974) reported that 45 species of Tephritidae from 18 genera participated in courtship and mating behavior on their host plants. Moreover, Drew and Lloyd (1987) recorded mating pairs of *Bactrocera tryoni* in a fruiting peach tree over a 14 day period. *Bactrocera cacuminata*, a monophagous species in eastern Australia, has been recorded mating in its host plant *Solanum mauritianum* Scop. In the peach tree study, Drew and Lloyd (1987) recorded a semipermanent population of *B. tryoni* with a range of behavioral patterns that were dependent on the host plant, namely, adult feeding, courtship and mating, oviposition, and larval feeding (refer in Drew and Romig, 2001b).

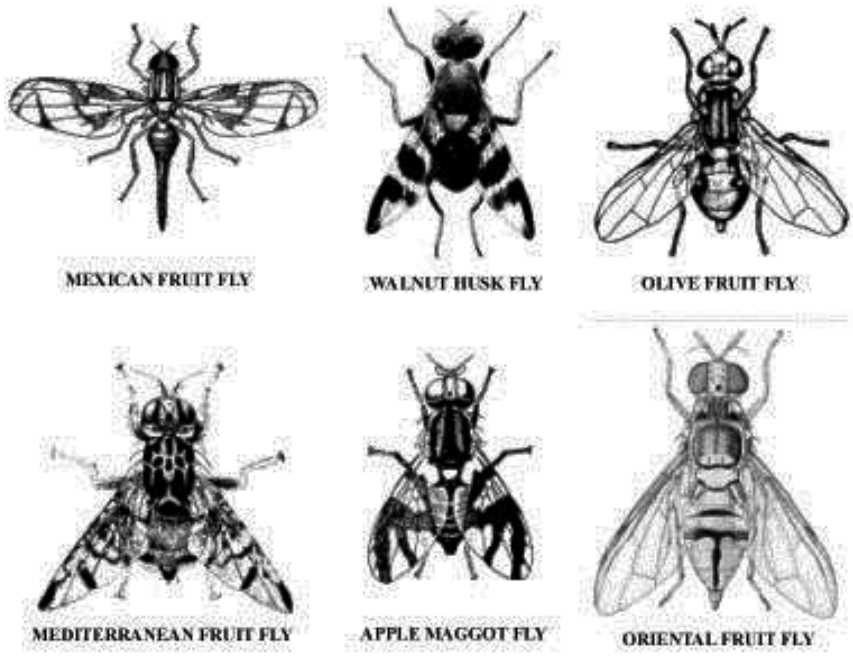


Figure 2.6 The structure of fruit fly adult
(from: http://www.faculty.ucr.edu/~legneref/cover/fruitfly.htm)

2.3 Distribution of the fruit flies

Although there are approximately 4,500 known species in 27 tribes of the family Tephritidae worldwide, the tribe Dacini is very important because it contains the most genera which are serious pests of crops (White and Elson-Harris, 1992). Dacini contains approximately 770 described fruit fly species in 4 genera such as *Bactrocera, Dacus, Ichneumonopsis,* and *Monacrostichus* (Drew 1989; Drew and Hancock, 2000). They distribute in the tropical and subtropical rain forests around the world such as in West Africa, coastal East Africa, Madagascar and the Mascarene Islands, southwest India, Southeast Asia from Nepal to southern China in the north to the Indonesian islands in the south, Papua New Guinea, northeastern Australia, and some South Pacific islands (Drew and Handcock, 2001a). In general, the rain forests of Southeast Asia possess the greatest species richness, while those of Melanesia (e.g., Solomon Islands) are less rich and there is increasing floristic poverty eastward into the Pacific (Whitmore, 1986). In contrast, there are no extremely floristically rich rain forests in Africa (e.g. those of Ghana, Nigeria, and Mauritius; Whitmore, 1986). The distribution

of species in each of the generais listed in Table 2.3. Approximately 68% belong to *Bactrocera* and 32% to *Dacus*. It is noteworthy that the greatest speciation in genus *Dacus* has occurred in Africa while prolific speciation in genus *Bactrocera* has occurred in Southeast Asia and Papua New Guinea (Drew and Hand cock, 2001a).

In Southeast Asia and the Pacific region the major fruit fly pest species are the genus *Bactrocera* (Drew 1989; Drew and Hancock, 2000; Kittayapong *et al*., 2000; Plant Health Australia, 2011). The important pest species that damage to most fruit and vegetable crops such as *Bactrocera dorsalis* complex (*B. dorsalis, B. carambolae, B. occipitalis, B. papayae, B. philippinensis, B. pyrifoliae, B. caryeae, B. kandiensis*), *B. cucurbitae*, *B. correcta*, *B. latifrons*, *B. zonata*, *B.tau* are distributed throughout these areas (Drew and Romig, 1996).

In Thailand and bordering countries about 221 Dacini fruit fly species have been reported.Of these 182 species belong to genus *Bactrocera*. The most important member of this genus is *B. dorsalis* complex which composed of at least 51 species (Drew and Hand cock, 2001a; Clarke *et al*., 2001). Clarke *et al*. (2001) studied distribution of seven *Bactrocera* species (i.e. *B. dorsalis*, *B. correcta*, *B. papayae*, *B. carambolae*, *B. cucurbitae*, *B. latifrons* and *B. umbrosa*), which are important pest species, in Thailand and Peninsular Malaysia. The result revealed that *B. dorsalis* and *B. correcta* were found in northern and central Thailand, *B. papaya*e, *B. carambolae* and *B. umbrosa* were restricted to southern Thailand and Malaysia, while *B. cucurbitae* was widespread, although more abundant in the northern. For *B. latifrons*, they were not trapped.

The distribution and abundance of the fruit fly species depended on various factor (e.g. seasonal, temperature, distribution of host plants etc.). The polyphagous species usually have wider geographic distribution than monophagous species (Drew and Romig, 2001b).

Table 2.3 Worldwide geographic distribution of species of Dacini in each of the four genera.

Areas	**Total No. of Species**	**No. of Species of *Bactrocera***	**No. of Species of *Dacus***	***Ichneumonopsis***	***Monacrostichus***
Africa (including Madagascar and Mascarene Islands)	182	10	172	0	0
Southeast Asia	229	182	44	1	2
Papua New Guinea	168	155	13	0	0
Australia	87	75	12	0	0
Solomons (including Bougainville)	56	54	2	0	0
Vanuatu	13	12	1	0	0
New Caledonia	11	10	1	0	0
Fiji	4	4	0	0	0
Tonga	6	6	0	0	0
Samoa	7	7	0	0	0
Niue	2	2	0	0	0
Cook Islands	2	2	0	0	0
Austral Islands	2	2	0	0	0
Society Islands	2	2	0	0	0
Marquesas Islands	1	1	0	0	0
Tuamotu Archipelago	2	2	0	0	0
Micronesia/N. Pacific	2	2	0	0	0

(From: Drew and Handcock, 2001a)

2.4 Pest status and economic impact of the fruit flies

Because the life cycle of the fruit fly required host fruit for their development thus, they are very important pest of crops. Most fruit flies have courtship, mating and oviposition behavior at their host plants (Drew and Lloyd, 1987). After the eggs were fertilized, female fruit fly punches the target fruit to lays its eggs. The target fruit, which is selected by the female, is considered from physical characteristics for example, there was an attraction to the leeward side of fruit, shade rather than sunlight, soft rather than hard areas, rough rather than smooth surfaces and a preference for cracks and broken fruit surfaces, and the oviposition holes of the other flies (Bateman, 1972). There has to be doubt over the choice of prior oviposition holes as some observations have indicated that females avoid such encounters (Drew and Romig, 2001b). When the fruit was pierced by the ovipositor, the bacteria in the ovipositor and around the surface of fruit are pushed into the fruit and cause fruit decay, providing a medium in which the larvae feed. Moreover, after the larvae hatched they feed on the internal tissue of fruit. This renders the fruit unsuitable for consumption, leading to a loss of economic revenue (Drew and Lloyd, 1987; White and Elson-Harris, 1992; Allwood *et al*., 1999; Clarke *et al*., 2005).

Fruit flies are cause of the export barrier limiting a potential multimillion dollar worldwide trade. They impose a significant cost on horticultural production every year. The world market for fresh fruit has been estimated at US$ 772 billion in 1995 (Armstrong and Jang, 1996). Especially the genus *Bactrocera* cause economic losses from direct fruit damage as well as from quarantine regulations that restrict the movement of fruits and vegetables from infested areas. The hosts of *Bactrocera* spp. belong to a wide variety of plant families including many major commercial crops such as citrus, mango, apples, cucurbits, tomatoes, and many others (Cugola and Mangana, 2009).

Several countries that have the horticultural industry, loss of economic revenue. For example, in Hawaii the direct impact of fruit flies was 15 million dollars (Nakahara *et al*., 1977), which did not include the costs or impacts of insecticide use to control these pests. Twenty-four years later, Staples and Cowie (2001) reported potential impacts of 300 million dollars due to fruit flies in Hawaii (Jang, 2007).

For South Africa, the export was dropped by 80% in 2008 (Ekesi *et al*., 2009). Especially in the Southeast Asia and Pacific region which the genus *Bactrocera* is the dominant species, the economic cost of fruit flies to Australia alone is estimated to be $4.8 billion (Plant Health Australia, 2011). Only in South Australia, there are over three outbreaks of the Queensland fruit fly and one to two outbreaks of the Medfly each year (Bailey and Cartwright, 1994).

Southeast Asian countries such as Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Singapore, Thailand and Vietnam are also a center for tropical fruit production, with approximately 400 edible tropical fruit and nut species being grown (Verheij and Coronel, 1992). These countries are the center of distribution of the *B. dorsalis* complex, with 51 of the 75 species being found there and other species. *Bactrocera* species in Southeast Asia such as *B. dorsalis*, *B. papaya*e, *B. carambolae*, *B. cucurbitae*, *B. latifrons*, *B. minax*, *B. occipitalis*, *B. philippinensis*, *B. tau*, *B. umbrosa*, *B. zonata*, *B. irvingiae*, *B. pyrifoliae*, *B. raiensis*and *B. trivialis* have been reared from 27 commercial fruits (Table 2.4) (Clarke *et al*., 2005; Ferrar, 2010).

In Thailand and bordering countries the fruit crops are attacked, especially the mango, guava and starfruit which are major commercial crops (Mahmood, 2004; Aemprapa, 2007; Orankanok *et al*.,2007). In Malaysia fruit flies incur severe damage to certain potential fruit crops like starfruit and guava. Fruit crops may suffer 100% damage if not protected owing to the fact that these are the polyphagous pests and losses can run several million dollars annually (Singh, 1991). In Thailand, although mangoes are cultivated in up to 300,000 hectares, most of the fruit product is currently lost due to fruit fly infestation. The consequences are a significant reduction to yields and market shares, leading to an estimated loss of millions of dollars annually (Orankanok *et al*., 2006; 2007).

Table 2.4 Fruits of economic importance to South-east Asia and the members of the *Bactrocera* species that infest them.

Fruit species (common name)	Countries in which crop is economically important[a]	Fly species recorded to infest fruit in SE Asia[b]
Abelmoschus esculentus (okra)	I	-
Actinidia chinensis (kiwi fruit)	i, n	-
Ananas comosus (pineapple)	c, d, g, h, i, m, n, q, r	-
Annona spp. (custard apple)	m, q	car, dor, pap
Artocarpus altilis (breadfruit)	c, i, g, m, n, q	car, dor, pap, phi, rai, umb
Artocarpus heterophyllus (jackfruit)	c, i, g, m, n, q, r	car, dor, irv, pap, umb
Averrhoa carambola (carambola)	I	car, dor, pap
Capsicum spp. (chillie)	c, e, f, g, i, k, m, n, o, p, q, r	car, dor, lat, pap, tri
Carica papaya (papaya)	g, i, m, n, q, r	dor, pap, phi
Chrysophyllum spp. (star apple)	m, r	car, dor, pap
Citrullus lanatus (watermelon)	c, f, g, i, k, m, n, o, p, q, r	cuc
Citrus spp. (orange, lemon, lime, etc.)	c, d, g, h, i, m, n, q, r	car, dor, occ, pap, tri
Cucumis melo (cantaloupe)	g, h, i, m, n, q	dor
Cucumis sativus (cucumber)	b, c, d, e, f, g, h, i, k, m, n, o, p, q, r	cuc, dor, pap, tau
Cucurbita spp. (pumpkin, gourd)	g, i, m, q	-
Dimocarpus longan (longan)	q, r	dor
Durio zibethinus (durian)	g, i, m, q, r	-
Ficus carica (fig)	G	-
Fragaria spp. (strawberry)	g, i, n, q	-
Garcinia mangostana (mangosteen)	g, i, m, q, rı	car, pap
Litchi chinensi s(litchi)	q, r	dor
Lycopersicon esculentum (tomato)	g, i, m, n, q	car, pap
Malus domestica (apple)	g, i, m, n, q	dor
Mangifera indica (mango)	g, h, i, m, n, q, r	car, dor, occ, pap, phi
Manilkara zapota (sapodilla)	i, m, q	car, dor, pap
Musa spp. (banana and plantain)	b, c, d, e, f, g, h, i, j, k, m, n, p, q, r	dor, pap, tua
Nephelium lappaceum (rambutan)	g, i, m, q, r	dor, pap
Passiflora edulis (passion fruit)	I	pap
Persea americana (avocado)	g, i, m, n, q	car, dor, pap
Phoenix dactylifera (date)	g, i, n	-
Pouteria sapota (sapote)	R	pap

Table 2.4 (Continued).

Fruit species (common name)	Countries in which crop is economically important[a]	Fly species recorded to infest fruit in SE Asia[b]
Prunus armeniaca (apricot)	g, n	-
Prunus avium (cherry)	g, i, n	dor
Prunus domestica (plum)	i, n, q	dor
Prunus persica (peaches, netcarine)	f, h, i, k, n, p, q, r	dor, pap, pyr, tri, zon
Rubus idaeus (raspberry)	M	-
Solanum melongena (aubergine)	g, i, m, q	pap
Tamarindus indica (tamarind)	m, r	-
Vitis vinifera (grape)	g, i, m, n, q, r	-

[a] Location only includes countries within each species' natural range. *Country Codes*: a, Australia; b, Bhutan; c, Brunei; d, Cambodia; e, China; f, India; g, Indonesia; h, Laos; i , Malaysia; j, Myanmar; k, Nepal; l, Papua New Guinea; m, Philippines; n, Singapore; o, Sri Lanka; p, Taiwan; q, Thailand; r, Vietnam.

[b]*Bactrocera* species infesting South-east Asian fruit derived from. car – *B. carambolae*; cuc - *B. cucurbitae*; dor – *B. dorsalis*; irv – *B. irvingiae*; lat - *B. latifrons*; occ – *B. occipitalis*; pap – *B. papayae*; phi – *B. philippinensis*; pyr - *B. pyrifoliae*; rai - *B. raiensis*; tau - *B. tau*; tri – *B. trivialis*; umb -*B.umbrosa*; zon – *B. zonata*.

2.5 Fruit flies management

Because the high economical impact of fruit flies several agencies for example, Australian Centre for International Agricultural Research (ACIAR), Regional Management of Fruit Fly Project (RMFFP) funded by the United Nations Development Programme, the Food and Agriculture Organization (FAO) of the United Nations were support the regional fruit fly management projects. The projects include identification, development of the control methods and management and quarantine systems for fruit flies. These projects capably help several countries (e.g. Fiji, Samoa, Vanuatu, Tonga etc.) to solve the fruit fly problems and can export commercial crops again (Mcleod, 2005).

Integrated pest management (IPM) was employed to control many pest species, especial fruit flies, which damage the vegetable and fruit crops. IPM is environmental friendly methods that reduce insecticides problems because they integrated physical control, biological control and chemical control to find the best proportion for pest control and protect the crops. The common techniques used for the fruit flies IPM (Allwood, 2000; Kumar *et al*., 2011) such as:

Biological control: The eggs and larvae are target stage. Hymenopteran parasitoids are commonly employed. However, biological control alone does not provide high degree of control on sustainable basis.

Crop hygiene/sanitation: removal of fallen fruits/old crops; each fruit can produce up to 400 fruit fly adults. Removal and destruction is very import for fruit fly IPM; collected fruits should be buried 6 inches deep in soil; some part of China achieved good success in reducing population of fruit flies using sanitation.

Bagging/ netting: young fruits should be completely bagged; bags must not have any holes; prevent oviposition. Initially labor intensive; increases cosmetic value of fruits; age of bagging of different fruits varies.

Insecticides: Not recommended in IPM as there are other robust tools available; however in citrus fruits fruit flies can be suppressed by a single spray; limited use of pesticides in protein baits.

Bait sprays: adult fruit fly needs protein for their reproductive functions; beer waste based protein baits or other mixed with insecticide have been successfully used in Vietnam.

Early harvesting: Due to color preferences for oviposition, some fruits at early stage are not host, in such cases this method could be employed; e.g. Green mango are not hosts of fruit flies.

Male annihilation: using lures (methyl eugenal) and cuelures; large number of traps are needed; traps are excellent tools for ministering flies population.

Sterile Insect Technique (SIT): available in some countries like in Thailand; good when working with a low population; can also be used in combination of other methods.

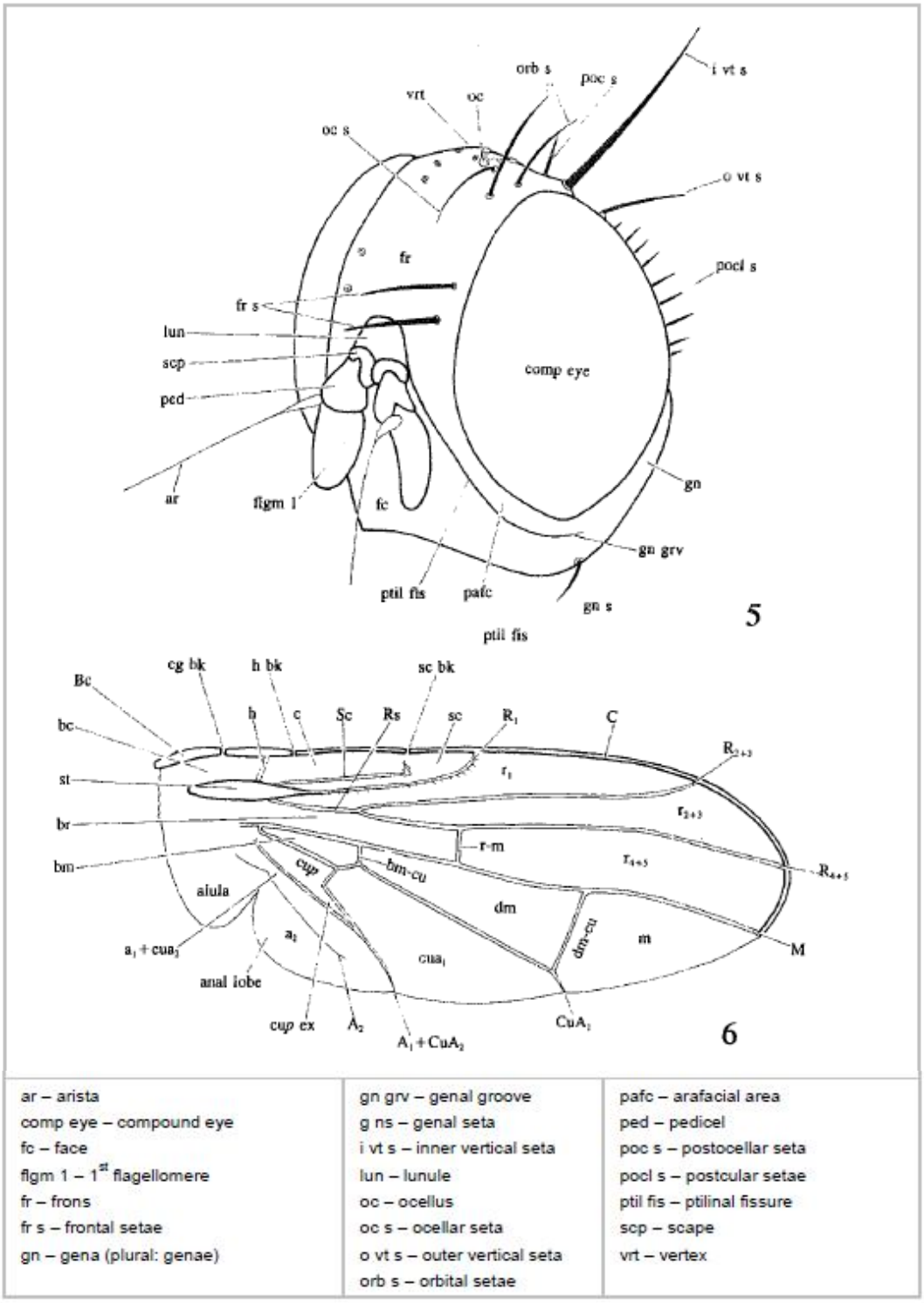
In Thailand, area wide integrated pest management (AW-IPM) using SIT was used to control fruit flies, *Bactrocera dorsalis* and *B. correcta* which are considered to be the key insect pests of fruit production in Thailand. The result revealed that in Ratchaburi Province (western of Thailand) the integrated approach has been effective in controlling fruit flies by reducing damage from over 80% before programme implementation to an average of less than 3.6% in the five years period (2000 to 2004). In Pichit Province (northern of Thailand) where the control programme has been carried out for only two year (2003 and 2004), the infestation has been reduced from 42.9 to 15.5%. These clearly shows that fruit fly control in Thailand using an integrated area-wide approach with an SIT component could be expanded to other production areas with significant economic returns (Orankanok *et al*., 2007).

2.6 Identification of the fruit flies

The identification of the fruit flies can separate in three levels including morphological level, cytological level and molecular level. These levels have different efficiency and different limitation.

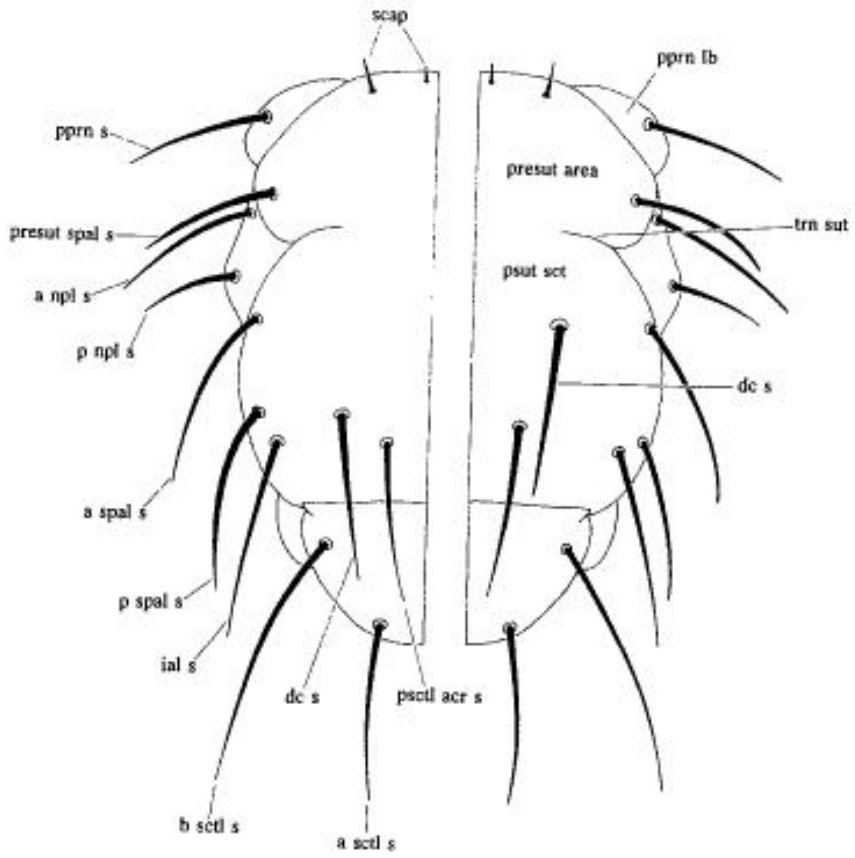
2.6.1 Morphological identification

Approximately 90% of the dacinepest species can be identify using microscopic examination of the adult morphology. The key features (Fig. 2.7, Fig. 2.8, Fig. 2.9, Fig. 2.10) used for the morphological diagnosis of adult fruit flies include wing morphology and infuscation, presence or absence of various setae and relative setae size, overall color and color patterning, and presence, shape and color of thoracic vittae (band or stripe of color) (Plant Health Australia, 2011). However, several species that are closely related, especial *Bactrocera dorsalis* complex, are morphological very similar and difficult to differentiate morphologically (Clarke *et al*., 2005; Ferrar, 2010; Liu *et al*., 2011). Moreover, the number of specialists is very limited worldwide and the immature stages (egg, larva and pupa) were difficult to identify morphologically (Houdt *et al*., 2010; Asokan *et al*., 2011). A key to known fruit fly larvae has been given by White and Elson-Harris (1992), and some information on the larvae of four Asian species (*B. cucurbitae*, *B. dorsalis*, *B. umbrosa*, *B. tau*) has been given by Rohani (1987) (Ferrare, 2010).



ar – arista	gn grv – genal groove	pafc – arafacial area
comp eye – compound eye	g ns – genal seta	ped – pedicel
fc – face	i vt s – inner vertical seta	poc s – postocellar seta
flgm 1 – 1st flagellomere	lun – lunule	pocl s – postcular setae
fr – frons	oc – ocellus	ptil fis – ptilinal fissure
fr s – frontal setae	oc s – ocellar seta	scp – scape
gn – gena (plural: genae)	o vt s – outer vertical seta	vrt – vertex
	orb s – orbital setae	

Figure 2.7 Adult morphology: head (top) and wing (bottom) (White and Elson-Harris 1992)

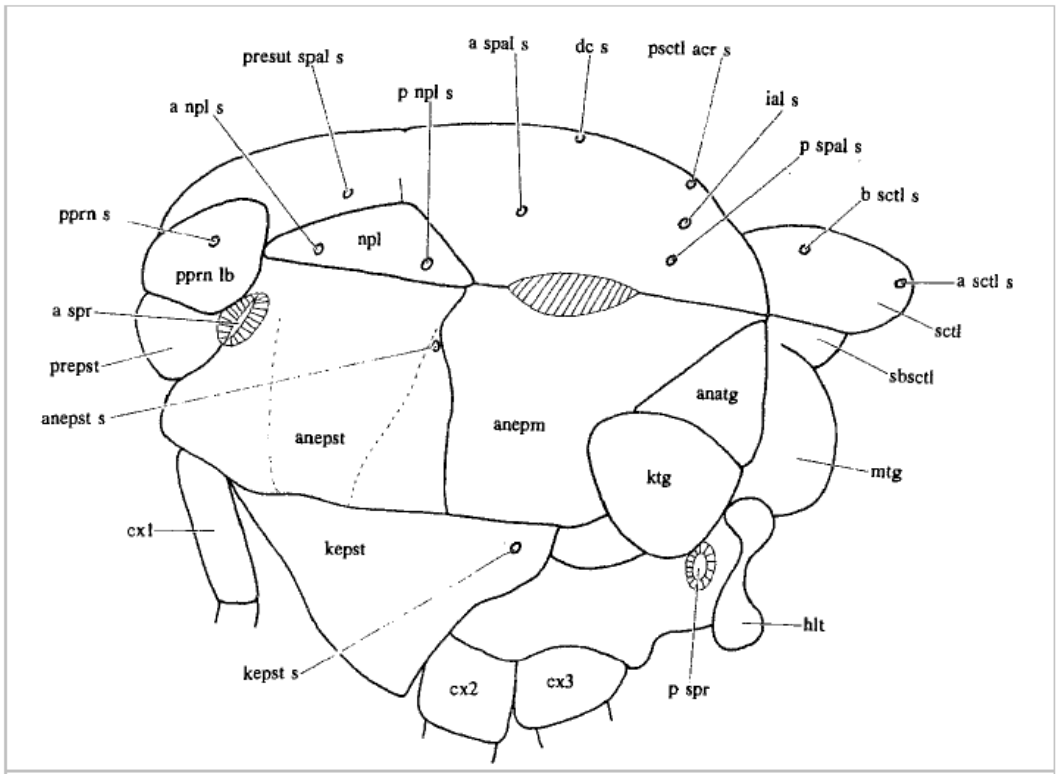


a npl s – anterior notopleural seta
a sctl s – apical scutellar seta
a spal s – anterior supra-alar seta
a spr – anterior spiracle
anatg – anatergite
anepm – anepimeron
anepst – anepisternum
anepst s – upper anepisternal seta
b sctl s – basal scutellar seta
cx – coax
dc s – dorsocentral seta

hlt – halter or haltere
ial s – intra-alar seta
kepst – katepisternum
kepst s – katepisternal seta
ktg – katatergite
npl – notopleuron
p npl s – posterior notopleural seta
p spal s – posterior supra-alar seta
p spr – posterior spiracle
pprn lb – postpronotal lobe
pprn s – postporontal seta

prepst – propisternum
presut area – presutural area
presut spal s – preutural supra-alar seta
psctl acr s – prescutellar acrostichal seta
psut sct – postcutural scutum
sbsctl – subscutellum
scape – scapula setae
sctl – scutellum
trn sut – transverse scuture

Figure 2.8 Adult morphology, Thorax: Dorsal features (White and Elson-Harris 1992)



See Figure 2.7 for abbreviations

Figure 2.9 Adult morphology, thorax: lateral features (White and Elson-Harris 1992)

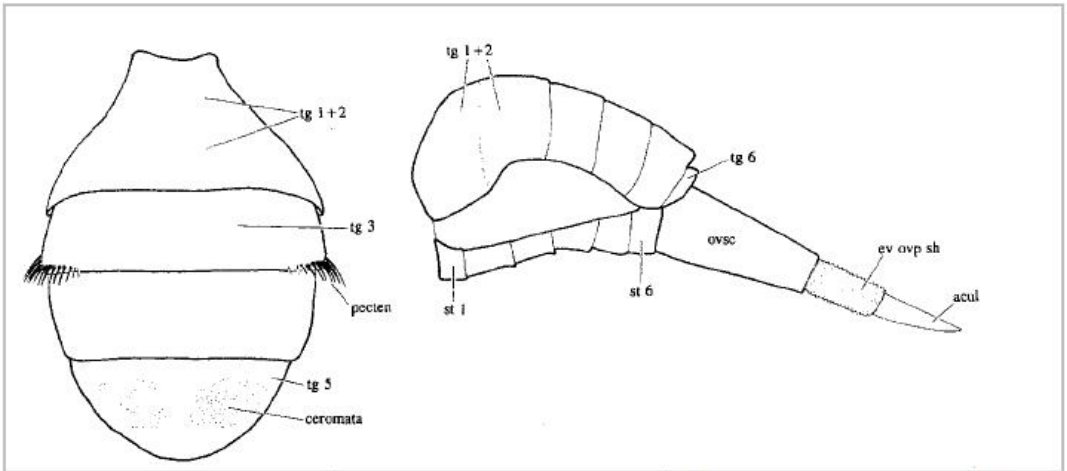


Figure 2.10 Adult morphology, abdomen: male with features of typical dacini (left), Female, with extended ovipositor (right) (White and Elson-Harris 1992)

2.6.2 Cytological identification

In general, the polytene chromosomes are an excellent material for studying chromosome structure and identification of many Dipteran species because they have stable and specific banding patterns. Moreover, the chromosome inversions of the polytene chromosomes can be used to assess phylogenetic relationships among closely related species or to distinguish members of a species-complex group (Zhimulev *et al*., 2004). For example, several species in common fruit fly (Drosophilidae), black fly (Simuliidae), non-biting midge (Chironomidae) (e.g. Brncic *et al*., 1971; Bedo, 1975; Michailova, 1996: Hamada and Adler, 1999; Michailova *et al*., 2001; Kuvangkadilok *et al*., 2008; Jitklang *et al*., 2008; Pramual *et al*., 2008; Tangkawanit *et al*., 2009 etc.). Although several polytene chromosome studies of the true fruit fly have been reported (Bedo, 1986; 1987; Zacharopoulou *et al*., 1990; 1992; Mavragani *et al*., 1992; Zambetaki *et al*., 1995; Zhao *et al*., 1998; Gariou Papalexiou *et al*., 2002), polytene chromosomes aredifficult to prepare and of the low quality. Therefore, polytene chromosomes are excluded or non popular for fruit flies identification. Baimai (2010) presumed that the confusion of fruit fly polytene chromosomes as a results of high inversion and/or translocation between chromosomes. However, many reports indicated that the high efficiency of the mitotic metaphase chromosome for fruit fly identification and revealing sibling species of a species-complex group. Bush (1962) use the mitotic chromosomes for identification of fruit flies genus *Anastrepha*. Baimai *et al*. (1999a, b; 2000a, b) differentiate sibling species of *Bactrocera dorsalis* complex and *B. tau* complex in Thailand according to variation in heterochromatin in mitotic chromosomes. The results revealed that the patterns of mitotic karyotype are useful as diagnostic characters for separation of these closely related species (Bush, 1962; Baimai *et al*., 1995; 1996d; 1999a, b; 2000a, b). Although the mitotic chromosomes are an excellent material for fruit flies identification, this method required specialists, experiences and specimens are limited only in third instar larva stage.

2.6.3 Molecular identification

Molecular identification was proposed as a solution to the limitation of traditional taxonomy. The advantage of using molecular identification are listed as that it is fast, suitable for use with a little material (in insect may be use only head, leg, antenna or other tissues) and can be used even with stored, dry, frozen or old samples.

In addition, all developmental stages can be identified using molecular techniques (e.g. Leh, 2004; Floyd *et al*., 2010; Asoka *et al*., 2011; Stur, 2011 etc.). The molecular identification include with molecular markers. There is a wide range of molecular markers available for entomological studies (Loxdale and Lushai, 1998). The protein markers are represented using allozymes or isozymes, while DNA markers can be represented using nuclear DNA or mitochondrial DNA (Leh, 2004).

2.6.3.1 Protein markers

Protein markers are employed before the presence of DNA markers. However, the protein markers have many limitations such as some markers may be tissue-specific. Isozymes are also phenotypic markers and its efficiency can be affected by the tissue, growth stage and conditions of the organisms. Moreover, in the oftentimes polymorphism of isozymes is so low and difficult to detect, as a result closely related species may not be differentiated (Feraday and Leonhardt, 1989; Godwin *et al*., 2001). In tephritid fruit flies both success and not success for detection of polymorphism have been reports. For example, electrophoretic data was used initially with limited success to distinguish between five species of the *Bactrocera dorsalis* complex, with *B. dorsalis*, *B. carambolae* and *B. papaya* (Yong, 1993), while allozyme electrophoresis success to distinguish complex species within the *B. tau* group in Thailand (Saelee *et al*., 2006).

2.6.3.2 DNA markers

DNA marker may be come from nuclear DNA, ribosomal DNA, mitochondrial DNA (in the animal), chloroplast DNA (in the plants) or the other genetic materials. The species identification and evolution studies of the organisms are revealed using genetic divergences base on these markers. The choosing genetic marker depends on propose of the study, property of the marker and molecular techniques. However, several reports revealed that DNA marker used to identify the animal species from mitochondrial DNA and some ribosomal DNA regions.

In tephritid fruit flies the first is a draft molecular genetic marker protocol developed by McKenzie *et al*. (2004). Restriction Fragment Length Polymorphism (RFLP) tests of internal transcribed spacer region (ITS1), part of the nuclear rRNA gene cluster, were used to identify the fruit fly species. The result revealed that the ITS1 products which were digested using various restriction enzymes

can be used for identification of at least 30 fruit fly species (McKenzie *et al*., 2004). Moreover, in 1997 the 18S and the ITS1 genes were tests by PCR – RFLP, similar the first time, by Armstrong *et al*. (1997). The result revealed that at least 31 economically significant fruit fly species can be identified. Several reports revealed about identification of fruit flies using PCR – RFLP based on both ribosomal DNA and mitochondrial DNA (e.g. Nakahara *et al*., 2000; 2001; Muraji and Nakahara, 2001; 2002). Although PCR – RFLP as a powerful method for fruit flies identification, some closely related species or complex species were not success e.g. *Bactrocera carambolae* and *B. papayae*, *B. dorsalis* complex species etc. (Muraji and Nakahara, 2002; Clarke *et al*., 2005). One molecular technique which popular and was developed to solve the problems of identification is DNA barcoding (see the details in 2.7).

2.7 DNA barcoding

DNA barcode is a short nucleotide sequence (500 – 600 bp) which can be used for species identification. It was developed to solve the limitation of traditional taxonomy (Hebert *et al*., 2003a). Intraspecific and interspecific genetic divergences of the DNA sequences are used to identify species. The genetic distances between the sequence of an unknown specimen and a collection of well-identified reference sequences in DNA barcode library are compared and assigning to the query species name of the reference sequence with the smallest genetic distance (Hebert, 2007). Because the efficiency of identification base on DNA barcode depends on the distinction between intraspecific and interspecific genetic divergences thus, the standard sequence or gene region which is used to be barcoding sequence must suitable and has sufficient variation to identify specie. Each type of organism has different standard sequence or gene region for DNA barcoding. In the animal the mitochondrial cytochrome c oxidase subunit I (COI gene) is employed while the internal transcribed spacer (ITS) region of ribosomal DNA, the ribulose-bisphosphate carboxylase (rbcL) and maturase K (matK) genes of chloroplast DNA are used as the barcode of fungal and plant, respectively (http://www.boldsystems.org /index. php/IDS_OpenIdEngine; 10 April 2013). Although DNA barcode is the powerful method for species identification, some reports revealed unsuccessful of this technique. The ambiguous taxonomy bases

on DNA barcode as a result from overlapping of intraspecific and interspecific genetic divergence values which are influenced by two major factors such as time since species and effective population size (Avise, 2000; Meyer and Paulay, 2005; Pramual *et al*., 2011b).

DNA barcode of the animals, using COI gene, was used to identify animal species throughout the world. Several reports revealed the successful of DNA barcode based on COI sequences to identify many groups of animals, for examples , springtails, mayflies, spiders, fish, mosquitos, birds, blackflies, etc. (Hogg and Hebert, 2004; Ball *et al*., 2005; Barrett and Hebert, 2005; Ward *et al*., 2005; Cywinska *et al*., 2006; Kerr *et a*l., 2007; Rivera and Currie, 2009). However, at present (10 April 2013) DNA barcode data in Barcode of Life Data Systems (BOLD), which is online databases, have only 2,093,306 specimens from 177,105species which less than 10% of all species in the world. This could leads to taxonomy limitation in some species using DNA barcoding.

In tephritid fruit flies, Armstrong and Ball (2005) studied the efficiency of DNA barcode for fruit flies identification. One hundred and ninety three sequences, representing 60 species were used to create a tephritid COI reference profile. The result revealed the high rates of success for DNA barcode to differentiate fruit fly species, but also mentioned some difficulties with the identification of few species (e.g., *Bactrocera dorsalis*, *B. tryoni*, *Anastrepha fraterculus*), where the occurrence of cryptic species and high levels of geographic differentiation might complicate identification. After that several organizations and researchers interest to study fruit flies identification base on DNA barcode. The Tephritid Barcoding Initiative (TBI) was initiated in 2006 by the Consortium for the Barcode of Life (CBOL). The TBI aims to barcode 10,000 specimens representing 2,000 species of fruit flies, including all taxa (about 350 species) of major and minor economic importance. (Houdt *et al*., 2010; http://www.bolinfonet.org /casestudy/index.php/display/study/30; 21 september, 2012). Several trials have started to develop DNA barcodes for three of the main pest genera occurring in Africa: *Bactrocera*, *Ceratitis* and *Dacus* (Houdt *et al*., 2010).

DNA barcode of the fruit flies are continuously studied especially economic important species. Asokan *et al*. (2011) employed COI sequence to identify and study phylogenetic of *Bactrocera* species. The results revealed that *B. dorsalis*, *B. tau*, *B. correcta* and *B. zonata* can identify using DNA barcode. Phylogenetic analysis showed

that the subgenus *Bactrocera* is monophyletic. Samie and Fiky (2011) success to use DNA barcode for *B. zonata* identification. Moreover, DNA barcode revealed the high efficiency identification of *B. invadens*, a new species of fruit fly reported in the year 2005 belongs to the *B. dorsalis* complex. This species difficult to diagnose based on morphological characters (Liu *et al*., 2011). However, DNA barcode is limited when dealing with species complexes of tephritid fruit flies; where it appears that insufficient lineage sorting of COI variation and/or inadequate current taxonomic knowledge hampers molecular identification of currently recognized species within several tephritid complexes (Armstrong and Ball, 2005). Recent study, the problems of Queensland fruit flies (*B. tryoni*) identification were revealed (Blacket *et al*., 2012) because *B. tryoni* is a closely related species in *B. tryoni* complex (i.e. *B. tryoni*, *B. neohumeralis*, *B. aquilonis*, *B. melas*) which have broad host ranges (Hancock *et al*., 2000) as a result of overlapping of intraspecific and interspecific genetic divergence values and polyphyly were found in this group. Moreover, a nuclear copy (a numt pseudogene) of the barcoding region of COI was found (Blacket *et al*., 2012). DNA barcode database of fruit fly species is very limit compared with other organisms. Only 7,113 specimens within 747 Tephritidae species and 1,597 DNA barcodes specimens in 163 *Bactrocera* species are presented (http://www.barcodinglife.com/index.php/ Taxbrowser_Taxonpage?taxid=6515; 10 April, 2013).

In Thailand, there is only one report of DNA barcode of fruit fly. Nopparat *et al*. (2011) reported COI gene sequences from five larvae of fruit fly which collected in guava fruit. The result shown that these samples of fruit fly larvae were identified as *B .correcta* (Nopparat *et al*., 2011).

2.8 Mitochondrial cytochrome oxidase subunit I (COI) gene

Mitochondrial DNA (mtDNA) of animals is a close circular molecule. It contains 37 genes including13 protein coding genes, 2 ribosomal genes, a non – protein coding control region and several tRNAs (Fig. 2.11) (Boore, 1999). The mtDNA has been used widely as a genetic marker for species identification and evolutionary studies because it lacks of repetitive DNA, transposable elements, pseudogene and

intron so gene organization is sample. The mtDNA evolve rapidly because it has high mutation rate. Moreover, there is not recombination or other genetic rearrangement in almost mtDNA (Avise *et al*., 1987). These characters are advantages of mtDNA to use as DNA barcode marker causeit requires a gene that haslow level of intraspecific but high interspecific genetic divergences (Hebert *et al*., 2003a).

COI gene encodes protein products which are play important role in both electron transport and the associated translocation of proton across mitochondrial membrane (Saraste, 1990; Gennis, 1992; Lent *et al*., 1996; refer in Pramual, 2006). The nucleotide composition of this gene is A – T bias; for example, in insects have the A – T content of 68 – 76% (Lent *et al*., 1996; refer in Pramual, 2006). The COI gene contains both highly conserve site and variable region. Its nucleotides reveal enough variation to differentiate between species. Moreover, less than 10% of intraspecific variation has been detected in this gene (Blaxter, 2004).

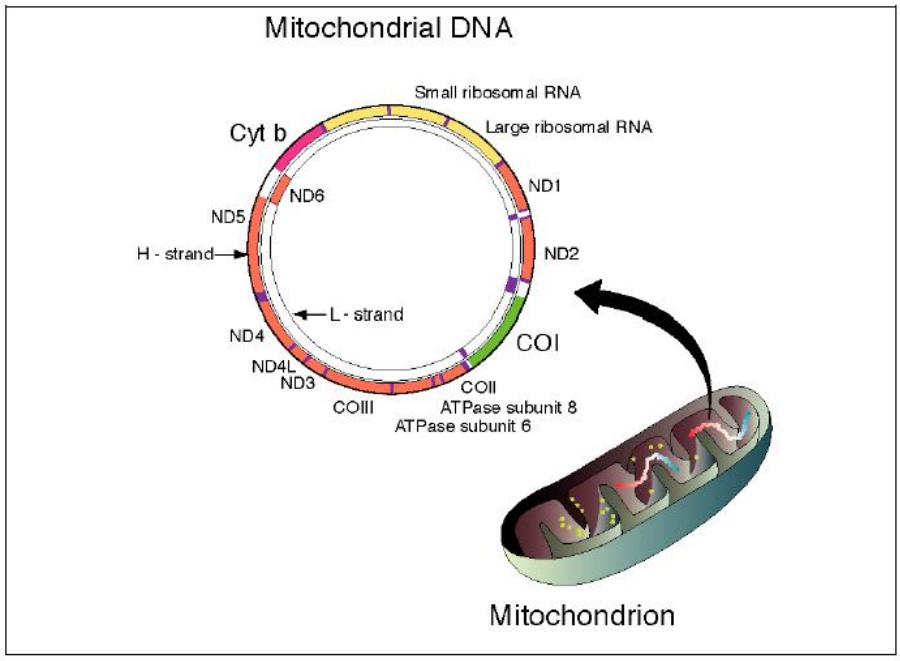


Figure 2.11 The mitochondrial genome of a eukaryote (from: Wauhh, 2011)

2.9 *Bactrocera latifrons* (Hendel)

Solanum fruit fly, *Bactrocera latifrons* belongs to the subgenus *Bactrocera*. *Bactrocera latifrons* is a medium sized species; face fulvous with a pair of large oval black spots; postpronotal lobes and notopleura yellow; scutum dull black; lateral postsutural vittae present; medial postsutural vitta absent; mesopleural stripe extending to anterior *npl*. seta dorsally; scutellum yellow; wing with a narrow fuscous costal band overlapping R2+3 and expanding into a small spot around apex of R4+5, a medium width fuscous anal streak; cells bc and c colorless; microtrichia in outer corner of cell c only; all abdominal terga entirely dark orange-brown, posterior lobe of male surstylus short; female with apex of aculeus trilobed (Plant Health Australia, 2011). The adult morphology of *B. latifrons* shown in Figure 2.12. *Bactrocer alatifrons* is native to South and Southeast Asia (White and Elson-Harris, 1992) and recently invaded other geographic regions such as Japan, Hawaii, Tonga and Tanzania (Kaneta *et al*., 1985; Vargas and Nishida 1985; Wang, 1996; Mwatawala *et al*., 2007). *Bactrocera latifrons* is a major pest of the important commercial crops in the family Solanaceae such, as chili (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), turkey berry (*Solanum torvum* Sw.) and tomato (*Lycopersicon esculentum* M.)(Vijaysegran and Osman, 1991; Liquido*et al*., 1994; Harris *et al*., 2001; McQuate *et al*., 2007). Especially in chill crops, 60% to 80% were infested in Malaysia (Vijaysegaran and Osman, 1991).

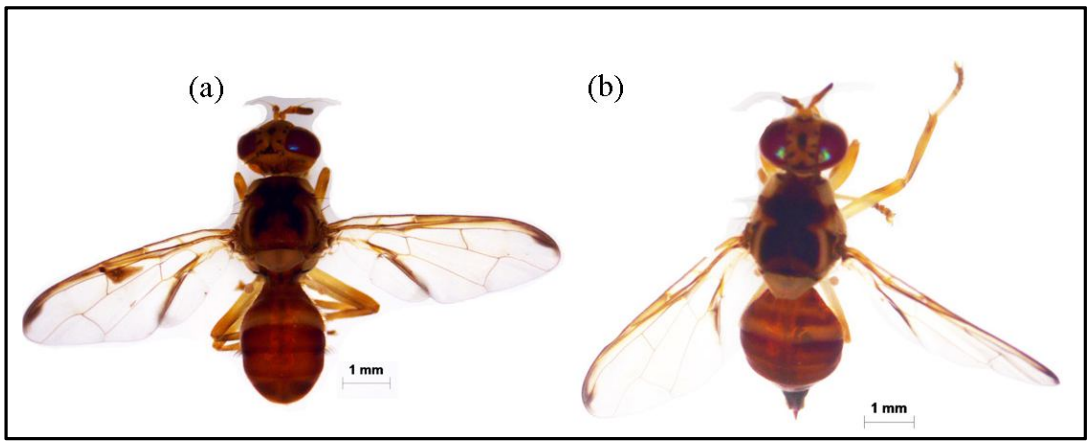


Figure 2.12 Adults of *Bactrocera latifrons*: (a) male (b) female

2.10 Genetic variation of *Bactrocera latifrons* (Hendel)

Thelittle information on genetic variation and genetic structure of this species is available. To the best of our knowledge, there is only one report on population genetics of this species from Peninsular Malaysia based on allozyme electrophoresis (Yong, 1993). Yong (1993) recovered *B. latifrons* from four solanaceous fruits including *Capsicum annuum L., Lycopersicon esculentum Mill., Solanum pseudocapsicum* L. and *Solanum melongena* L. and analyzed for a total of 15 gene-enzyme systems comprising 21 loci. The results revealed 11 loci including *aAdh, Aldox, Ald, Est-F, Est-S, Hk-F, Ldh, cMdh, Me, Pep-A* and *Pep-C*were invariant. The polymorphic loci, cathodal alcohol dehydrogenase, glucose phosphate isomerase, glycerol-3-phosphate dehydrogenase, hydroxybutyrate dehydrogenase, isocitrate dehydrogenase, anodal malate dehydrogenase and phosphoglucomutase were represented by two alleles each, while hexokinase-S, peptidase-B and phosphogluconate dehydrogenase were represented by three alleles each. The proportion of polymorphic loci ranged from 0.28 to 0.33, while the mean heterozygosity ranged from 0.04 to 0.13.This study found comparable levels of genetic variation in *B. latifrons* in Malaysia with *B. cucurbitae* but these were lower than those of *B. caudata* and *B. dorsalis* complexes. And different levels of genetic variations of *B. latifrons* from different host plants were reported.

Chapter 3

Research Methodology

3.1 Sample collections

Both adults and larvae of tephritid fruit fly (*Bactrocera* spp.) were collected from natural habitats throughout northeastern Thailand, which covers more than 168,000 km^2. The fruit fly samples were collected from 19 host plant species including Baby Jackfruit (*Momordica cochinchinensis* Lour.),Carambola (*Averrhoac arambola* L.), Chilli (*Capsicum annuum* L.), Eggplant (*Solanum melongena* L.), Golden apple (*Diospyrosdecandra* Lour.), Guava (*Psidium guajava* L.), Ivy gourd (*Cocciniagrandis* L.), Japanese Cucumber (*Cucumis sativas* L.), Jujube (*Ziziphus mauritiana* Lam.), Kayu (*Irvingia malayana* Oliv. Ex A. Benn.), Keledang (*Artocarpus lanceifolius* Roxb.), Mango (*Mangifera indica* L.), Pumpkin (*Cucurbita moschata* Decne.), Rose apple (*Syzygium*sp.), Snake gourd (*Trichosanthes cucumerina* L.),*Solanum trilobatum*, Sugar apple (*Annona squamosa* L.), *Trichosanthes tricuspidata* and Turkey berry (*Solanum torvum* Sw.), 18 locations, in 12 provinces (Fig. 3.1, Table 3.1).Geographic location (latitude, longitude, and altitude) were recorded. Some adult flies were also collected from natural habitats using sweep net. Adult flies were also obtained from fruit fly reared in laboratory. The ripen or rotten fruits which were pierced by female were harvested to find fruit fly larvae in laboratory. The larval samples were collected by hand from fruits only one or two larvae from each fruit to avoid potential sampling of sibling individuals (Fig. 3.2). The larval and adult specimens were preserved in 80% ethanol and were maintained at a low temperature (≈ - 20 to 4 °C) for further studies. The remaining larvae were reared using the ripe fruits from their natural habitats. Fruits were placed on sawdust in a plastic box which covered by calico (Fig. 3.3). To reveal population genetic structure of *B. latifrons*. Additional of *B. latifron* specimens were also obtained fromchili (*Capsicum annuum* L.), turkey berry (*Solanum torvum*Sw.) and *S. trilobatum* L. from four populations in northern Thailand. The sample collection sites (Figure 3.4), host plant species and number of COI sequences of *B. latifrons* were shown in Table 3.2.

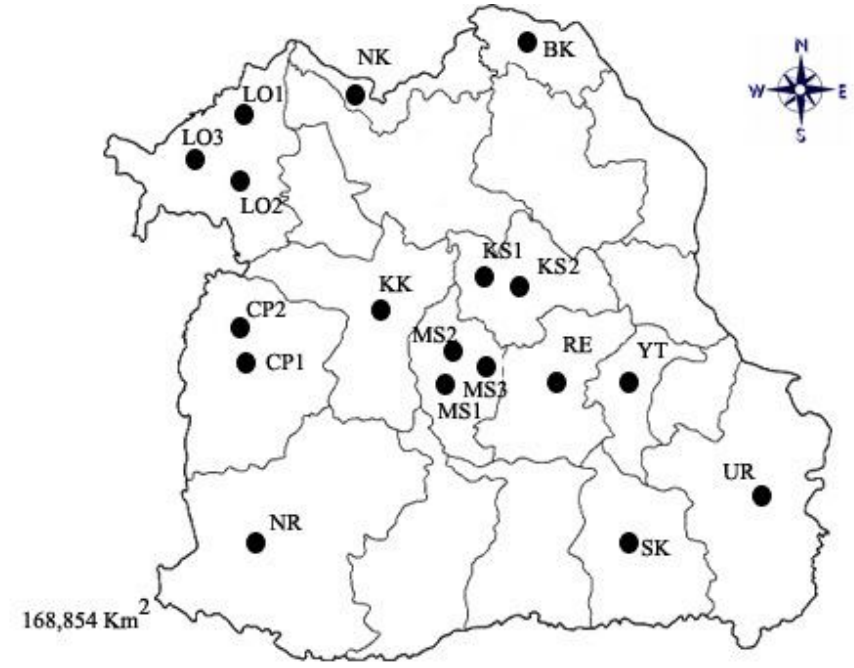


Figure 3.1 Sampling locations of the fruit flies in northeastern Thailand: BK – Bueng Kan, CP – Chaiyaphum, KK – Khon Kean, KS – Kalasin, LO – Loei, MS – Maha Sarakham, NK – Nong Khai, NR – Nakhon Ratchasima, RE – Roi Et, SK – Si Sa Ket, UR – Ubon Ratchathani, YT – Yasothon (details in Table 3.1)

Figure 3.2 Collection larva samples of fruit flies using forceps

Figure 3.3 Fruit flies were reared by put the infested fruit in the plastic box that contained sawdust at the bottom and covered by calico. The plastic box was kept under the room condition.

Table 3.1 Sample collection sites and host plant species in this study.

Location	**Code**	**Host plant species** **Common name (Scientific name: Familly)**	**Latitude/** **longitude**	**Altitude** **(m)**	**Collection date**
So Phisai, Bueng Kan	BK	- Chilli (*Capsicum annuum* L.: Solanaceae)	18°04′42′′N 103°26′36′′E	167	02/ 10/ 2012
		- Eggplant (*Solanum melongena* L.: Solanaceae)	18°04′42′′N 103°26′36′′E	167	02/ 10/ 2012
Kaset Sombun, Chaiyaphum	CP1	- Carambola/ star fruit (*Averrhoa carambola* L.: Oxalidaceae)	16°22′55′′N 101°58′21′′E	234	21/ 07/ 2012
		- Rose apple (*Syzygium*sp.: Myrtaceae)	16°22′55′′N 101°58′21′′E	234	21/ 07/ 2012
Khonsan, Chaiyaphum	CP2	- Keledang (*Artocarpus lanceifolius* Roxb.: Moraceae)	16°36′25′′N 101°54′35′′E	260	19/ 01/ 2013
Kham Muang, Kalasin	KS1	- Jujube (*Ziziphus mauritiana* Lam.: Rhamnaceae)	16°55′24′′N 103°37′54′′E	201	13/ 08/ 2012
Tha Khantho, Kalasin	KS2	- Kayu (*Irvingia malayana* Oliv. Ex A. Benn.: Irvingiaceae)	16°56′24′′N 103°14′40′′E	171	14/ 08/ 2012

Table 3.1 (Continued).

Location	Code	Host plant species Common name (Scientific name: Familly)	Latitude/ longitude	Altitude (m)	Collection date
Mueang Khon Kaen, Khon Kaen	KK	- Baby Jackfruit (*Momordica cochinchinensis* Lour.: Cucurbitaceae)	16°26′18′′N 102°50′20′′E	158	24/ 09/ 2012
Chiang Khan, Loei	LO1	- Carambola/ star fruit (*Averrhoa carambola* L.: Oxalidaceae)	17°53′53′′N 101°39′59′′E	213	06/ 08/ 2012
Na Haeo, Loei	LO2	- Pumpkin (*Cucurbita moschata* Decne.: Cucurbitaceae)	17°28′29′′N 101$^{0°}$58′32′′E	629	19/ 11/ 2012
		- *Trichosanthes tricuspidata*Lour. (Cucurbitaceae)	17°28′29′′N 101$^{0°}$58′32′′E	629	19/ 11/ 2012
Pak Chom, Loei	LO3	- Chilli (*Capsicum annuum* L.: Solanaceae)	18°01′18′′N 101°53′18′′E	209	31/ 10/ 2012
Kantharawichai, Maha Sarakham	MS1	- Carambola/ star fruit (*Averrhoa carambola* L.: Oxalidaceae)	16°14′58′′N 103°15′52′′E	166	19/ 07/ 2012
		- Chilli (*Capsicum annuum* L.: Solanaceae)	16°14′58′′N 103°15′52′′E	166	18/ 07/ 2012 26/ 07/ 2012 17/ 08/ 2012

Table 3.1 (Continued).

Location	Code	Host plant species Common name (Scientific name: Familly)	Latitude/ longitude	Altitude (m)	Collection date
Kantharawichai,	MS1	- Guava	16°14′58′′N	166	28/ 07/ 2012
Maha Sarakham		(*Psidium guajava* L.: Myrtaceae)	103°15′52′′E		20/ 08/ 2012
		- Ivy gourd	16°16′46′′N	150	31/ 07/ 2012
		(*Coccinia grandis* L.: Cucurbitaceae)	103°14′36′′E		
			16°19′22′′N	148	13/ 12/ 2012
			103°17′48′′E		
		- Mango	16°15′17′′N	150	19/ 07/ 2012
		(*Mangifera indica* L.: Anacardiaceae)	103°13′50′′E		20/ 07/ 2012
		- Sugar apple	16°14′58′′N	166	11/ 07/ 2012
		(*Annona squamosa* L.: Annonaceae)	103°15′52′′E		
		- Turkey berry	16°14′58′′N	166	15/ 07/ 2012
		(*Solanum torvum*Sw.: Solanaceae)	103°15′52′′E		01/ 08/ 2012
		- Japanese Cucumber	16°14′45′′N	150	19/ 12/ 2012
		(*Cucumis sativas* L.: Cucurbitaceae)	103°14′52′′E		
Mueang Maha	MS2	- Ivy gourd	16°11′03′′N	146	13/ 12/ 2012
Sarakham, Maha		(*Coccinia grandis* L.: Cucurbitaceae)	103°18′04′′E		
Sarakham			16°08′32′′N	158	13/ 12/ 2012
			103°18′30′′E		

Table 3.1 (Continued).

Location	Code	Host plant species Common name (Scientific name: Familly)	Latitude/ longitude	Altitude (m)	Collection date
Mueang Maha Sarakham, Maha Sarakham	MS2	- Rose apple (*Syzygium*sp.: Myrtaceae) - *Solanum trilobatum* L. (Solanaceae)	16°08′06′′N 103°18′34′′E 16°12′39′′N 103°18′24′′E	157 149	13/ 03/ 2013 18/ 11/ 2012
Na Dun, Maha Sarakham	MS3	- Kayu (*Irvingia malayana* Oliv. Ex A. Benn.: Irvingiaceae)	15°42′50′′N 103°13′37′′E	162	08/ 08/ 2012
Si Chiang Mai, Nong Khai	NK	- Chilli (*Capsicum annuum* L.: Solanaceae) - Eggplant (*Solanum melongena* L.: Solanaceae)	17°57′23′′N 102°35′22′′E 17°57′23′′N 102°35′22′′E	172 172	26/ 08/ 2012 30/ 10/ 2012 26/ 08/ 2012
Mueang Nakhon Ratchasima, Nakhon Ratchasima	NR	- Mango (*Mangifera indica* L.: Anacardiaceae) - Sugar apple (*Annona squamosa* L.: Annonaceae)	14°58′16′′N 102°05′59′′E 14°58′16′′N 102°05′59′′E	197 197	09/ 09/ 2012 16/ 07/ 2012

Table 3.1 (Continued).

Location	Code	Host plant species Common name (Scientific name: Familly)	Latitude/ longitude	Altitude (m)	Collection date
Nong Phok, Roi Et	RE	- Eggplant (*Solanum melongena* L.: Solanaceae)	16°18′35′′N 104°12′11′′E	179	06/ 08/ 2012
		- Mango (*Mangifera indica* L.: Anacardiaceae)	16°18′35′′N 104°12′11′′E	179	06/ 08/ 2012
Prang Ku, Si Sa Ket	SK	- Carambola/ star fruit (*Averrhoa carambola* L.: Oxalidaceae)	14°48′59′′N 104°04′00′′E	139	11/ 08/ 2012
		- Chilli (*Capsicum annuum* L.: Solanaceae)	14°49′53′′N 104°03′38′′E	173	11/ 08/ 2012
		- Eggplant (*Solanum melongena* L.: Solanaceae)	14°48′59′′N 104°04′00′′E	139	11/ 08/ 2012
		- Golden apple (*Diospyros decandra*Lour.: Ebenaceae)	14°49′48′′N 104°03′38′′E	138	12/ 08/ 2012
		- Ivy gourd (*Cocciniagrandis* L.: Cucurbitaceae)	14°48′59′′N 104°04′00′′E	139	11/ 08/ 2012
		- Jujube (*Ziziphus mauritiana* Lam.: Rhamnaceae)	14°50′26′′N 104°03′32′′E	139	11/ 08/ 2012
		- Kayu (*Irvingia malayana*Oliv. Ex A. Benn.: Irvingiaceae)	14°49′48′′N 104°03′38′′E	138	11/ 08/ 2012

Table 3.1 (Continued).

Location	Code	Host plant species Common name (Scientific name: Familly)	Latitude/ longitude	Altitude (m)	Collection date
Mueang Ubon	UR	- Guava	15°13´44´´N	121	04/ 08/ 2012
Ratchathani**,** Ubon		(*Psidium guajava* L.: Myrtaceae)	104°51´15´´E		
Ratchathani		- Rose apple	15°13´44´´N	121	04/ 08/ 2012
		(*Syzygium*sp.: Myrtaceae)	104°51´15´´E		
		- Sugar apple	15°13´44´´N	121	04/ 08/ 2012
		(*Annona squamosa* L.: Annonaceae)	104°51´15´´E		
PaTew, Yasothon	YT	- Chilli	15°49´35´´N	140	30/ 08/ 2012
		(*Capsicum annuum* L.: Solanaceae)	104°21´50´´E		
		- Snake gourd (*Trichosanthes cucumerina* L.:	15°49´35´´N	140	30/ 08/ 2012
		Cucurbitaceae)	104°21´50´´E		
		- Turkey berry	15°49´35´´N	140	30/ 08/ 2012
		(*Solanum torvum*Sw.: Solanaceae)	104°21´50´´E		

Solanum trilobatum L. and *Trichosanthes tricuspidata* Lour. were not reported common name .

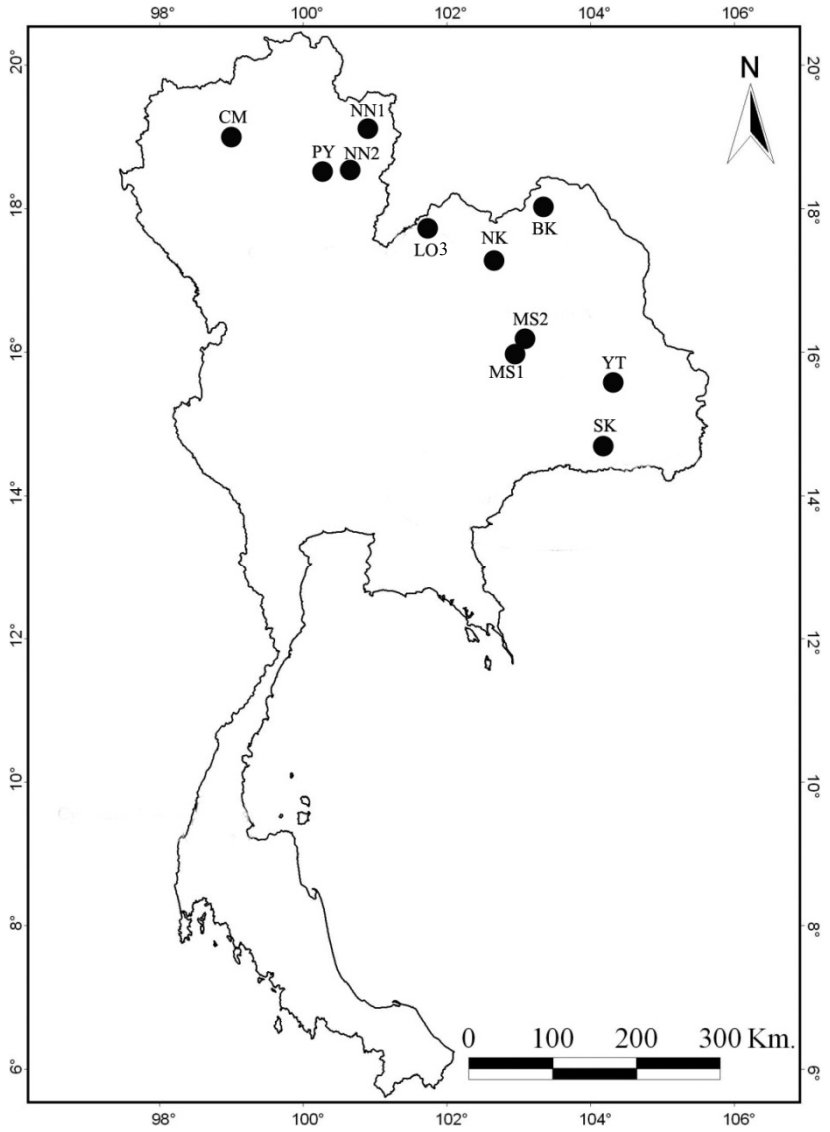


Figure 3.4 Collection sites of 11 populations of *Bactrocera latifrons* from Thailand: Details of the sampling locations are given in Table 3.2.

Table 3.2 Sample collection sites, host plant species and number of COI sequences of *Bactrocera latifrons* from Thailand.

Location	Code	Latitude/ longitude	Altitude (m)	Host plant species	N	Collection date
	CM	19°01′01′′N 99°18′14′′E	618	*Solanum torvum*	6	24 November 2012
Chiang Muan, Phayao	PY	18°55′11′′N 100°12′04′′E	401	*S. trilobatum*	10	21 November 2012
Phu Kha, Pua, Nan	NN1	19°11′19′′N 101°04′40′′E	1,142	*Capsicum annuum*	10	20 November 2012
Ban Luang, Nan	NN2	18°54′42′′N 100°27′49′′E	382	*C. annuum*	5	21 November 2012
Si Chiang Mai, Nong Khai	NK	17°57′23′′N 102°35′22′′E	172	*C. annuum*	4	26 August 2012
				C. annuum	2	30 October 2012
				S. melongena	3	26 August 2012
Pak Chom, Loei	LO3	18°01′18′′N 101°53′18′′E	209	*C. annuum*	4	31 October 2012
So Phisai, Bueng Kan	BK	18°04′42′′N 103°26′36′′E	167	*C. annuum*	7	2 October 2012
				S. melongena	1	2 October 2012
Kantharawichai, Maha Sarakham	MS1	16°14′58′′N 103°15′52′′E	166	*C. annuum*	2	18 July 2012
				C. annuum	4	26 July 2012
				C. annuum	3	17 August 2012
				S. torvum	2	15 July 2012
				S. torvum	3	1 August 2012
Mueang, Maha Sarakham	MS2	16°12′39′′N 103°18′24′′E	149	*S. trilobatum*	10	18 November 2012
Prang Ku, Si Sa Ket	SK	14°49′53′′N 104°03′38′′E	173	*C. annuum*	9	11 August 2012
				S. melongena	2	11 August 2012
Pa Tew, Yasothon	YT	15°49′35′′N 104°21′50′′E	140	*C. annuum*	4	30 August 2012
				S. torvum	2	30 August 2012

3.2 Identification of the fruit flies

Morphological criteria, includingwing morphology, setae, overall color and color patterns, shape and color of thoracic vittae, were employed for species identification. Only adult specimens were used for morphological identification because other stages (egg, larva and pupa) are very difficult or cannot be used to identify based on morphological characters (Houdt*et al*., 2010; Asokan*et al*., 2011). Adult specimens were identified to the species using the keys of Drew and Hancock (1994a) and Plant Health Australia (2011).

3.3 DNA extraction, polymerase chain reaction (PCR) and DNA sequencing

DNA samples were obtained from both adults and larvae. DNA was extracted using the GF-1 Tissue DNA Extraction Kit (Vivantis, Malaysia) followed the manufacturing protocol. DNA samples were stored at -20º C until use. The mitochondrial cytochrome oxidase I (COI) gene were amplified by polymerase chain reaction (PCR). PCR primers developed by Folmer *et al*. (1994) were used to amplify about 700-bp long target region of the COI gene. These same primers are considered the standard for DNA barcoding by Hebert *et al*. (2003a): LCO1490 (5′ GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAG GGTGACCAAAAAATCA-3′). A total volume of 50 µl PCRs reaction contained 2 µl of DNA template 2 µl of each primer (10 µM), 3 µl of 50 mM $MgCl_2$, 5 µl of 10X PCR buffer, 1.6 µl of 10 µM dNTPs, 0.4 µl of *Taq* DNA polymerase (5 U/ µl). Temperature profile as follow; an initial denaturation at 96 °C for 1 min followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1.5 min with the final extension at 72 °C for 7 min.

PCR products were checked by 1% argarose gel electrophoresis which contained 0.5 µg/ml of ethidium bromide (Sambrook *et al*., 1987). Five microliters of PCR products were mixed with 1 µl of 6× loading buffer. The mixture was carefully loaded into a well of the submerged (in 0.5× TBE buffer) agarose gel using a disposable micropipette tip. The outermost wells were loaded with a DNA marker (Invitrogen).

A voltage of 100 volts was applied for 30 – 40 minutes and the gel was visualized under ultraviolet (UV) light using UV – transilluminator. PCR products were purified by using the HiYield Gel/PCR DNA Fragments Extraction Kit (RBC BIOSCIENCE, Xindian City, Taiwan) followed the manufacturing protocol. The purified DNA was sequenced using the same primers as in the PCR by Macrogen sequencing service (Seoul, Korea).

3.4 Data analysis

3.4.1 Genetic diversity

Haplotype diversity and nucleotide diversity were calculated for each species using ARLEQUIN 3.5.1.2 (Excoffier and Lischer, 2010). The haplotype diversity (h) was calculated following the equation:

$$h = \frac{n}{n-1}\left(1 - \sum_{i=1}^{k} P_i^2\right)$$

with the variance:

$$V(h) = \frac{2}{n(n-1)}\left[2(n-2)\left[\sum_{i=1}^{k} P_i^3 - \left(\sum_{i=1}^{k} P_i^2\right)^2\right] + \sum_{i=1}^{k} P_i^2 - \left(\sum_{i=1}^{k} P_i^2\right)^2\right]$$

where n is the number of gene copies in the sample, k is the number of haplotypes and P_i is the sample frequency of the i-th haplotype (Nei, 1987; Arlequin 3.5 Manual).

The nucleotide diversity (π_n) was calculated using the equation:

$$\pi_n = \frac{\sum_{i=1}^{k}\sum_{j<1} P_i P_j \hat{d}_{ij}}{L}$$

with the variance:

$$V(\pi_n) = \frac{n+1}{3(n-1)L}\pi_n + \frac{2(n^2+n+3)}{9n(n-1)}\pi_n^2$$

where $\hat{d}_{ij}$ is the number of mutations having occurred since the divergence of haplotype i and j, k is the number of the haplotypes, P_i is the frequency of the haplotype i (Tajima, 1993; Arlequin 3.5 Manual).

Intraspecific and interspecific sequence divergences, based on Kimura's 2-Parameter (K2P) model, were calculated using MEGA 5 (Tamura *et al*., 2011).

3.4.2 Phylogenetic analysis

The neighbor – joining (NJ), maximum parsimony (MP), and Bayesian analysis were used to construct the phylogenetic relationships between species of the Tephritidae in northeastern Thailand. The NJ and MP trees were estimated in PAUP 4.0b10 (Swofford, 2002). For Bayesian analysis MRBAYES 3.0.4b (Huelsenbeck and Ronquist, 2001) was used. The Kimura's 2- Parameter (K2P) model was used for NJ and Bayesian analyses. The COI sequences of *Anastrepha luden* and *Ceratitis capitata* from GenBank under accession numbers DQ116207 and DQ116368 were use as outgroups.

3.4.3 Fruit fly and host plant species association

Chi – square goodness of fit test (χ^2-test) was used to analyze the relationship between fruit fly and host plant species. The present or absent of a species in a host plant was expressed on a binary scale (0 = species absent, 1 = species present). These tests were used to test the appearance of fruit fly species is random or non-random with respected to the host plant species:

H_O: The appearance of fruit fly species with host plant species is randomization.

H_A: The appearance of fruit fly species with host plant species is non –randomization.

The chi – square value (χ^2) was calculated using SPSS statistic package (SPSS Inc) by the equation:

$$\sum_{i=1}^{k} \frac{(O-E)^2}{E}$$

where$O$ is the frequency of the appearance of fruit fly species from observation, E is the frequency of the appearance of fruit fly species from expectation and k is the number of the fruit fly species.

The expected frequency is calculated by:

$$E = \big(F(Y_u) - F(Y_l)\big)K$$

where$F$ is the cumulative distribution function for the distribution being tested, Y_u is the upper limit for class i, Y_l is the lower limit for class i, and K = the sample size.

The χ^2 value can be compared to the chi-squared distribution to determine the goodness of fit. In order to determine the degrees of freedom of the chi-squared distribution, one takes the total number of observed frequencies and subtracts one. The test statistic follows, approximately, a chi-square distribution with $(k-c)$ degrees of freedom where k is the number of non-empty cells and c is the number of estimated parameters (including location and scale parameters and shape parameters) for the distribution + 1(Pearson, 1990).

3.4.4 Genetic variation at population level: a case study of *Bactrocera latifrons*in Thailand

Haplotype diversity (h) and nucleotide diversity (π) were calculated using Arlequin 3.5.1.2 (Excoffier and Lischer, 2010).The median joining (MJ) network (Bandelt *et al*., 1999) was used toestimate the genealogical relationships of the haplotypes. In addition to 93 sequences from this study, 11 sequences reported in Genbank (GenBank accession no. GQ154140-42 from Japan; GQ154144, GQ154146 from Tanzania; GQ154143, FJ009203 from Kenya; DQ116296-97, GQ154138 from Hawaii, U.S.A.; and FJ903490 from Malaysia) and one in the Barcode of Life Database

(www.boldsystems.org) (Sequence ID: MVTBI006-08 from Tanzania) were included in the haplotype network analysis. The MJ network was calculated using NETWORK ver. 4.6.1.0 (www.fluxus-engineering.com).

Genetic structure was estimated by population pairwise F_{ST}. The significance of test statistic was obtained by 1023 permutations. The sequential Bonferroni correction (Rice, 1989) was applied for the multiple tests. Analysis of molecular variance (AMOVA)was used to test the genetic differentiation among groups of populations from different host plants. Both population pairwise F_{ST} and AMOVA analysis were performed in Arlequin using the Kimura's 2-parameter model (K2P). A Mantel test (1967) was used to determine the relationship between genetic distance (F_{ST} from Arlequin) and geographical distance (km) to test an isolation-by-distance model. The Mantel test was implemented in IBD 1.52 (Bohonak, 2002) using 1000 randomizations.

Mismatch distribution was used to test the signature of population expansion. Populations that have undergone recentpast demographic expansion showna unimodal mismatch distribution (Roger and Harpending, 1992). The sum-of-squares deviation and Harpending's raggedness index (Harpending, 1994) were used to test deviation from the sudden expansion model. Mismatch distribution was estimated using Arlequin. If the mismatch distribution revealed a signature of population expansion, the expansion time was calculated from $\tau = 2ut$ (where $u = m_T\mu$ and m_T are the length of nucleotides sequences under study, μ is the mutation rate per nucleotide and t is the generation time; Roger and Harpending, 1992), assuming a divergence rate of 2.3% per million years for insectmt DNA (Brower, 1994).Fu's F_S test (Fu, 1997) and Tajima's D (Tajima, 1989) statistical tests were used to test the population equilibrium. Large negative values from these tests were expected for the demographics of population expansion.

Chapter 4

Results

4.1 Species diversity and host plants of tephritid fruit flies in northeastern Thailand

A total of 940 fruit flies represent nine species were obtained. These fruit flies infested 19 host plant species from 10 families (Table 4.1). All fruit flies belong to the genus *Bactrocera* Macquart which had been reported in previous studies including *B. caryeae* (Kapoor), *B. correcta* (Bezzi), *B. cucurbitae* (Coquillett), *B. dorsalis* (Hendel) complex, *B. invadens* (Drew), *B. latifrons* (Hendel), *B. occipitalis* (Bezzi), *B. philippinensis* (Drew and Hancock) and *B. tau* (Walker) complex (Fig. 4.1 – 4.12). According to the morphological variation based on thoracic vittae and abdomen color patterns, *B. correcta* was further divided into four forms (A, B, C, D) (Fig. 4.2 – 4.5).

Bactrocera correcta A and *B. invadens* were the most geographically widespread. These species was found in nine locations, *B. correcta* A was found in 13 locations whereas *B. invadens* was found in 19 locations (Table 4.1). *Bactrocera correcta* A was found in Chaiyaphum, Kalasin,Maha Sarakham, Roi Et, Si Sa Ket and Ubon Ratchathani provinces. *Bactrocera invadens* was found in Chaiyaphum, Kalasin, Loei, Maha Sarakham, Nakhon Ratchasima, Si Sa Ket and Ubon Ratchathani provinces. *Bactrocera occipitalis* was the most limited in geographic distribution. This species was found in only two locations one in Si Sa Ket and one in Ubon Ratchathani provinces.

A total of 19 host plant species from 10 families were found infested by fruit flies (Table 4.1 Figure 4.13).

Numbers of host plant species infested by fruit fly species range from two in *B. occipitalis* to eight in *B. correcta* A, *B. dorsalis* and *B. invadens* (Table 4.1). *Bactrocera correcta* infested highest number of host plants. This species was found in nine host plant species from eight families. *Bactrocera correcta* A infested eight host plant species from seven families including *Artocarpus lanceifolius* Roxb. (Moraceae), *Averrhoa carambola* L. (Oxalidaceae), *Diospyros decandra* Lour. (Ebenaceae), *Irvingia malayana* Oliv. Ex A. Benn. (Irvingiaceae), *Mangifera indica* L.

(Anacardiaceae), *Psidium guajava* L. (Myrtaceae), *Syzygium sp.* (Myrtaceae) and *Ziziphus mauritiana* Lam. (Rhamnaceae).

Bactrocera correcta B infested six host plants from five families, *B. correcta* C and *B. correcta* D infested four host plants from three families. *Bactrocera correcta* B shared host plants with *B. correcta* A except *Artocarpus lanceifolius* Roxb. (Moraceae), *Irvingia malayana* Oliv. Ex A. Benn. (Irvingiaceae) and *M. indica* L. (Anacardiaceae), which found infested only by *B. correcta* A. In contrast, one individual of *B. correcta* B was found infested *Annona squamosa* L. (Annonaceae) where *B. correcta* A was not found in this plant. *Bactrocera correcta* C was detected in *A. carambola* L. (Oxalidaceae), *P. guajava* L. (Myrtaceae), *Syzygium*sp. (Myrtaceae) and *Z. mauritiana* Lam. (Rhamnaceae) while *B. correcta* D was found in *D. decandra* Lour. (Ebenaceae), *P. guajava* L. (Myrtaceae), *Syzygium*sp. (Myrtaceae) and *Z. Mauritiana* Lam. (Rhamnaceae).

Bactrocera caryeae was detected in five host plants from five families including *A. squamosa* L. (Annonaceae), *A. carambola* L. (Oxalidaceae), *M. indica* L. (Anacardiaceae), *P. guajava* L. (Myrtaceae) and *Z. mauritiana* Lam. (Rhamnaceae).

Bactrocera cucurbitae, *B. latifrons*, *B. occipitalis* and *B. tau*were found in limited host plants. *Bactrocera cucurbitae* and *B. tau* were found only in plants family Cucurbitaceae. *Bactrocera cucurbitae* was found in three host plant species including *Coccinia grandis* L., *Cucumis sativas* L. and *Trichosanthes cucumerina* L. while *B. tau* was found in *Cucurbita moschata* Decne., *Momordica cochinchinensis* Lour. and *Trichosanthe stricuspidata* Lour..

Bactrocera latifrons was found in four host plant species all were from family Solanaceae including *Capsicum annuum* L., *Solanum melongena* L., *Solanum trilobatum* L. and *Solanum torvum* Sw. *Bactrocerao ccipitalis* was detected in limit numbers only seven individuals were obtained in this study. This species was found infested *A. squamosa* L. (Annonaceae) and *I. malayana* Oliv. Ex A. Benn. (Irvingiaceae) (Table 4.1).

Bactrocera caryeae, *B. correcta*, *B. dorsalis*, *B. invadens* and *B. philippinensis* shared the major host plants. For examples, *B. dorsalis* and *B. invadens* shared all host plant species. Both species found infested eight species from seven families, including *A. squamosa* L. (Annonaceae), *A. carambola* L. (Oxalidaceae),

D. decandra Lour. (Ebenaceae), *I. malayana* Oliv. Ex A. Benn. (Irvingiaceae), *M. indica* L. (Anacardiaceae),*P. guajava* L. (Myrtaceae), *Syzygium*sp. (Myrtaceae)and *Z. mauritiana* Lam. (Rhamnaceae). *Bactrocera philippinensis* shared six host plant species from five families with *B. dorsalis* and *B. invadens*.

Although many host plants were shared by several fruit fly species, some host plants did not. Analysis of the association between host plants and fruit fly species revealed that the fruit flies which are polyphagous are not specifically associated to host plants including *B. correcta* A (χ^2 = 0.474, *P* = 0.491), *B. correcta* B (χ^2 = 2.579, *P* = 0.108), *B. dorsalis* (χ^2 = 0.474, *P* = 0.491), *B. invadens* (χ^2 = 0.474, *P* = 0.491) and *B. philippinensis* (χ^2 = 2.579, *P* = 0.108) (Table 4.2). In contrast, fruit fly species which are oligophagous shown some degree of association to host plant species including *B. caryeae* (χ^2 = 4.263, *P* = 0.039), *B. correcta*C (χ^2 = 6.368, *P* = 0.012), *B. correcta*D (χ^2 = 6.368, *P* = 0.012),*B. cucurbitae* (χ^2 = 8.895, *P* = 0.003), *B. latifrons* (χ^2 = 6.368, *P* = 0.012),*B. occipitalis* (χ^2 = 11.842, *P* = 0.001) and *B. tau* (χ^2 = 8.895, *P* = 0.003) (Table 4.2).

Table 4.1 Fruit fly species and their host plant species in northeastern Thailand. The details of location code were shown in Table 3.1.

Fruit fly species	Host plant Family	Host plant species	Location code
Bactrocera caryeae	Anacardiaceae	Mango (*Mangifera indica* L.)	MS1 (3)
	Annonaceae	Sugar apple (*Annona squamosa* L.)	NR (2)
	Myrtaceae	Guava (*Psidium guajava* L.)	MS1 (7) UR (1)
	Oxalidaceae	Carambola/ star fruit (*Averrhoa carambola* L.)	CP1 (2)
	Rhamnaceae	Jujube (*Ziziphus mauritiana* Lam.)	KS1 (2) SK (16)
Total	**5 families**	**5 species**	**n = 33**
Bactrocera correcta A	Anacardiaceae	Mango (*Mangifera indica* L.)	RE (1)
	Ebenaceae	Golden apple (*Diospyros decandra* Lour.)	SK (4)
	Irvingiaceae	Kayu (*Irvingia malayana* Oliv. Ex A. Benn.)	MS3 (3) SK (10)
	Moraceae	Keledang (*Artocarpus lanceifolius* Roxb.)	CP2 (1)
	Myrtaceae	Guava (*Psidium guajava* L.) Rose apple (*Syzygium*sp.)	MS1 (11) UR (3) CP1 (10) MS2 (10)
	Oxalidaceae	Carambola/ star fruit (*Averrhoa carambola* L.)	CP1 (2) SK (7)

n, represent number of adult fruit fly.

Table 4.1 (Continued).

Fruit fly species	Host plant Family	Host plant species	Location code
Bactrocera correcta A	Rhamnaceae	Jujube (*Ziziphus mauritiana* Lam.)	KS1 (33) SK (61)
Total	**7 families**	**8 species**	**n = 156**
Bactrocera correcta B	Annonaceae	Sugar apple (*Annona squamosa* L.)	MS1 (1)
	Ebenaceae	Golden apple (*Diospyros decandra* Lour.)	SK (1)
	Myrtaceae	Guava (*Psidium guajava* L.)	MS1 (4) UR (13)
		Rose apple (*Syzygium*sp.)	MS2 (8)
	Oxalidaceae	Carambola/ star fruit (*Averrhoa carambola* L.)	SK (12)
	Rhamnaceae	Jujube (*Ziziphus mauritiana* Lam.)	KS1 (18) SK (9)
Total	**5 families**	**6 species**	**n = 68**
Bactrocera correcta C	Myrtaceae	Guava (*Psidium guajava* L.)	MS1 (3)
		Rose apple (*Syzygium*sp.)	MS2 (4)
	Oxalidaceae	Carambola/ star fruit (*Averrhoa carambola* L.)	SK (2)
	Rhamnaceae	Jujube (*Ziziphus mauritiana* Lam.)	KS1 (3) SK (1)
Total	**3 families**	**4 species**	**n = 13**

n, represent number of adult fruit fly.

Table 4.1 (Continued).

Fruit fly species	Host plant Family	Host plant species	Location code
Bactrocera correcta			
D	Ebenaceae	Golden apple (*Diospyros decandra* Lour.)	SK (4)
	Myrtaceae	Guava (*Psidium guajava* L.)	MS1 (13)
		Rose apple (*Syzygium* sp.)	MS2 (4)
	Rhamnaceae	Jujube (*Ziziphu smauritiana* Lam.)	KS1 (15) SK (24)
Total	**3 families**	**4 species**	**n = 60**
Bactrocera cucurbitae	Cucurbitaceae	Ivy gourd (*Coccinia grandis* L.)	MS1 (98) MS2 (18) SK (30)
		Japanese Cucumber (*Cucumis sativas* L.)	MS1 (12)
		Snake gourd (*Trichosanthes cucumerina* L.)	YT (1)
Total	**1 family**	**3 species**	**n = 159**

n, represent number of adult fruit fly.

Table 4.1 (Continued).

Fruit fly species	Host plant Family	Host plant species	Location code
Bactrocera dorsalis	Anacardiaceae	Mango (*Mangifera indica* L.)	MS1 (5) NR (6) RE (2)
	Annonaceae	Sugar apple (*Annona squamosa* L.)	MS1 (2) NR (2)
	Ebenaceae	Golden apple (*Diospyros decandra* Lour.)	SK (10)
	Irvingiaceae	Kayu (*Irvingia malayana* Oliv. Ex A. Benn.)	MS3 (1) SK (4)
	Myrtaceae	Guava (*Psidium guajava* L.)	MS1 (1) UR (14)
		Rose apple (*Syzygium* sp.)	CP1 (1)
	Oxalidaceae	Carambola/ star fruit (*Averrhoa carambola* L.)	CP1 (1) MS1 (4)
	Rhamnaceae	Jujube (*Ziziphus mauritiana* Lam.)	SK (8)
Total	**7 families**	**8 species**	**n = 61**
Bactrocera invadens	Anacardiaceae	Mango (*Mangifera indica* L.)	MS1 (5) NR (3)
	Annonaceae	Sugar apple (*Annona squamosa* L.)	MS1 (3) NR (4) UR (4)
	Ebenaceae	Golden apple (*Diospyros decandra* Lour.)	SK (17)

n, represent number of adult fruit fly.

Table 4.1 (Continued).

Fruit fly species	Host plant Family	Host plant species	Location code
Bactrocera invadens	Irvingiaceae	Kayu (*Irvingia malayana*	KS2 (2)
		Oliv. Ex A. Benn.)	MS3 (5)
			SK (4)
	Myrtaceae	Guava	MS1 (22)
		(*Psidium guajava* L.)	UR (47)
		Rose apple	CP1 (13)
		(*Syzygium* sp.)	UR (1)
	Oxalidaceae	Carambola/ star fruit	CP1 (9)
		(*Averrhoa carambola* L.)	LO1 (3)
			MS1 (15)
			SK (4)
	Rhamnaceae	Jujube (*Ziziphus*	KS1 (1)
		mauritiana Lam.)	SK (16)
Total	**7 families**	**8 species**	**n = 178**
Bactrocera latifrons	Solanaceae	Chilli	BK (26)
		(*Capsicum annuum* L.)	MS1 (15)
			LO3 (4)
			NK (13)
			SK (33)
			YT (5)
		Eggplant	BK (1)
		(*Solanum melongena* L.)	RE (1)
			SK (2)
			NK (3)
		Solanum trilobatum L.	MS2 (15)
		Turkey berry	MS1 (15)
		(*Solanum torvum* Sw.)	YT (2)

n, represent number of adult fruit fly.

Table 4.1 (Continued).

Fruit fly species	Host plant Family	Host plant species	Location code
Total	**1 family**	**4 host plant species**	**n = 135**
Bactrocera occipitalis	Annonaceae	Sugar apple (*Annona squamosa* L.)	UR (6)
	Irvingiaceae	Kayu (*Irvingia malayana* Oliv. Ex A. Benn.)	SK (1)
Total	**2 families**	**2 species**	**n = 7**
Bactrocera philippinensis	Anacardiaceae	Mango (*Mangifera indica* L.)	MS1 (4) NR (6)
	Annonaceae	Sugar apple (*Annona squamosa* L.)	MS1 (6) NR (1)
	Irvingiaceae	Kayu (*Irvingia malayana* Oliv. Ex A. Benn.)	MS3 (2) SK (1)
	Myrtaceae	Guava (*Psidium guajava* L.)	MS1 (3) UR (9)
		Rose apple (*Syzygium*sp.)	CP1 (3)
	Oxalidaceae	Carambola/ star fruit (*Averrhoa carambola* L.)	LO1 (1) MS1 (7) SK (5)
Total	**5 families**	**6 species**	**n = 48**

n, represent number of adult fruit fly.

Table 4.1 (Continued).

Fruit fly species	Host plant Family	Host plant species	Location code
Bactrocera tau	Cucurbitaceae	Baby Jackfruit (*Momordica cochinchinensis* Lour.)	KK (5)
		Pumpkin (*Cucurbita moschata* Decne.)	LO2 (14)
		Trichosanthes tricuspidata Lour.	LO2 (3)
Total	**1 family**	**3 species**	**n = 22**
Total	**10 families**	**19 species**	**n = 940**

n, represent number of adult fruit fly.

Table 4.2 Chi – square goodness of fit test (χ^2-test) to reveal the relationship between fruit fly and host plants species in the northeastern Thailand.

Fruit fly species	χ^2 value	*P* - value	Result[a]
Bactrocera caryeae	4.263	0.039*	non - random
Bactrocera correcta	0.053	0.819	random
Bactrocera correcta A	0.474	0.491	random
Bactrocera correcta B	2.579	0.108	random
Bactrocera correcta C	6.368	0.012*	non - random
Bactrocera correcta D	6.368	0.012*	non - random
Bactrocera cucurbitae	8.895	0.003*	non - random
Bactrocera dorsalis	0.474	0.491	random
Bactrocera invadens	0.474	0.491	random
Bactrocera latifrons	6.368	0.012*	non - random
Bactrocera occipitalis	11.842	0.001*	non - random
Bactrocera philippinensis	2.579	0.108	random
Bactrocera tau	8.895	0.003*	non - random

*$P < 0.05$.

[a] random = The appearance of fruit fly species with host plant species is randomization. non - random = The appearance of fruit fly species with host plant species is non - randomization.

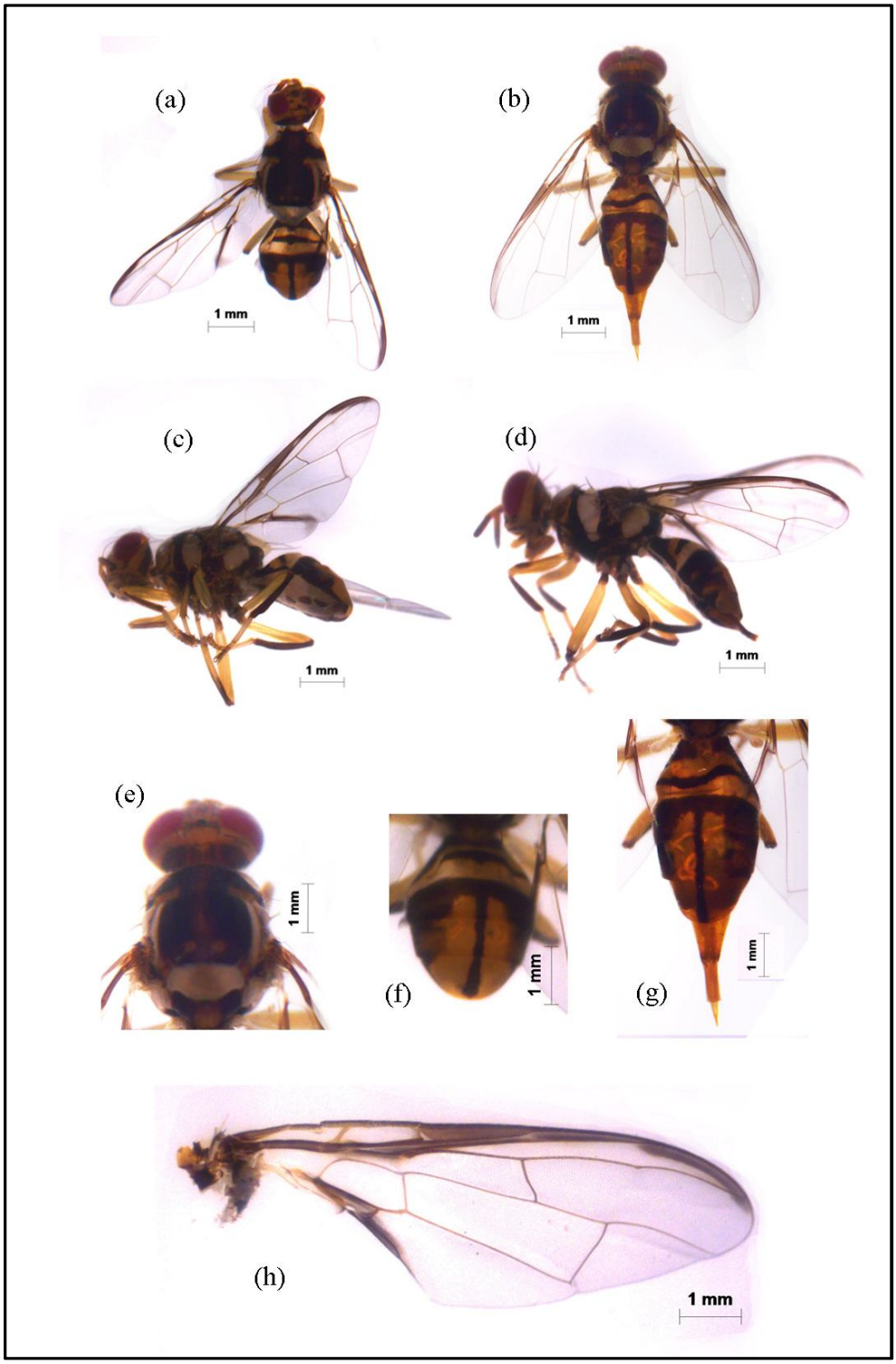


Figure 4.1 *Bactrocera caryeae*: (a) male (dorsal view), (b) female (dorsal view), (c) male (lateral view), (d) female (lateral view), (e) thorax, (f) male's abdomen, (g) female's abdomen, and(h) right wing

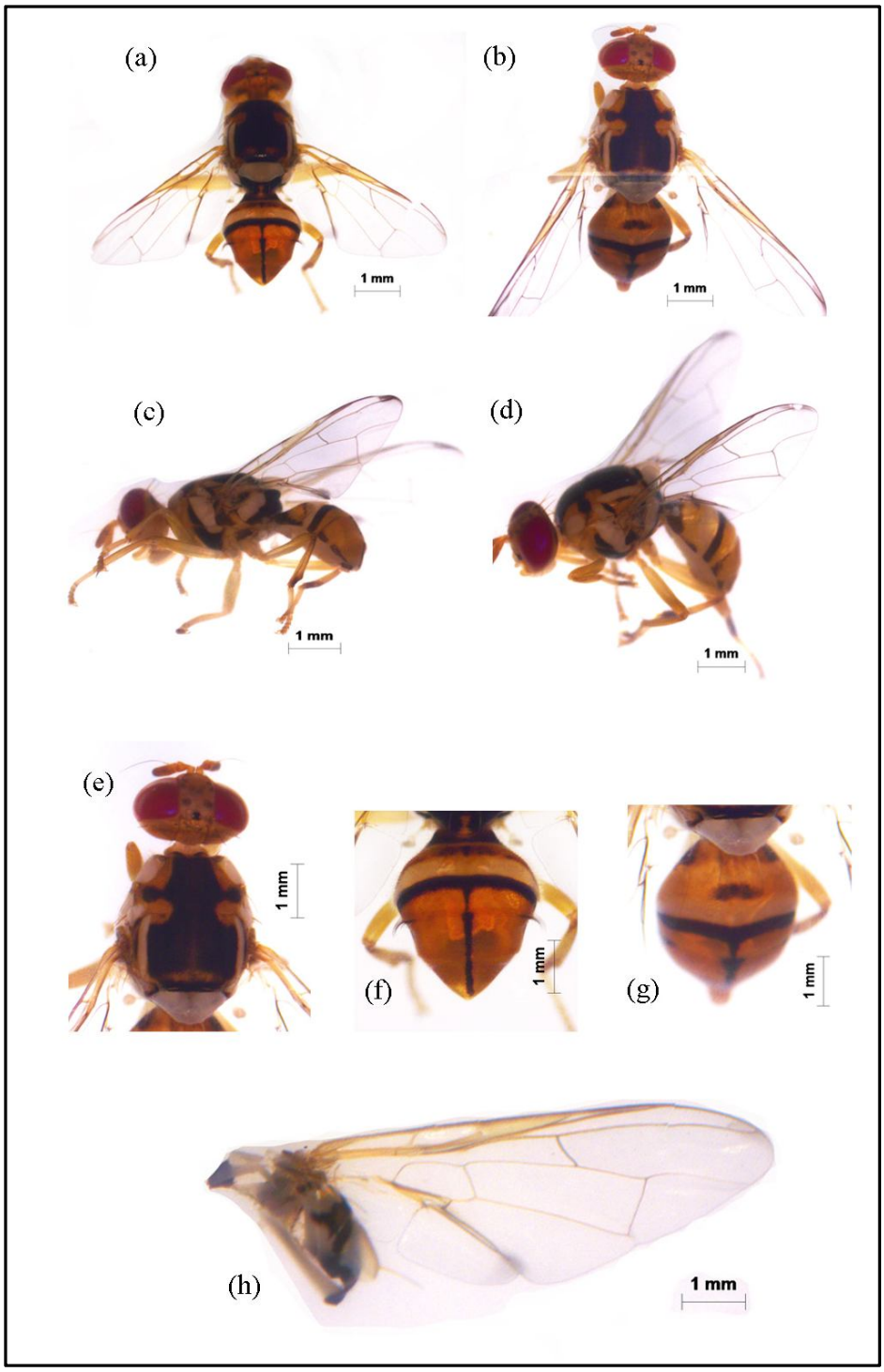


Figure 4.2 *Bactrocera correcta* form A: (a) male (dorsal view), (b) female (dorsal view), (c) male (lateral view), (d) female (lateral view), (e) thorax, (f) male's abdomen, (g) female's abdomen, and(h) right wing

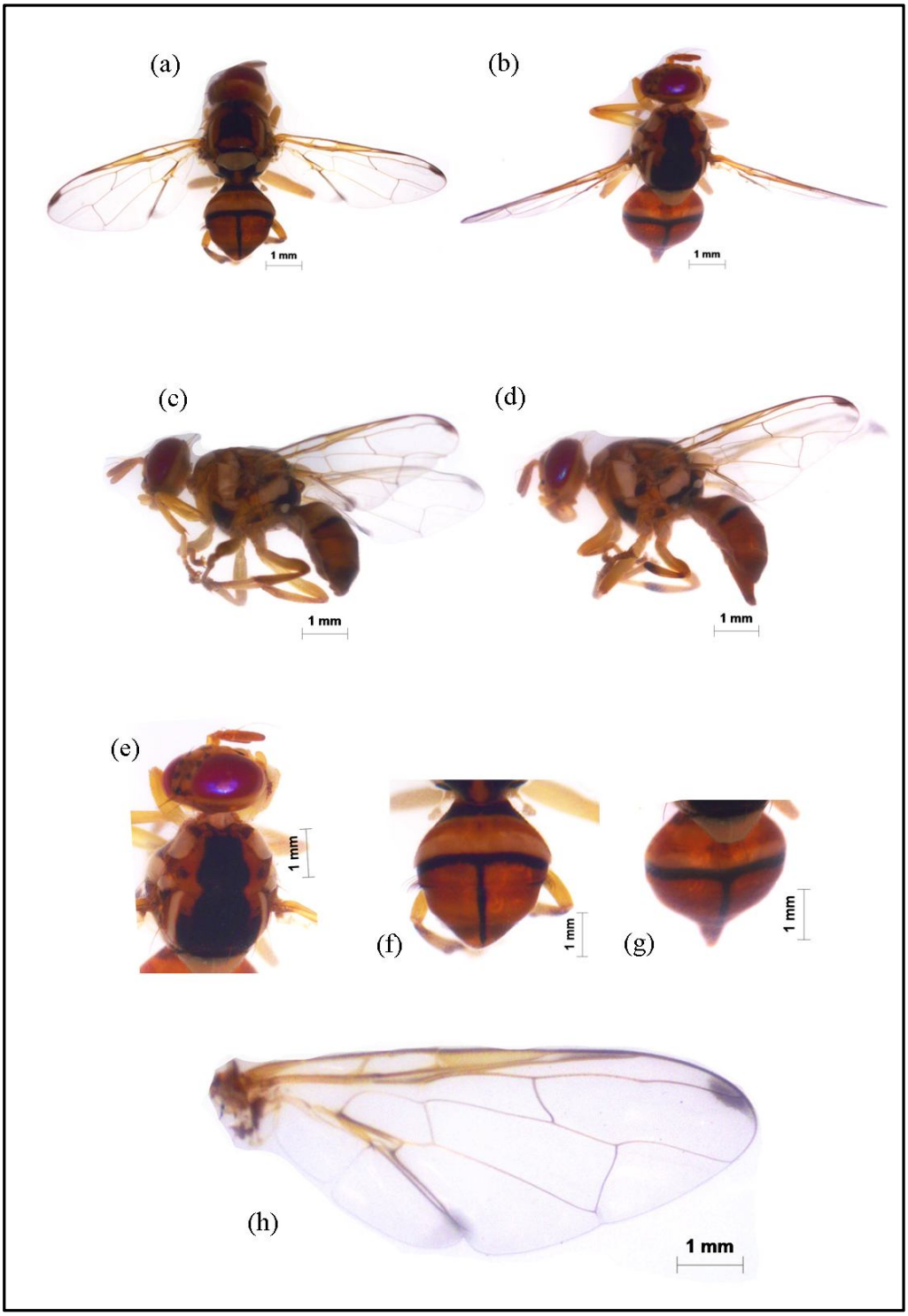


Figure 4.3 *Bactrocera correcta* form B: (a) male (dorsal view), (b) female (dorsal view), (c) male (lateral view), (d) female (lateral view), (e) thorax, (f) male's abdomen, (g) female's abdomen, and(h) right wing

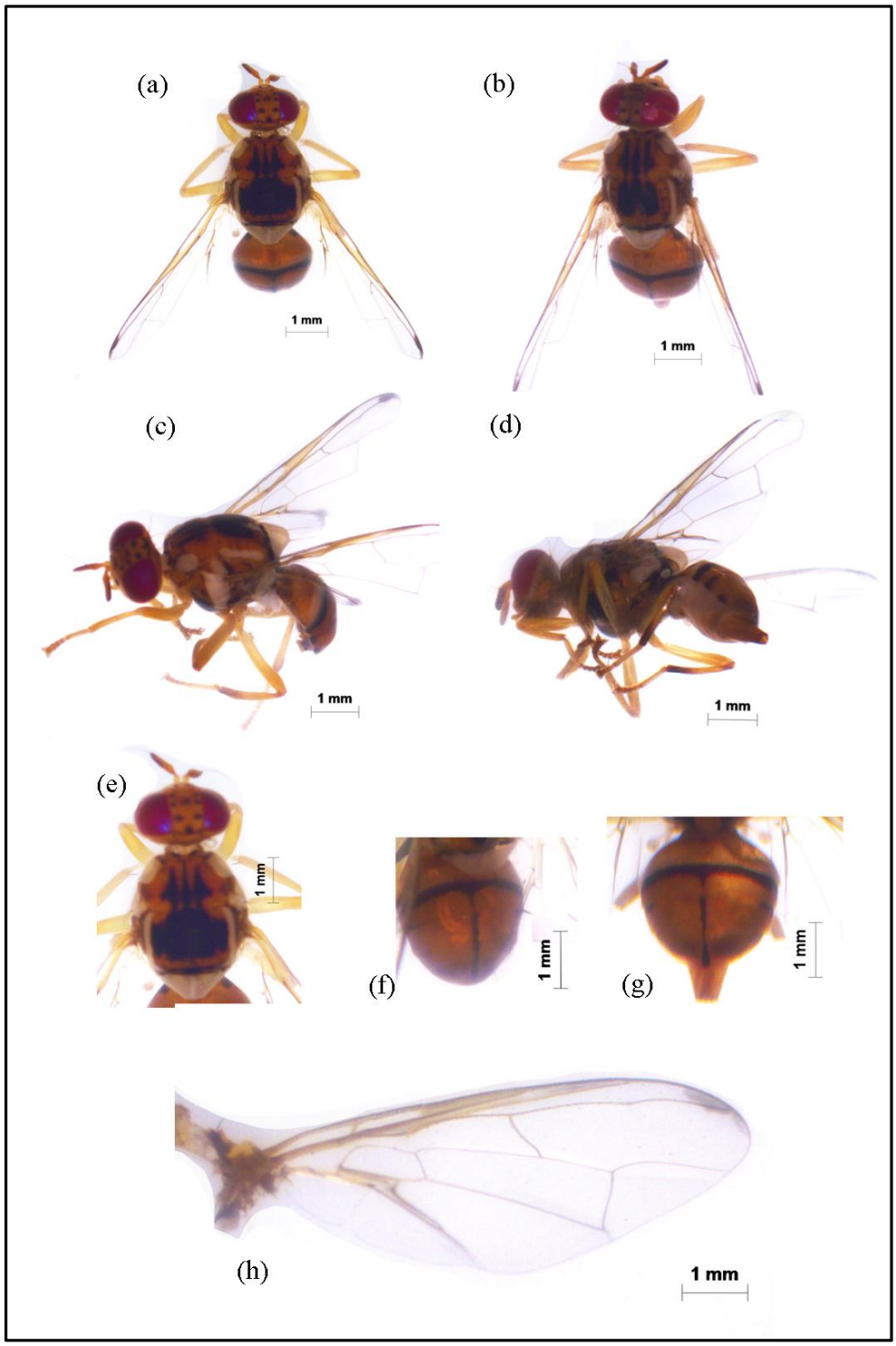


Figure 4.4 *Bactrocera correcta* form C: (a) male (dorsal view), (b) female (dorsal view) (c) male (lateral view), (d) female (lateral view), (e) thorax, (f) male's abdomen, (g) female's abdomen, and(h) right wing

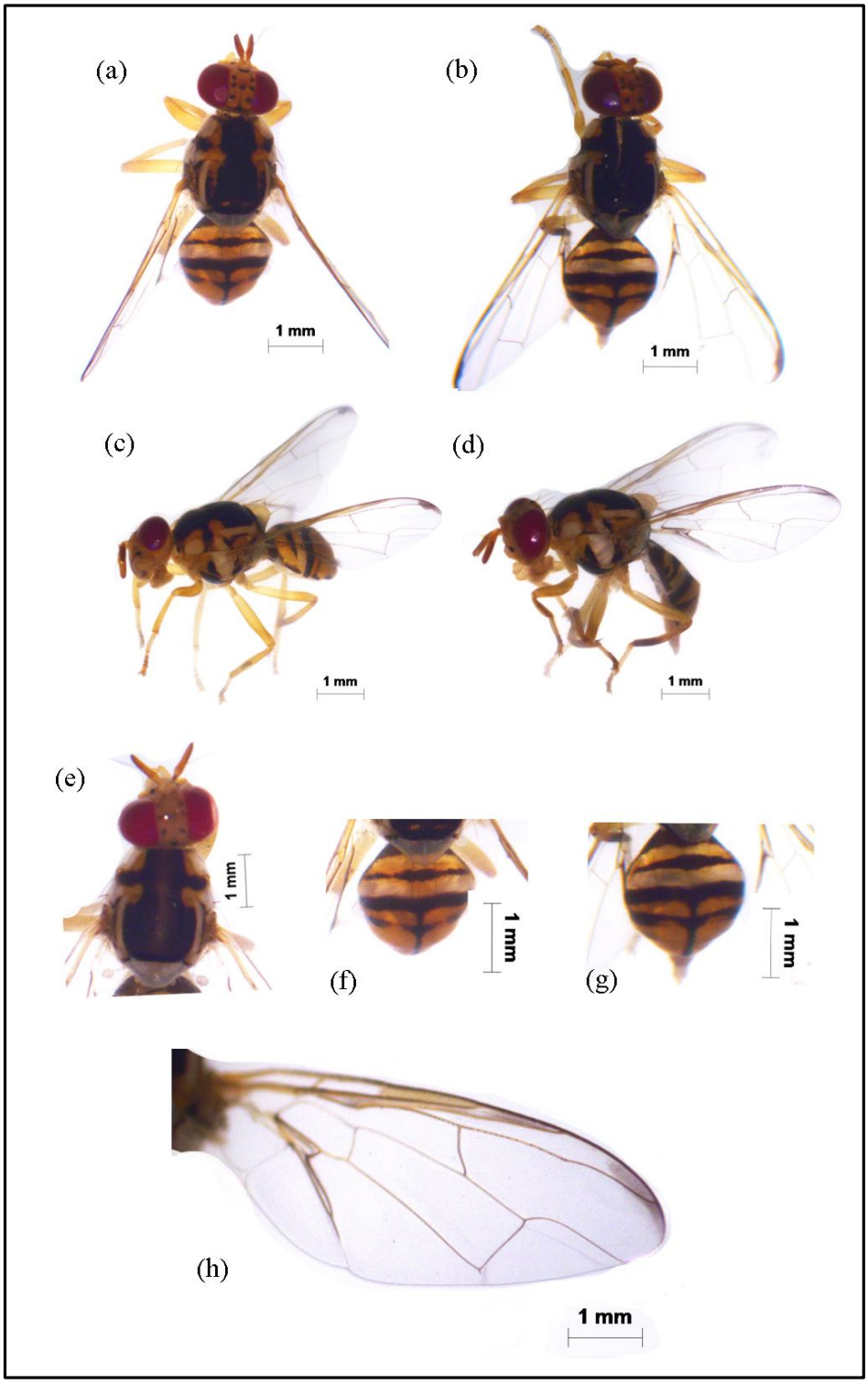


Figure 4.5 *Bactrocera correcta* form D: (a) male (dorsal view), (b) female (dorsal view), (c) male (lateral view), (d) female (lateral view), (e) thorax, (f) male's abdomen, (g) female's abdomen, and(h) right wing

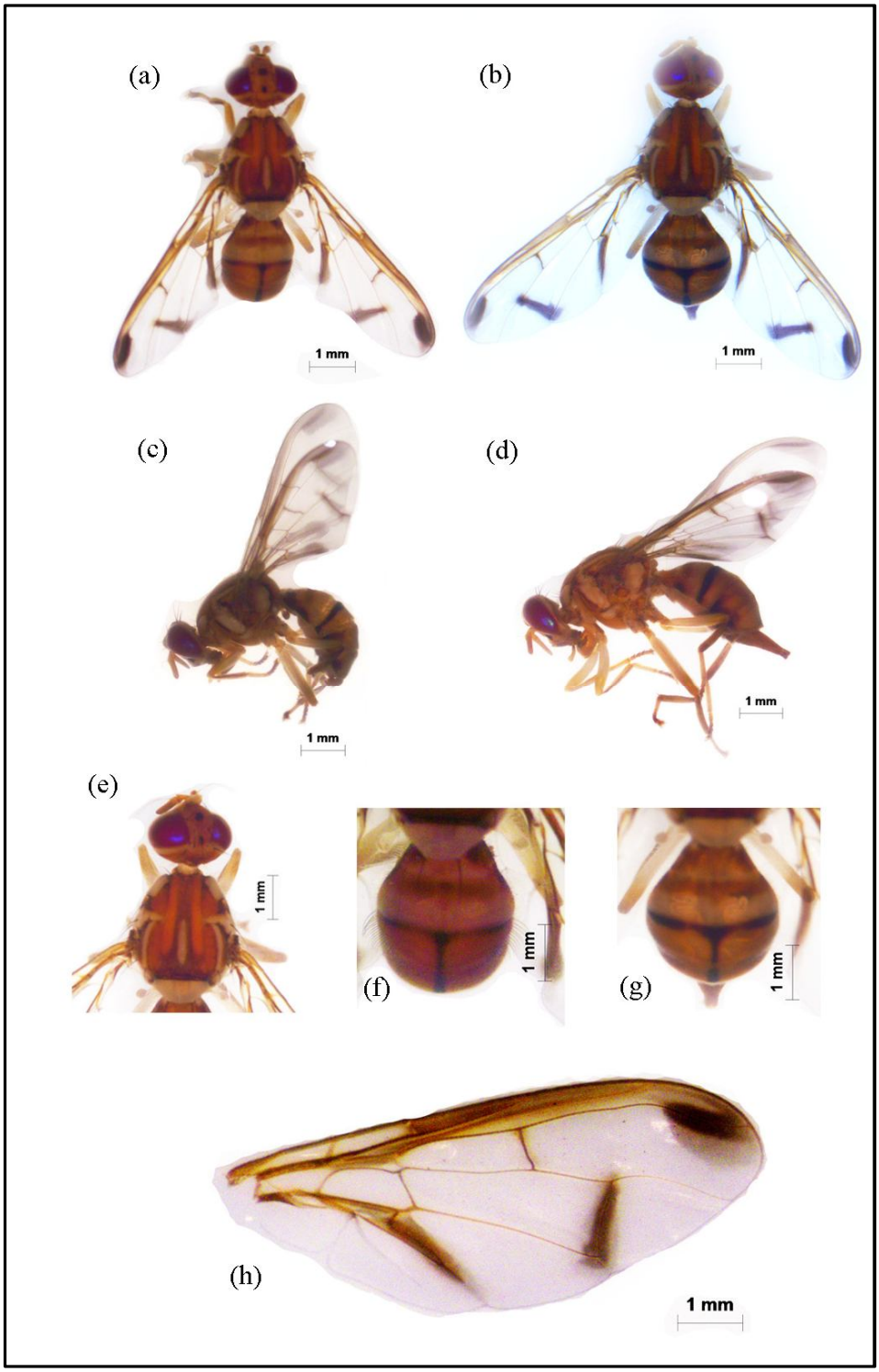


Figure 4.6 *Bactrocera cucurbitae*: (a) male (dorsal view), (b) female (dorsal view), (c) male (lateral view), (d) female (lateral view), (e) thorax, (f) male's abdomen, (g) female's abdomen, and(h) right wing

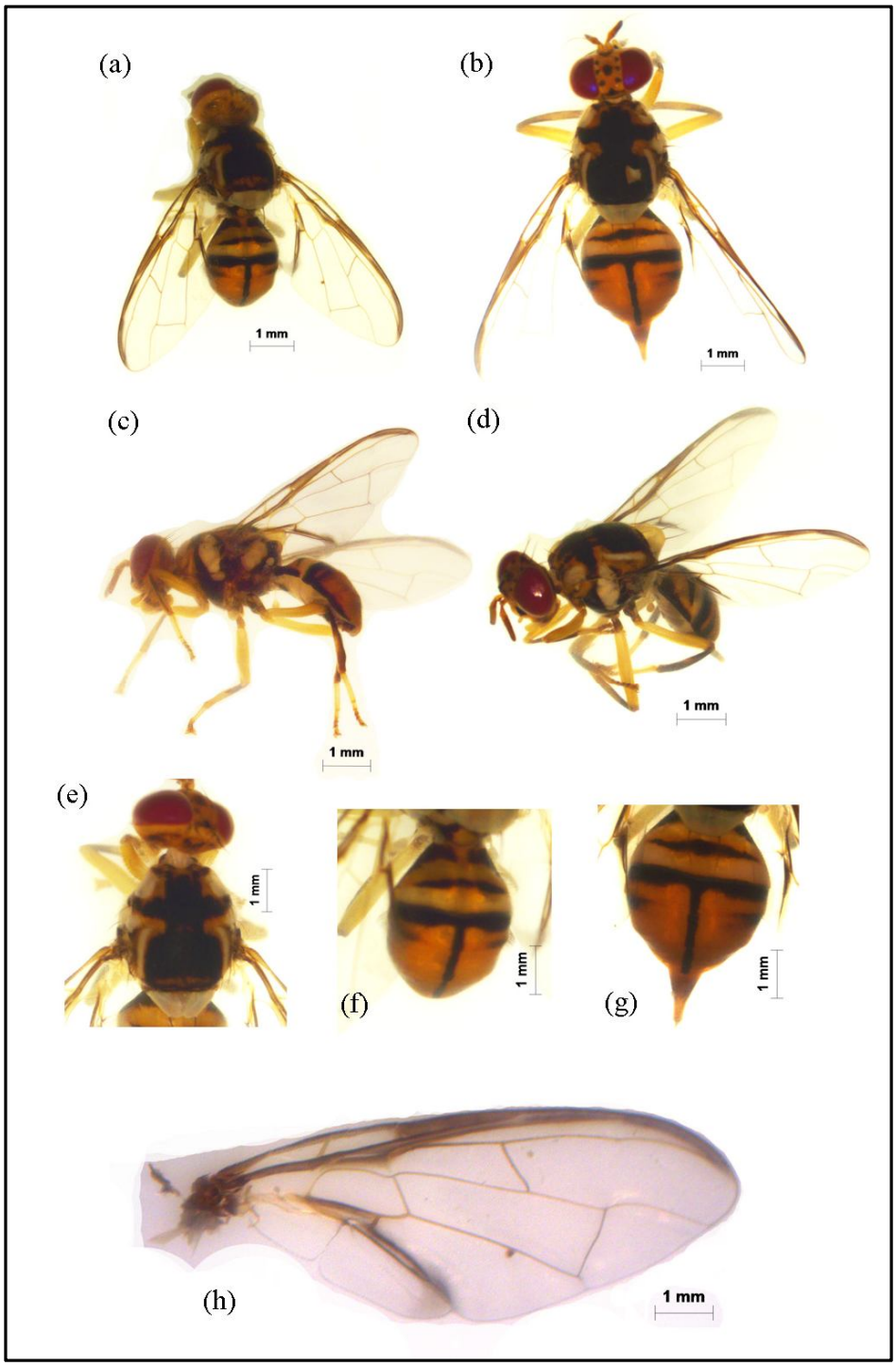


Figure 4.7 *Bactrocera dorsalis*: (a) male (dorsal view), (b) female (dorsal view), (c) male (lateral view), (d) female (lateral view), (e) thorax, (f) male's abdomen, (g) female's abdomen, and(h) right wing

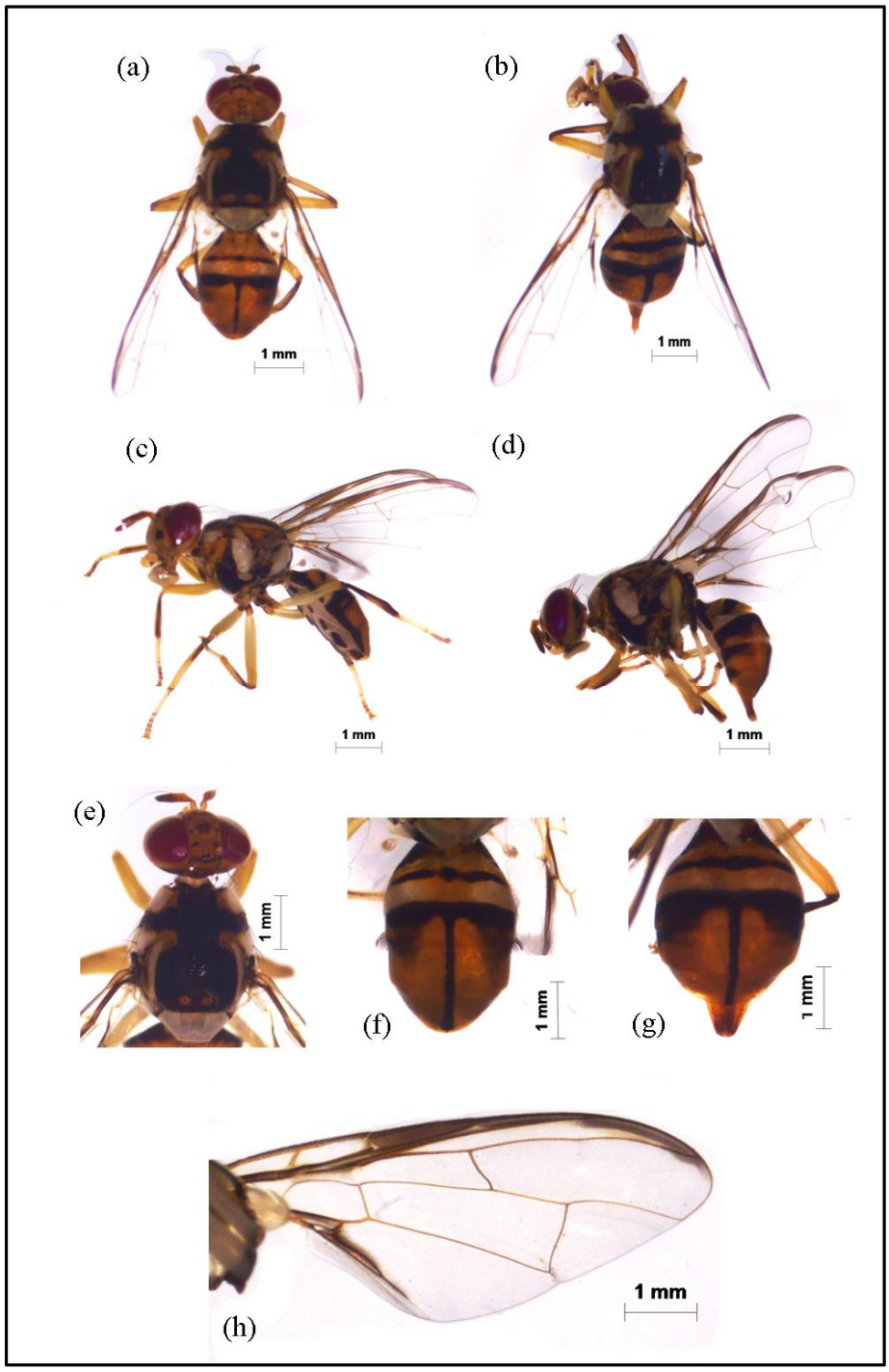


Figure 4.8 *Bactrocera invadens*: (a) male (dorsal view), (b) female (dorsal view), (c) male (lateral view), (d) female (lateral view), (e) thorax, (f) male's abdomen, (g) female's abdomen, and(h) right wing

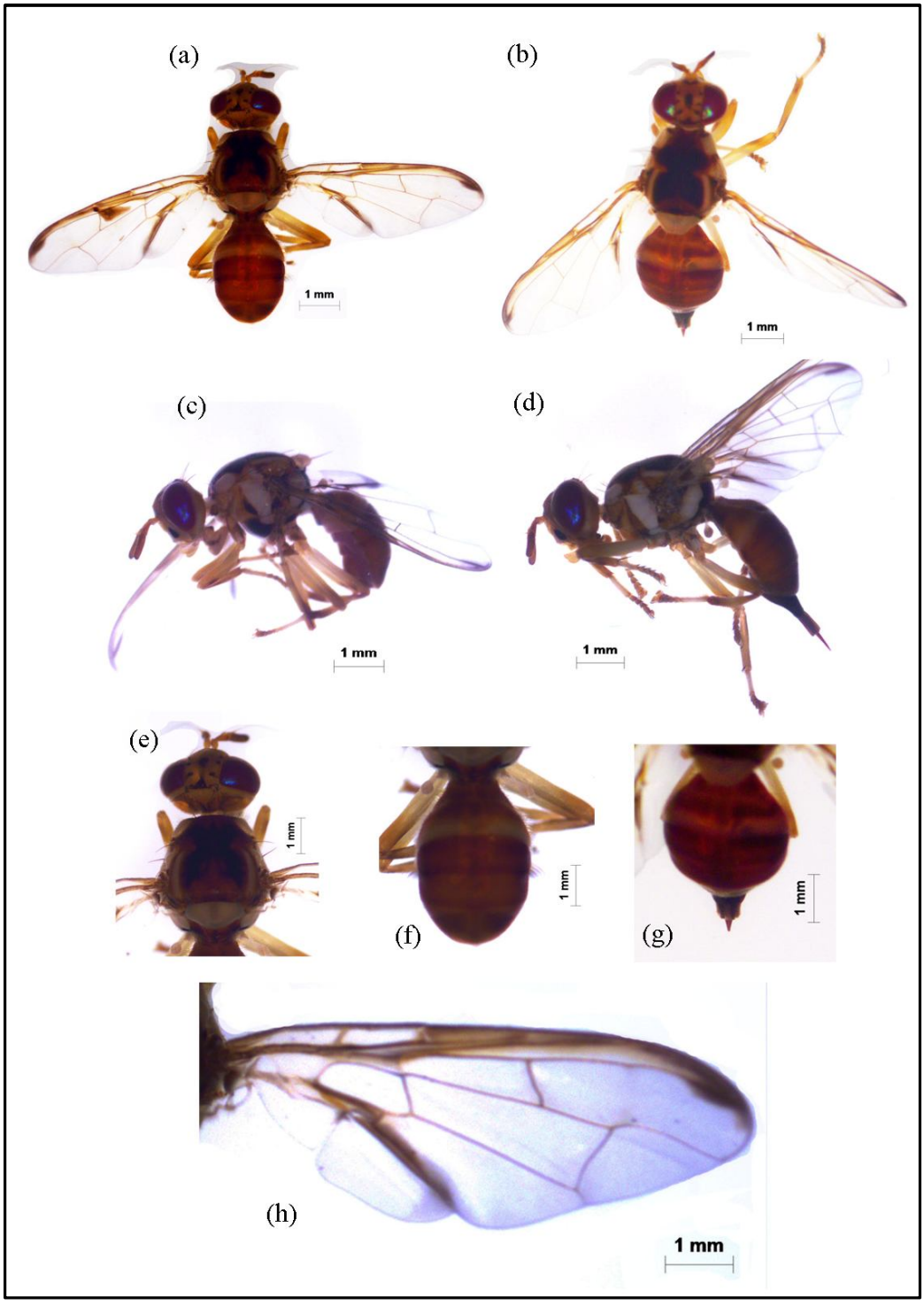


Figure 4.9 *Bactrocera latifrons*: (a) male (dorsal view), (b) female (dorsal view), (c) male (lateral view), (d) female (lateral view), (e) thorax, (f) male's abdomen, (g) female's abdomen, and(h) right wing

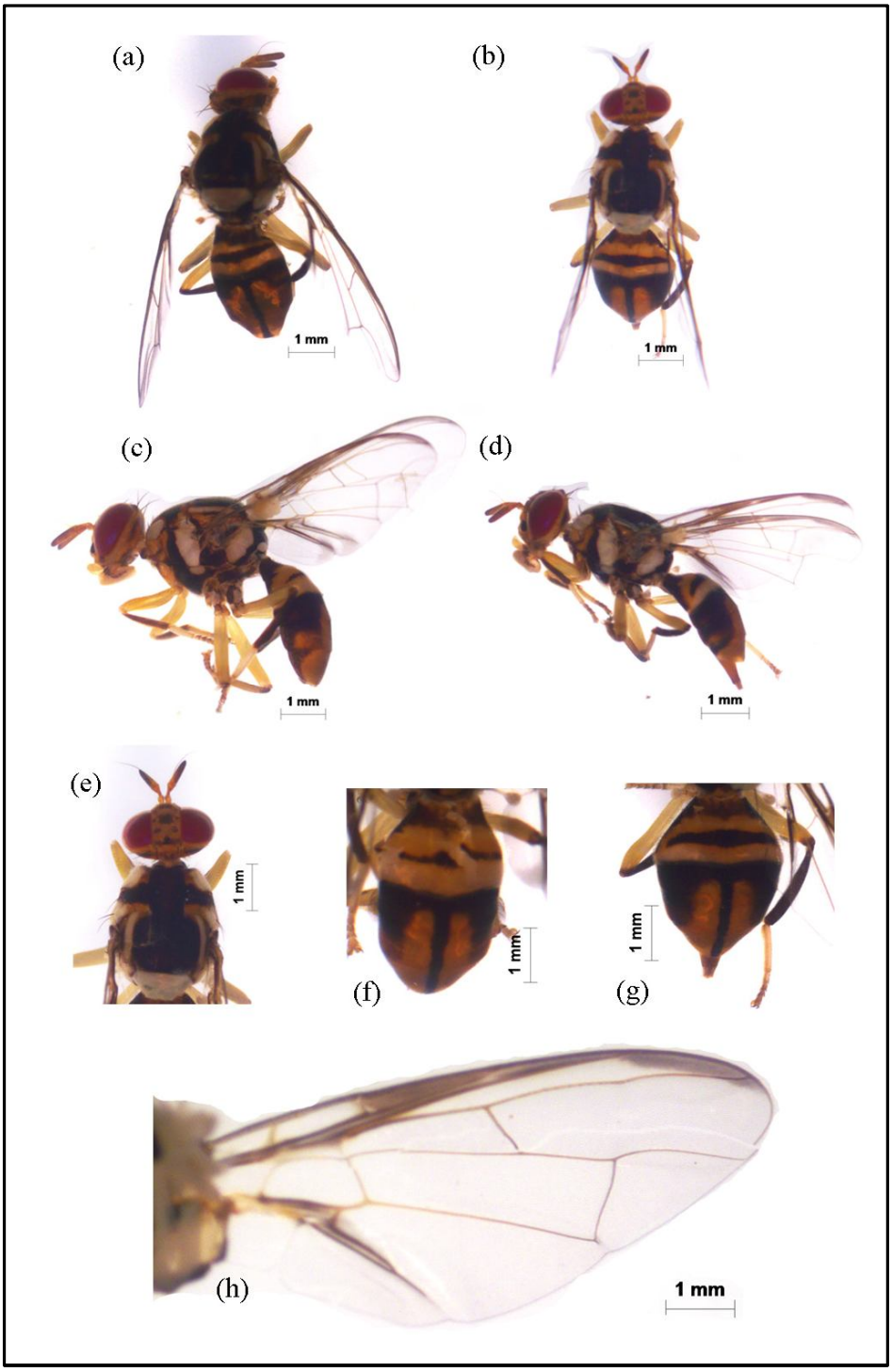


Figure 4.10 *Bactrocera occipitalis*: (a) male (dorsal view), (b) female (dorsal view), (c) male (lateral view), (d) female (lateral view), (e) thorax, (f) male's abdomen, (g) female's abdomen, and(h) right wing

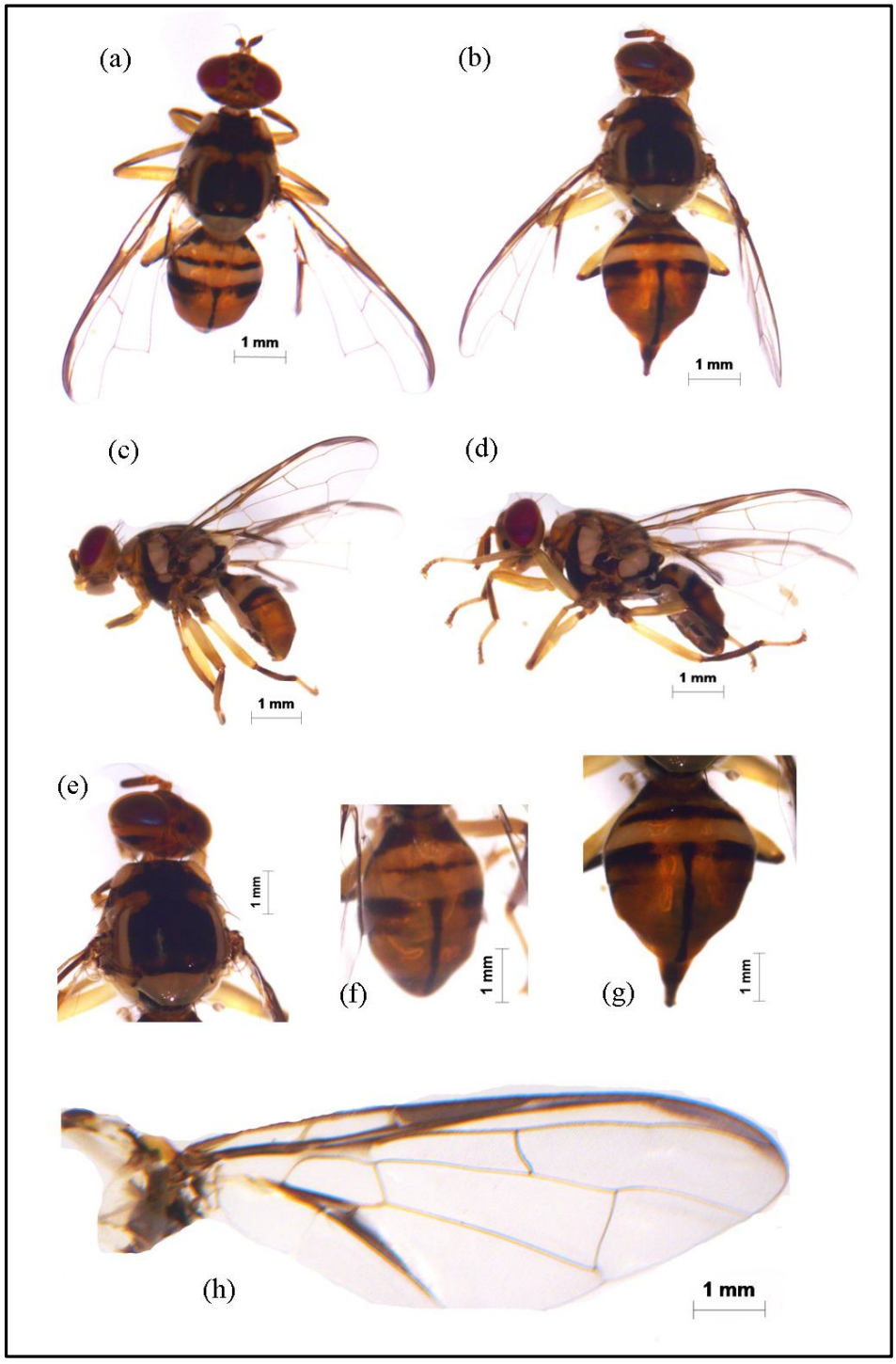


Figure 4.11 *Bactrocera philippinensis*: (a) male (dorsal view), (b) female (dorsal view), (c) male (lateral view), (d) female (lateral view), (e) thorax, (f) male's abdomen, (g) female's abdomen, and(h) right wing

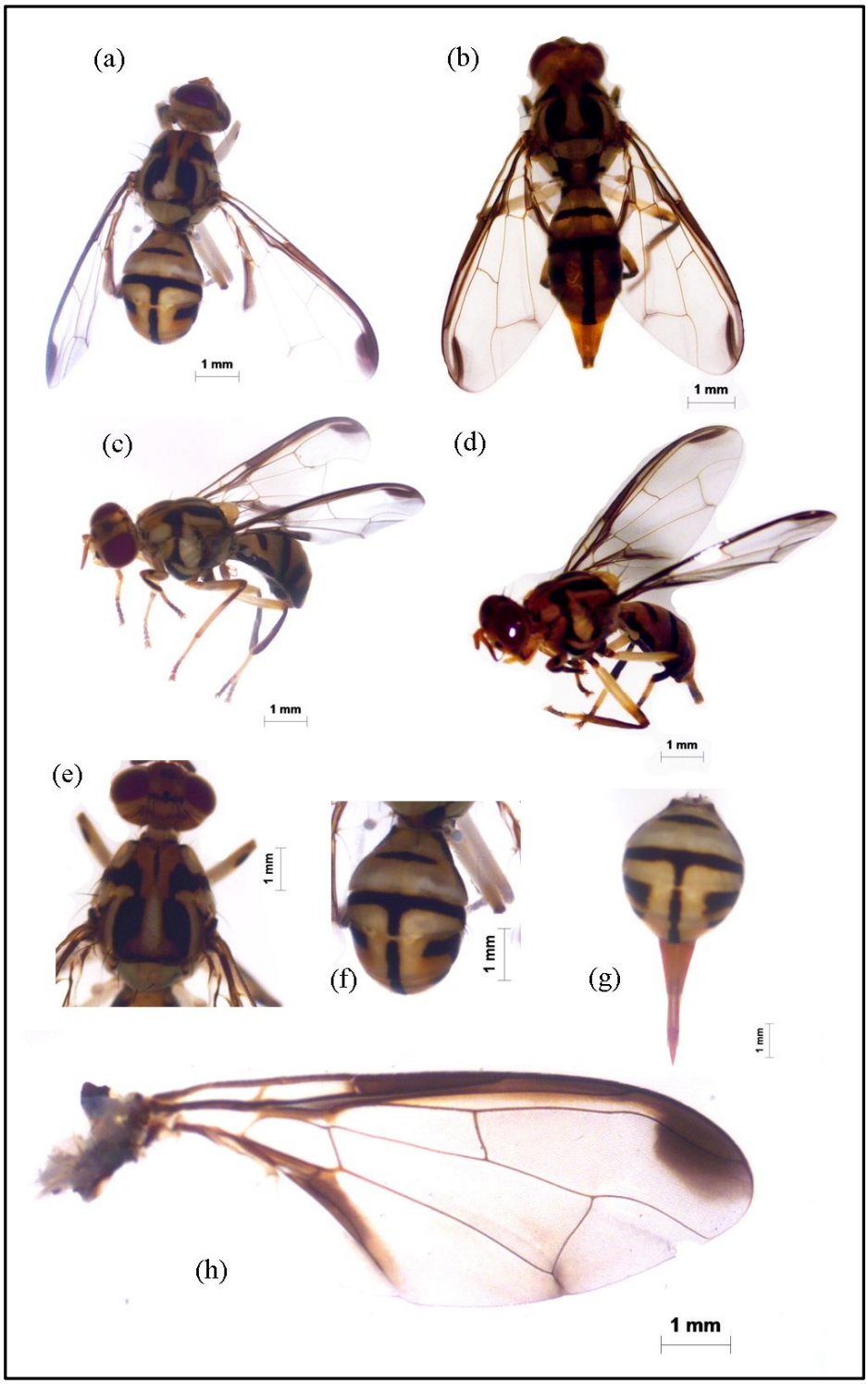


Figure 4.12 *Bactrocera tau*: (a) male (dorsal view), (b) female (dorsal view), (c) male (lateral view), (d) female (lateral view), (e) thorax, (f) male's abdomen, (g) female's abdomen, and(h) right wing

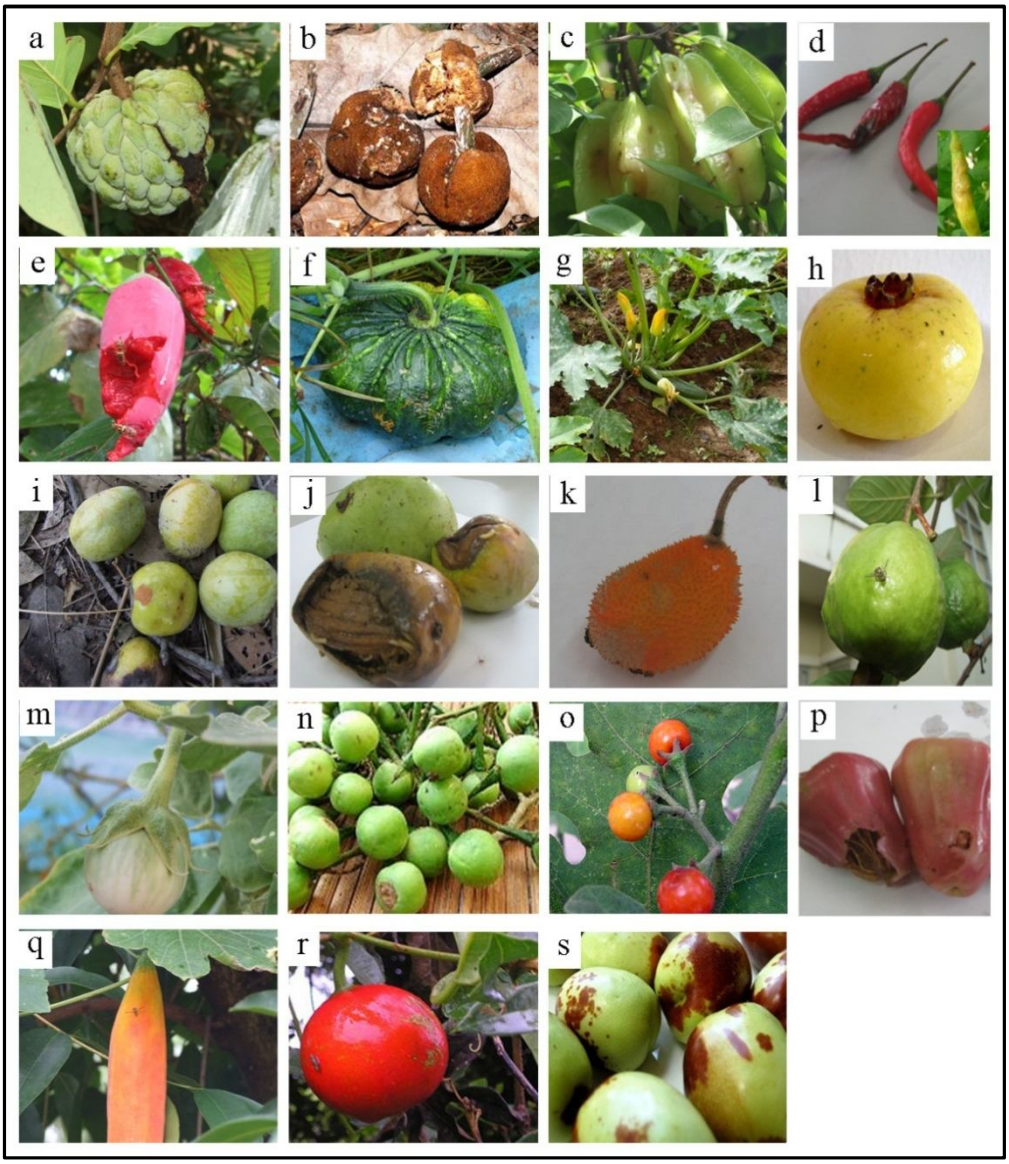


Figure 4.13 Host plant species infested by fruit flies: (a) *Annona squamosa* L., (b) *Artocarpus lanceifolius* Roxb.,(c) *Averrhoa carambola* L., (d) *Capsicum annuum* L., (e) *Cocciniagrandis* L., (f) *Cucurbita moschata* Decne., (g) *Cucumis sativas* L., (h) *Diospyros decandra*Lour., (i) *Irvingia malayana* Oliv. Ex A. Benn., (j) *Mangifera indica* L., (k) *Momordica cochinchinensis* Lour., (l) *Psidium guajava* L., (m) *Solanum melongena* L., (n) *Solanum torvum* Sw., (o) *Solanum trilobatum* L., (p) *Syzygium* sp., (q) *Trichosanthes cucumerina* L., (r) *Trichosanthes tricuspidata* Lour. and (s) *Ziziphus mauritiana* Lam.

4.2 COI sequences diversity

A total of 109 COI sequences were obtained from nine fruit fly species in northeastern Thailand. Additional of 31 COI sequences were also obtained from four populations of *Bactrocera latifrons* from northern Thailand (Table 4.3). Thus, there are 140 COI sequences of the nine *Bactrocera*s pecies included in this study. There are 217 variable sites, 178 are parsimony-informative characters and 39 areparsimony-uninformative. Seventy-seven haplotypes were identified. Haplotype diversity in each fruit fly species ranged from 0.834 in *B. latifrons* to 1.000 in *B. caryeae*, *B. cucurbitae*, *B. dorsalis*, *B. invadens* and *B. philippinensis* (Table 4.3). Nucleotide diversity in each fruit fly species ranged from 0.004 in *B. cucurbitae* to 0.018 in *B. invadens* (Table 4.3).

The average intraspecific genetic divergence based on K2P model ranges from 0.42% in *B. cucurbitae* to 1.84% in *B. invadens*. Species that show low intraspecific genetic divergence in addition to *B. cucurbitae* is *B. latifrons* (0.48%). Species that possess high intraspecific genetic divergence in addition to *B. invadens* including *B. caryeae* (1.22%), *B. correcta* (1.10%), *B. dorsalis* (1.00%), *B. occipitalis* (1.01%), *B. philippinensis* (1.14%) and *B. tau* (1.10%) (Table 4.3).

Interspecific genetic divergence between individual sequences ranged from 0% between *B. caryeae* and *B. invadens*, *B. dorsalis* and *B. occipitalis*, *B. dorsalis* and *B. philippinensis*, *B. occipitalis* and *B. philippinensis*to 22.3% between *B. cucurbitae* and *B. latifrons*with the average value of 11.2%. The average interspecific genetic divergence was lowest between *B. dorsalis* and *B. philipinensis* with the value of 0.90%. The greatest interspecific genetic divergence was a comparison between *B. latifrons* and *B. cucurbitae* with the value of 21.9%. Another comparison that reveals relatively high level of genetic divergence between species was *B. latifrons* and *B. tau* (21.5%). Interspecific genetic divergence between *B. cucurbitae* and other species often revealed high values (>17%) (Table 4.4). The only exception is comparison with *B. tau* with the interspecific genetic divergence of only 5.2%. *Bactroceralatifrons* also show high level of interspecific genetic divergences. Levels of genetic differentiation between *B. latifrons* and other species always greater than 14% indicated that this species is genetically distinct from other *Bactrocera* species included in this study. *Bactrocera tau* also show considerable interspecific genetic divergences. Comparisons between this

species and other often revealed the values greater than 17% genetic divergence. The exception is comparison with *B. cucurbitae* as pointed out above. Although most of the interspecific genetic divergence are high but these values are overlap with intraspecific genetic divergence in five species including *B. caryeae*, *B. dorsalis*, *B. invadens*, *B. occipitalis* and *B. philippinensis* (Fig. 4.14).

4.3 Phylogenetic relationships

A total of 77 haplotypes from nine species were used for phylogenetic analysis. The COI sequences of *Anastrepha luden* and *Ceratitis capitata* from GenBank under accession numbers DQ116207 and DQ116368 were use as outgroups. All three phylogenetic analysis methods (MP, NJ andBayesian) revealed similar tree topologies; thus, only the MP tree was showed (Fig. 4.15). Four fruit fly species were monophyletic where five species were not. *Bactrocera correcta*, *B. cucurbitae*, *B. latifrons* and *B. tau* were monophyletic with high bootstrap support (100%). Although *B. correcta* was divided into four forms (A, B, C, D) based on the morphological variation, they stilled group in one clade. *Bactroceracu curbitae* and *B. tau* were belong to *Zeugodacus* species group. These species were separated from other *Bactrocera* species group with high bootstrap support (100%). *Bactrocera caryeae*, *B. dorsalis*, *B. invadens*, *B. occipitalis* and *B. philippinensis* were polyphyletic species. All of these species were belong to *B. dorsalis* complex therefore, they were monophyletic with high bootstrap support (100%) in a wider sense (i.e. species complex).

Table 4.3 Fruit fly species, host plant species, number of cytochrome c oxidase subunit 1 (COI) sequences, haplotype diversity, nucleotide diversity and mean and maximum intraspecific genetic divergence based on Kimura 2-parameter.

Fruit fly species	**Host plant species (locality)**	**NO. of samples (haplotypes)**	**Haplotype diversity (*h*)**	**Nucleotide diversity (π)**	**Mean divergence (%) (max)**
Bactrocera caryeae	*Mangifera indica* L.(MS1)	2			
(Kapoor)	*Psidium guajava* L. (MS1)	1			
	Ziziphus mauritiana Lam. (SK)	2			
	Total	**5(5)**	**1.000 ± 0.126**	**0.012 ± 0.008**	**1.22 (1.90)**
Bactrocera correcta	*Syzygium* sp. (CP1)	3			
(Bezzi) From A	*Ziziphus mauritiana* Lam.(KS1, SK)	3 (2, 1)			
	Total	**6 (3)**	**0.733 ± 0.155**	**0.012 ± 0.007**	**1.03 (1.90)**
Bactrocera correcta	*Ziziphus mauritiana* Lam.(KS1)	2			
(Bezzi) From B	**Total**	**2 (2)**	**1.000 ± 0.500**	**0.012 ± 0.013**	**1.30 (1.30)**
Bactrocera correcta	*Psidium guajava* L. (MS1)	2			
(Bezzi) From C	Total	**2 (2)**	**1.000 ± 0.500**	**0.006 ± 0.007**	**0.42 (0.42)**
Bactrocera correcta	*Psidium guajava* L. (MS1)	3			
(Bezzi) From D	*Ziziphus mauritiana* Lam. (SK)	2			
	Total	**5 (5)**	**1.000 ± 0.127**	**0.010 ± 0.007**	**0.89 (1.70)**

Table 4.3 (Continued).

Fruit fly species	Host plant species (locality)	NO. of samples (haplotypes)	Haplotype diversity (*h*)	Nucleotide diversity (π)	Mean divergence (%) (max)
***Total of Bactrocera correcta* (Bezzi)**					
(From A - D)		**15 (12)**	**0.962 ± 0.040**	**0.015 ± 0.008**	**1.10 (2.30)**
Bactrocera cucurbitae (Coquillett)	*Coccinia grandis* L. (MS1)	2			
	Total	**2 (2)**	**1.000 ± 0.500**	**0.004 ± 0.005**	**0.42 (0.42)**
Bactrocera dorsalis (Hendel)	*Annona squamosa* L. (MS1)	1			
	Averrhoa carambola L. (MS1)	1			
	Mangifera indica L. (MS1)	2			
	Syzygium sp. (CP1)	1			
	Total	**5 (5)**	**1.000 ± 0.127**	**0.010 ± 0.006**	**1.00 (1.80)**
Bactrocera invadens (Drew)	*Diospyros decandra* Lour. (SK)	1			
	Mangifera indica L. (MS1)	2			
	Syzygium sp. (CP1)	1			
	Total	**4 (4)**	**1.000 ± 0.177**	**0.018 ± 0.013**	**1.84 (2.60)**

Table 4.3 (Continued).

Fruit fly species	Host plant species (locality)	NO. of samples (haplotypes)	Haplotype diversity (*h*)	Nucleotide diversity (π)	Mean divergence (%) (max)
Bactrocera latifrons[a] (Hendel)	*Capsicum annuum* L.(BK, LO3, NN1, NN2, MS1, NK, SK, YT)	54 (7, 4, 10, 5, 9, 6, 9, 4)			
	Solanum melongena L.(BK, NK, RE, SK)	7 (1, 3, 1, 2)			
	Solanum trilobatum L. (MS2, PY)	20 (10, 10)			
	*Solanum torvum*Sw. (CM, MS1, YT)	13 (6, 5, 2)			
	Total	**94 (41)**	**0.834 ± 0.040**	**0.005 ± 0.003**	**0.48 (2.95)**
Bactrocera occipitalis (Bezzi)	*Annona squamosa* L. (UR)	4			
	Irvingia malayana Oliv. Ex A. Benn. (SK)	1			
	Total	**5 (4)**	**0.900 ± 0.161**	**0.010 ± 0.007**	**1.01 (1.70)**
Bactrocera philippinensis (Drew and Hancock)	*Annona squamosa* L. (MS1)	2			
	Averrhoa carambola L. (MS1)	2			
	Psidium guajava L. (MS1)	1			
	Total	**5(5)**	**1.000 ± 0.127**	**0.011 ± 0.008**	**1.14 (2.60)**

Table 4.3 (Continued).

Fruit fly species	Host plant species (locality)	NO. of samples (haplotypes)	Haplotype diversity (*h*)	Nucleotide diversity (π)	Mean divergence (%) (max)
Bactrocera tau (Walker)	*Momordica cochinchinensis* Lour. (KK)	2			
	Trichosanthes tricuspidata Lour. (LO2)				
	Total	3			
		5 (4)	**0.900 ± 0.161**	**0.010 ± 0.007**	**1.10 (1.50)**
	Total	**140 (77)**			

Haplotypes were shared between some species (details shown in the results).
[a]Additional of 31 COI sequences were also obtained from populations of *B. latifrons* from 4 populations in northern Thailand : CM = Doisaket, Chiang Mai; PY = Chiang Muan, Phayao; NN1 = Pua, Nan and NN2 = Ban Luang, Nan.

Table 4.4 Average of interspecific genetic divergences between nine *Bactrocera* species in northeastern Thailand, based on Kimura 2-parameter. Values below diagonal are average percentage of interspecific genetic divergence and values above diagonal are standard error of the mean.

Species	*B. caryeae*	*B.correcta*	*B. cucurbitae*	*B. dorsalis*	*B. invadens*	*B. latifrons*	*B. occipitalis*	*B. philippinensis*
B. caryeae								
B. correcta	9.5 ± 1.3							
B. cucurbitae	18.3 ± 2.1	18.5 ± 2.0						
B. dorsalis	1.0 ± 0.2	9.2 ± 1.3	17.8 ± 2.0					
B. invadens	1.6 ± 0.3	9.6 ± 1.3	18.4 ± 2.1	1.5 ± 0.3				
B. latifrons	16.4 ± 1.9	14.3 ± 1.7	21.9 ± 2.2	15.8 ± 1.9	16.7 ± 1.9			
B. occipitalis	1.5 ± 0.4	9.0 ± 1.3	18.2 ± 2.0	1.2 ± 0.3	1.6 ± 0.4	15.9 ± 1.9		
B. philippinensis	1.2 ± 0.3	9.3 ± 1.3	18.1 ± 2.0	0.9 ± 0.2	1.7 ± 0.3	16.0 ± 1.9	1.3 ± 0.3	
B. tau	17.6 ± 2.1	18.4 ± 2.0	5.2 ±1.0	17.2 ± 2.0	17.7 ± 2.0	21.5 ± 2.2	17.4 ± 2.0	17.4 ± 2.0

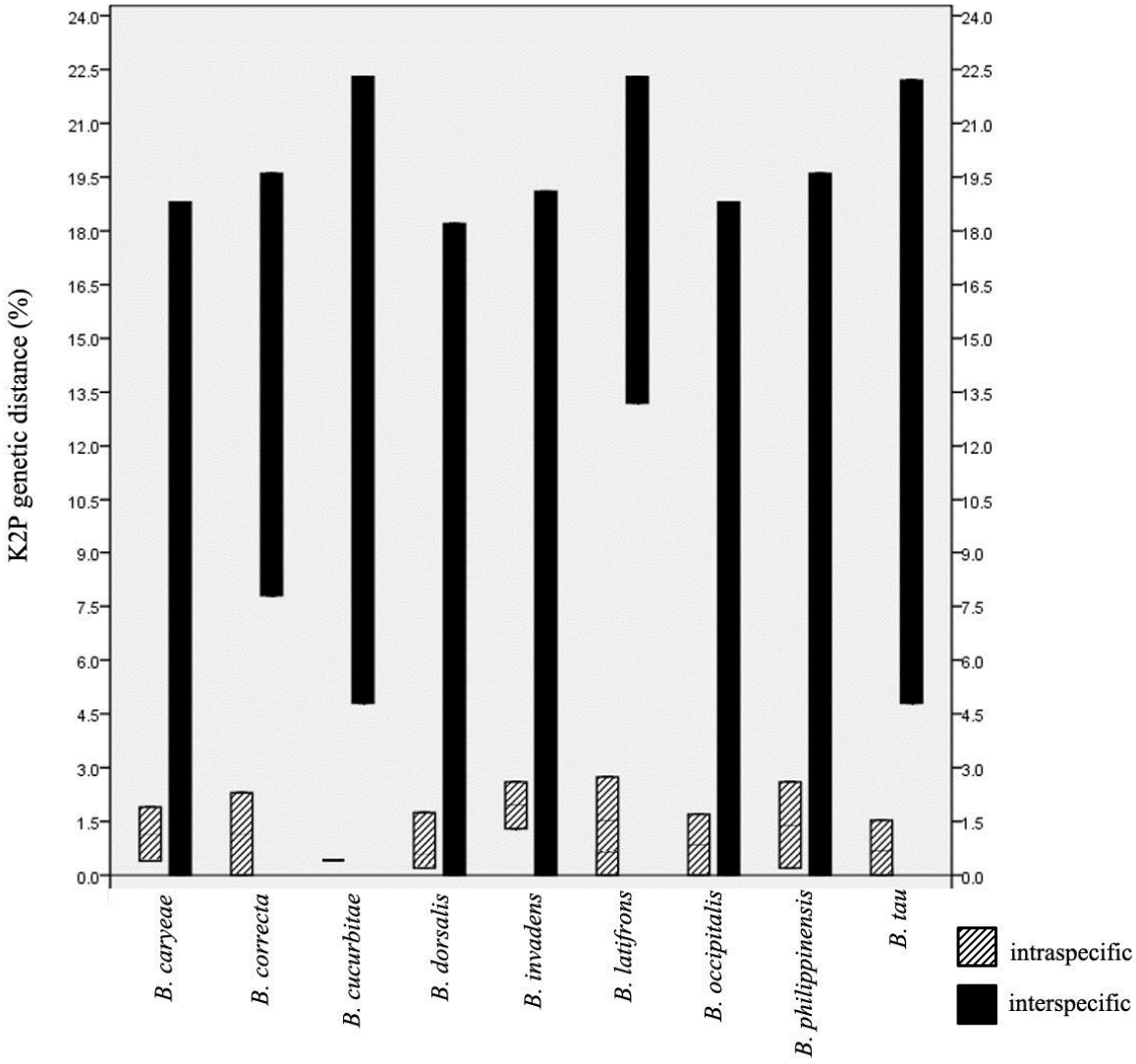


Figure 4.14 Range of intraspecific and interspecific genetic divergences based on Kimura 2-parameter of the cytochrome c oxidase subunit 1 (COI) sequences for nine species of fruit flies in northeastern Thailand

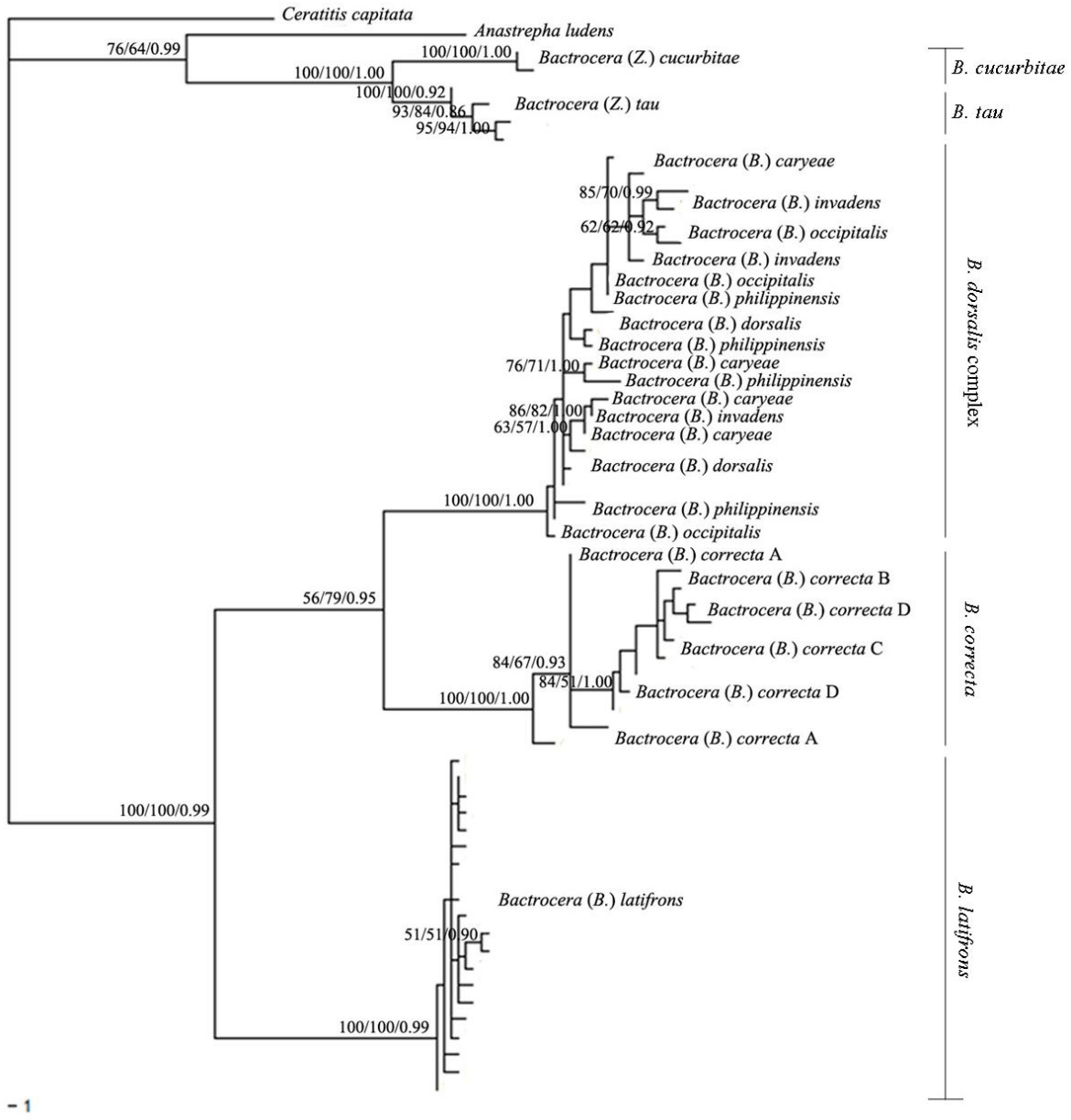


Figure 4.15 Maximum parsimony tree for cytochrome c oxidase subunit 1 (COI) barcoding sequences of nine fruit fly species in northeastern Thailand. Bootstrap values for maximum-parsimony, neighbor-joining and posterior probability from Bayesian analyses are shown above the branch.

4.4 DNA barcoding of tephritid fruit flies in northeastern Thailand

One hundred and forty COI barcoding sequences were obtained from nine fruit fly species. Of these, 109 sequences were from northeastern Thailand and 31 sequences were from *Bactrocera latifrons* in northern Thailand. The best match and best close match algorithms in TaxonDNA software (Meier *et al*., 2006) were used to identify species wiyh the sequence divergence threshold value of 3.0%. The results shown that the correct identifications according to "Best Match" were 124 sequences (88.57%), ambiguous identifications 3 sequences (2.14%) and incorrect identifications 13 sequences (9.28%). Similar results were obtained from the best close match method. The ambiguous and incorrect identifications were due to five species from *B. dorsalis* complex including *B. caryeae*, *B. dorsalis*, *B. invadens*, *B. occipitalis* and *B. philippinensis*. However, the best match and best close match revealed 100% correct identification when sequences of *B. dorsalis* complex were excluded.

To test the efficiency of the COI sequences for species identification of *Bactrocera*, 140 sequences obtained from this study were subjected to identify in Barcode of Life Data Systems (BOLD). The results revealed that all of 116 sequences from four species including *B. correcta*, *B. cucurbitae*, *B. latifrons* and *B. tau* were correctly identified. However, five species in *B. dorsalis* complex were unable to find the best match species in BOLD database. Five sequences of *B. caryeae* were identified as *B. dorsalis*, *B. invadens*, *B. irvingiae* and *B. papayea* with genetic similarity range between 99.02% to 99.49%. Five sequences of *B. dorsalis* were identified as *B. dorsalis*, *B. invadens*, *B. irvingiae*, *B. papayae*, *B. verbascifoliae* and *B. zonata* with genetic similarity range between 99.66 and 100%.Four sequences of *B. invadens* were identified as *B. dorsalis*, *B. invadens*, *B. irvingiae*, *B. papayae* and *B. verbascifoliae* with genetic similarity range between 98.33% and 99.33%. Five sequences of *B. occipitalis* were identified as *B. dorsalis*, *B. invadens*, *B. papayae*, *B. philippinensis*, *B. verbascifoliae* and *B. zonata* with genetic similarity range between 98.53% and 100%. Five sequences of *B. philippinensis* were identified as *B. dorsalis*, *B. invadens*, *B. papayae* and *B. philippinensis* with genetic similarity range between 98.33% and 100%.

4.5 Genetic variation at population level: a case study of *Bactrocera latifrons*in Thailand

4.5.1 Mitochondrial DNA sequence variation

A total of 93 sequences of the mitochondrial COI gene of *B. latifrons* were obtained from 11 populations in Thailand. Representative haplotypes were deposited in Genbank under accession numbers KC812832 – KC812871. There were 51 base substitutions including 33 transitions and 18 transversions. According to the DNA sequence variations, 40 haplotypes were identified. The most common haplotype was found throughout Thailand and also other geographic regions including Malaysia and Hawaii. Average haplotype diversity was 0.8308 (Table 4.5). Haplotype diversity in each population ranged from 0.25 in BK to 1.000 in CM and LO3 (Table 4.5). The average nucleotide diversity was 0.0048 with the range in each population between 0.0009 in BK and 0.0086 in N2 (Table 4.5). Average haplotype and nucleotide diversity were greater in the northern populations compare to the upper and lower northeastern populations (Table 4.5).

4.5.2 Mitochondrial DNA genealogy

A MJ network of 105 sequences (93 sequences from the present study and 12 sequences previously published) revealed no major divergence lineage (Fig. 4.16, 4.17). Most haplotypes connected with a short branch length. Haplotype clusters were associated neither with geographic origins (Fig. 4.16) nor with host plant species (Fig. 4.17). Overall, the network is a star-like shape, characteristic of population expansion (Slatkin and Hudson, 1991). The central haplotype has the highest frequency. Several geographically widespread populations and samples from all host plant species shared this haplotype (Fig. 4.16, 4.17).

The relationships between Thai *B. latifrons* and sequences from other geographic regions are as follows. Mitochondrial COI sequence from Malaysia belongs to the central haplotype. Sequences from Hawaii also shared the central haplotype but one was connected to the central haplotype by one mutation step. Five African (Tanzania and Kenya) specimens were made up of two haplotypes. One haplotype was shared with three specimens from Thailand and connected to the central haplotype by

one mutation step. Another haplotype was unique to Africa where it was shared by two individuals. A relatively divergent haplotype from Japan was connected to the central haplotype by six mutation steps. This haplotype was unique to Japan.

Table 4.5 Estimates of haplotype diversity (*h*) and nucleotide diversity (π) of 11 populations of *Bactrocera latifrons* in Thailand. Details of the sampling locations are given in Table 3.2.

Location	**Number of samples**	**Geographic region**	**Haplotype diversity (*h*)**	**Nucleotide diversity (π)**
CM	6	North	1.0000 ± 0.0962	0.0057 ± 0.0039
PY	10	North	0.9556 ± 0.059	0.0048 ± 0.0031
NN1	10	North	0.9778 ± 0.0540	0.0038 ± 0.0026
NN2	5	North	0.9000 ± 0.1610	0.0086 ± 0.0059
North	**31**		**0.9700 ± 0.0170**	**0.0066 ± 0.0040**
NK	9	Upper Northeast	0.8056 ± 0.1196	0.0029 ± 0.0021
LO3	4	Upper Northeast	1.0000 ± 0.1768	0.0060 ± 0.0046
BK	8	Upper Northeast	0.2500 ± 0.1802	0.0009 ± 0.0009
Upper Northeast	**21**		**0.6760 ± 0.1110**	**0.0027 ± 0.0020**
MS1	14	Lower Northeast	0.7692 ± 0.1198	0.0048 ± 0.0030
MS2	10	Lower Northeast	0.9333 ± 0.0620	0.0061 ± 0.0038
SK	11	Lower Northeast	0.4727 ± 0.1617	0.0012 ± 0.0011
YT	6	Lower Northeast	0.3333 ± 0.2152	0.0017 ± 0.0015
Lower Northeast	**41**		**0.7130 ± 0.0790**	**0.0041 ± 0.0030**
Total	**93**		**0.8308 ± 0.0400**	**0.0048 ± 0.0028**

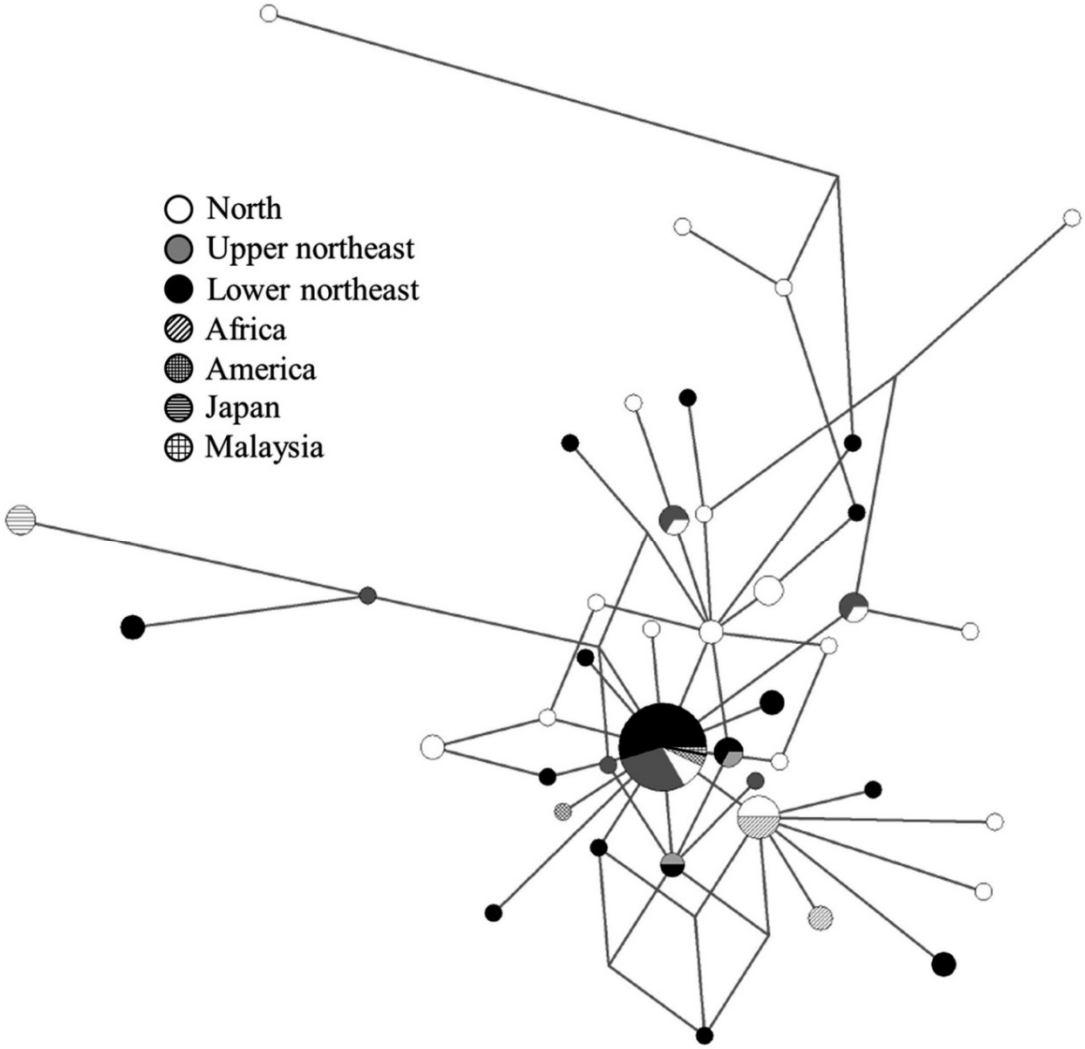


Figure 4.16 Median joining network of the 105 COI sequences (93 sequences from Thailand and 12 from other geographic regions) of *Bactrocera latifrons*. Circles represent haplotypes and sizes are relative to the number of individuals sharing the specific haplotype. Haplotypes labeled according to geographic origins.

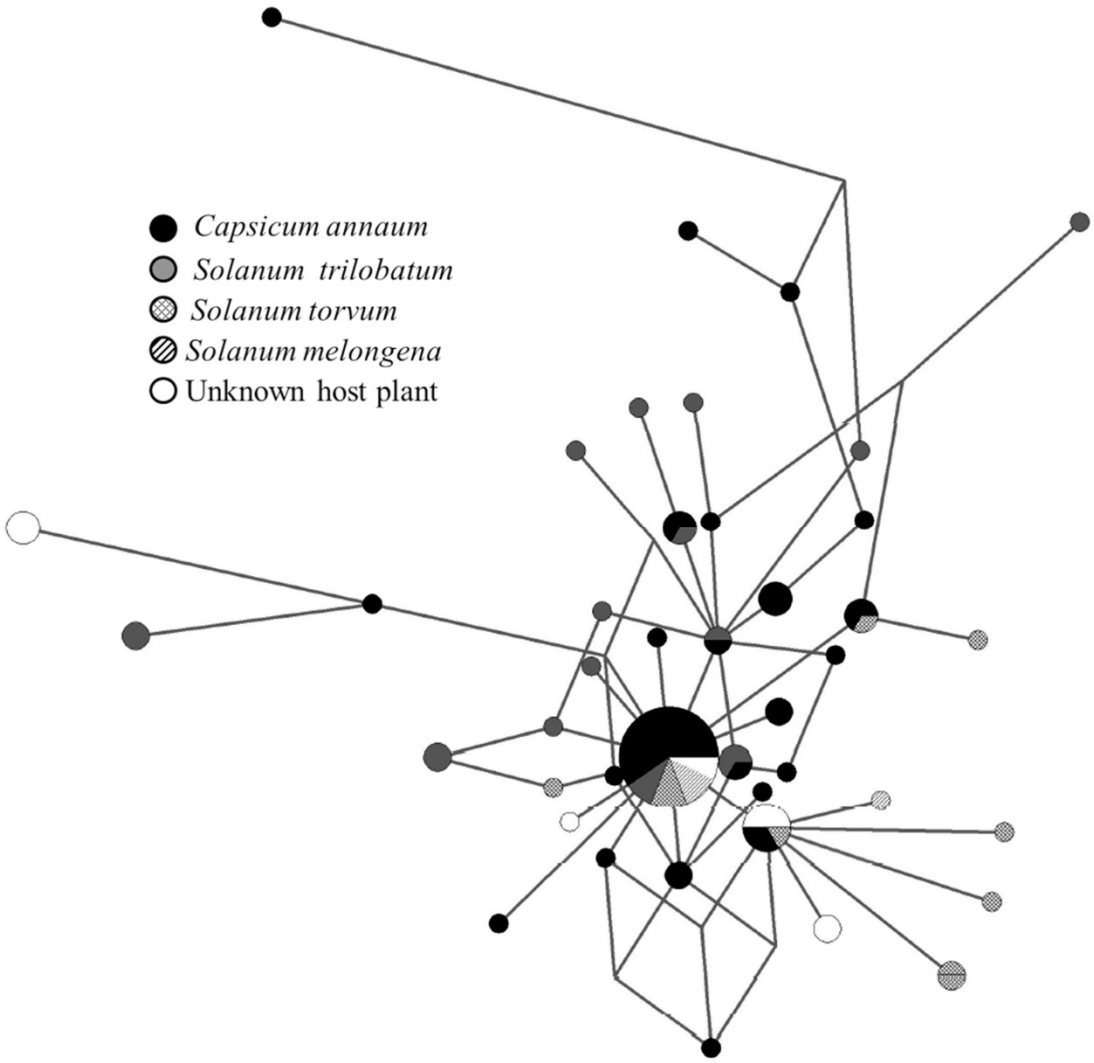


Figure 4.17 Median joining network of the 105 COI sequences (93 sequences from Thailand and 12 from other geographic regions) of *Bactrocera latifrons*. Circles represent haplotypes and sizes are relative to the number of individuals sharing the specific haplotype. Haplotypes labeled according to host plants.

4.5.3 Population genetic structure

Population pairwise F_{ST} analysis revealed that most (65%) populations were genetically not significantly different (Table 4.6). The exceptions to this were comparisons between NN2 with other populations where all comparisons were significantly different. Mantel's test revealed no significant relationships ($r^2 = 0.033$ $P = 0.135$) between genetic and geographic distances. AMOVA analysis by grouping populations according to the host plant species also revealed no significant genetic differentiation among groups ($F_{CT} = 0.032$, $P = 0.206$) (Table 4.7).

4.5.4 Demographic history

Mismatch distribution analysis revealed aunimodal mode of the mismatch graph (Fig. 4.18), a characteristic of recent population demographic expansion. This is consistent with the star-like shape of the mtDNA genealogy. Both sum-of-squares deviation (SSD = 0.0051, $P = 0.65$) and Harpending's raggedness index (0.0222, $P = 0.84$) were not significantly different from the simulated data under the sudden population expansion model (Fig. 4.18). Population expansion was also support by highly significant negative values of both Tajima's D (-2.2696, $P< 0.001$) and Fu's F_S(-26.6256, $P< 0.001$) tests. Population expansion time, estimated based on 2.3% sequence divergence for insect mitochondrial DNA (Brower, 1994) and assuming six generations per year for *B. latifrons* (Peck and McQuate, 2004), was estimated to be 16,000 years ago.

Table 4.6 Population pairwise F_{ST} between 11 populations of *Bactrocera latifrons* in Thailand. Details of the sampling sites are shown in Table 3.2.

Population	MS1	SK	NK	YT	LO3	BK	MS2	CM	PY	NN1	NN2
MS1	-										
SK	0.002	-									
NK	0.016	0.128	-								
YT	-0.071	0.001	0.075	-							
LO3	0.004	0.161	-0.028	0.057	-						
BK	-0.016	0.024	0.101	0.016	0.054	-					
MS2	0.153*	0.242*	0.081	0.167	-0.054	0.207	-				
CM	0.078	0.189*	0.182*	0.071	0.003	0.120	0.209*	-			
PY	0.140*	0.243*	0.128	0.173	0.070	0.194	0.116*	0.198	-		
NN1	0.079*	0.164	0.093	0.091	0.054	0.153	0.062	0.150	0.101	-	
NN2	0.461*	0.608*	0.477*	0.501*	0.333*	0.575*	0.326*	0.418*	0.404*	0.352*	-

*$P < 0.05$.

Table 4.7 Results of the AMOVA analyses of 11 populations of *Bactrocera latifrons* from Thailand, with grouping according to geographic origins and host plants.

Source of variation	**d.f.**	**SSD**	**Percentage of variation**	***F*-statistic**
Geographic origin				
Among groups	2	7.005	0.01	F_{CT}= 0.001
Among populations within group	8	26.660	18.47	F_{ST} = 0.185**
Within population	82	95.372	81.52	F_{SC} = 0.185**
Host plant species				
Among groups	3	9.639	3.16	F_{CT} = 0.032
Among populations within group	12	28.198	14.29	F_{ST} = 0.175**
Within population	77	91.200	82.54	F_{SC} = 0.148**

**$P < 0.001$.

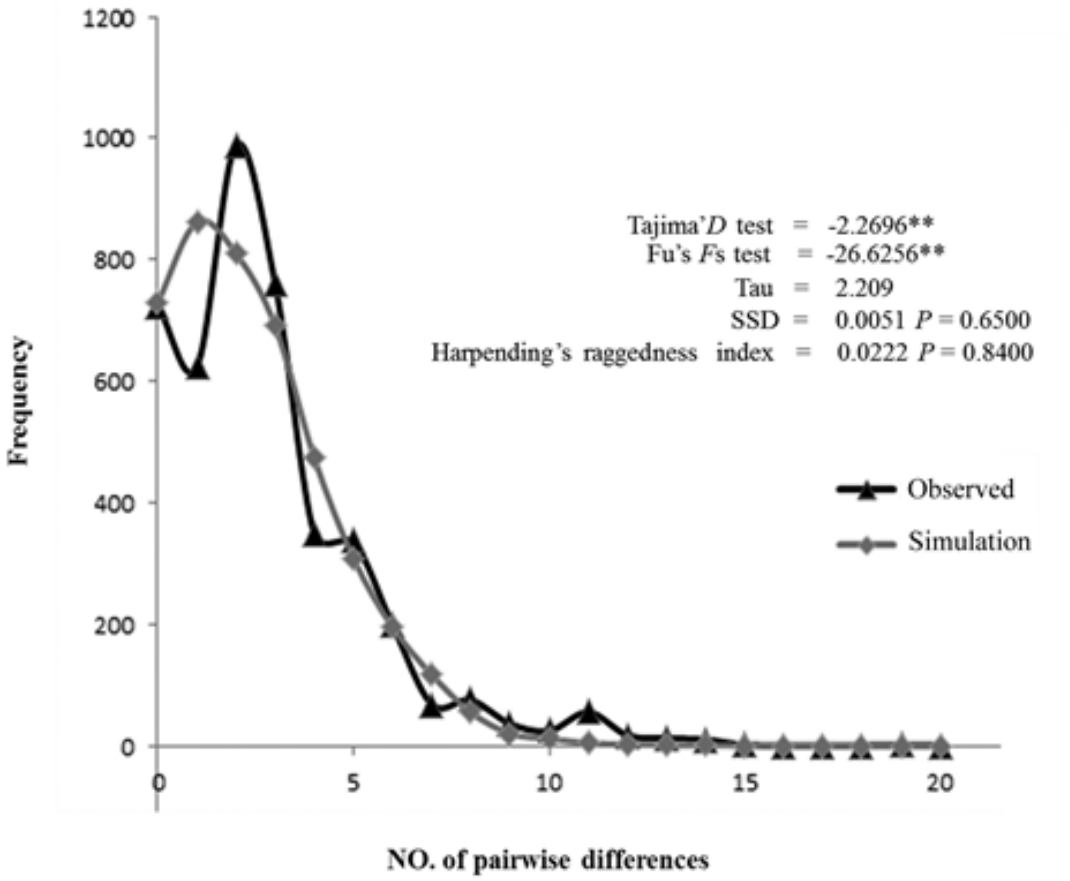


Figure 4.18 Mismatch distribution of 93 COI sequences of *Bactrocera latifrons* from Thailand representing the observed and expected pairwise differences under the sudden population expansion model. Mismatch distribution of *B. latifrons* was consistent with the sudden population expansion model (SSD = 0.0051, $P = 0.6500$; Harpending's raggedness index = 0.0222, $P = 0.8400$).

Chapter 5

Discussion

5.1 Species diversity and host plants relationship of tephritid fruit flies in northeastern Thailand

A total of nine species of tephritid fruit fly of the genus *Bactrocera* Macquart were detected from 19 host plant species. The genus *Bactrocera* was the most diverse fruit fly in the Pacific and Southeast Asia (Drew and Handcock, 2001a). In Thailand and bordering countries approximately 221 fruit fly species have been reported. Of these, 182 species were belong to genus *Bactrocera*. Baimai*et al*. (2002) reported 87 fruit fly species throughout Thailand. Among these, 18 *Bactrocera* species from 20 host plants were found in northeastern Thailand. The aim of the present study was concentrate on the species diversity of fruit fly in agricultural significant plants. A total of 19 host plant species were found. Fourteen species were agricultural crops including *Annona squamosa* L., *Averrhoa carambola* L., *Capsicum annuum* L.,*Cucurbita moschata* Decne.,*Cucumis sativas* L., *Diospyros decandra* Lour., *Mangifera indica* L., *Psidium guajava* L., *Solanum melongena* L., *Solanum trilobatum* L.,*Solanum torvum* Sw., *Syzygium* sp., *Trichosanthes cucumerina* L. and *Ziziphus mauritiana* Lam.. Five species were wild plants including *Artocarpus lanceifolius* Roxb.,*Coccinia grandis* L., *Irvingia malayana* Oliv. Ex A. Benn., *Momordica cochinchinensis* Lour. and *Trichosanthes tricuspidata* Lour..

Five fruit fly species belong to *Bactrocera dorsalis* complex including *B. caryeae*, *B. dorsalis*, *B. invadens*, *B. occipitalis*, *B. philippinensis*. These species are polyphagous, infest on plants of many families or even different orders (Aluja and Mangan, 2008). Among the members of *B. dorsalis* complex, *B. dorsalis* infested the greatest diverse host plants where 124 host plant species from 79 genera in 42 families were reported (Allwood *et al*., 1999; Clarke *et al*. 2005).

Bactrocera invadens attacks more than 44 host plant species from 13 families (Allwood*et al*., 1999; Clarke *et al*. 2005; Mwatawala *et al*., 2006). Although *B. invadens* originates from Asia, this species invaded to various part of Africa and other regions (Goergen *et al*., 2011). Eight plant species from seven families were found infested by *B. invadens* in the present study. Among these, five host plants including *A. squamosa* L., *A. carambola* L., *M. indica* L., *P. guajava* L. and *Syzygium* sp.were previously reported and three host plants including *D. decandra* Lour., *I. malayana* Oliv. Ex A. Benn. and *Z. mauritiana* Lam. were reported for the first time.

Bactrocera occipitalis infests three host plants from three families. This species was record in Brunei, Malaysia and Philippine from plants of the family Anacardiaceae, Myrtaceae and Rutaceae (Allwood *et al*., 1999; Clarke *et al*., 2005). In the present studyonly seven individuals of *B. occipitalis* was found from *A. squamosa* L. (Annonaceae) and *I. malayana* Oliv. Ex A. Benn. (Irvingiaceae).

Bactrocera philippinensis infests six host plants from five families (Allwood *et al*., 1999; Clarke *et al*. 2005; Mwatawala *et al*., 2006). *Bactrocera philippinensis* is the major pest of mango in Philippine. This species has not been reported in other geographic regions except Palau. This species was found in Anacardiaceae, Caricaceae, Moraceae, Myrtaceae and Sapotaceae (Allwood *et al*., 1999; Clarke *et al*., 2005; Plant Health Australia, 2011). The detection of *B. philippinensis* in this study, is the first report of this species in Thailand. A total of six host plant species from five families (Anacardiaceae, Annonaceae, Irvingiaceae, Myrtaceae and Oxalidaceae) were found infested by *B. philippinensis*. Among these plants, three families were first report as the host of *B. philippinensis*.

Bactrocera caryeae was reported to infest 10 host plant species from eight genera in six families. In this study *B. caryeae* was detected in five host plants from five families including *A. squamosa* L. (Annonaceae), *A. carambola* L.(Oxalidaceae), *M. indica* L. (Anacardiaceae), *P. guajava* L. (Myrtaceae) and *Z. mauritiana* Lam. (Rhamnaceae). Allwood *et al*. (1999) reported *B. caryeae* infests six host plant families including Anacardiaceae, Lecythidaceae, Malpighiaceae, Myrtaceae, Rutaceae and Sapotaceae and this fruit fly species distributes in southern India and Sri Lanka. Thus, this is first report of *B. caryeae* in Thailand. Three new host plant families (Annonaceae, Oxalidaceae, Rhamnaceae) were also report for the first time.

Although members of *B. dorsalis* complex infest diverse host plants but the major plant families include Anacardiaceae, Annonaceae, Clusiaceae, Lauraceae, Moraceae, Myrtaceae, Rutaceae, Sapotaceae and Solanaceae (Clarke *et al*., 2005). In this study fruit fly species in *B. dorsali s*complex and *B. correcta* shared the major host plants. A large number of adults *B. dorsalis* and *B. correcta* wer found in *Psidium guajava* L.. This result supports previous finding which found that *P. guajava* L. is the most favorite host of *B. dorsalis* complex (Allwood *et al*., 1999; Hancock *et al*., 2000; Clarke *et al*. 2001).

Bactrocera correcta had a very similar host use pattern to *B. dorsalis* complex in Thailand. Although this species was recorded from more than 25 plant families, the major host species restricted to only a few families including Anacardiaceae, Combretaceae, Myrtaceae and Rhamnaceae (Clarke *et al*., 2001). In this study an additional host plant species, *Ziziphus mauritiana* Lam. that has not yet been reported was found infested by *B. correcta* at high frequency (Table 4.1). According to thoracic vittae and abdomen color patterns the present study divided *B. correcta* into four forms (A, B, C, D) (Fig. 4.2 – 4.5). No association between the morphological form and host plant usage were revealed. All forms were found in *P. guajava* and *Syzygium* spp. which consistent with previous study (Clarke *et al*., 2001). However, if different morphological forms of *B. correcta* were test separately, form C and D shown some degree of association to host plant (Table 4.1, 4.2).

Chi – square goodness of fit test revealed that *B. dorsalis* complex species and *B. correcta* were not significantly associated to host plants (χ^2 = 0.474: *P* = 0.491, χ^2 = 0.053: *P* = 0.819). These results consist with previous host range record of these species as they were polyphagous which are not specifically associated to host plants (Clarke *et al*., 2001; 2005).

Bactrocera cucurbitae was found inthree host plant species of the family Cucurbitaceae including *Coccinia grandis* L., *Cucumis sativas* L. and *Trichosanthes cucumerina*L.. Thus, high degree of association to host plant species was revealed (χ^2 = 8.895, *P* = 0.003). Previous reports recorded more than 125 host plants infested by *B. cucurbitae*. Most host plant species were belong to the Cucurbitaceae and Solanaceae (Pinero *et al*., 2006; Hu *et al*., 2008). In Thailand almost all of *B. cucurbitae* were found in Cucurbitaceae (Baimai *et al*., 2002).

Bactrocera tau complex infested fruits of many species of the family Cucurbitaceae. Although they have also been found infesting fruits from other families, e.g., Leguminoseae (*Phaseolus vulgaris* L.), Moraceae (*Ficus racemosa* L.), Myrtaceae (*Psidium guajava* L.), Oleaceae (*Myxopyrum smilacifolium*), and Sapotaceae (*Manilkara zapota* L.), but in Southeast Asia Cucurbitaceae is the major host plant family (Drew and Romig, 1996; Allwood *et al.*, 1999). *Bactrocera tau* complex was cytologically divided into seven forms (A-G) (Baimai *et al*., 2000a). In addition recent reports revealed some cytoforms (e.g. cytoform A) of this species shown relationship with geographic origin and host plant species (Kitthawee and Dujardin, 2010; Dujardin and Kitthawee, 2013). In this study *B. tau* complex was not assigned into cytological forms. This species was collected from three host plants including *Cucurbita moschata* Decne., *Momordica cochinchinensis* Lour. and *Trichosanthes tricuspidata* Lour. (Table 4.1). This species shown highly significant association to host plant species (χ^2 = 8.895, *P* = 0.003).

Bactrocera latifrons is the major pest of plants in family Solanaceae and some species in Cucurbitaceae (Liquido *et al*., 1994; Harris *et al*., 2001; McQuate *et al*., 2007). This study found *B. latifrons* in four host plants including *Capsicum annuum* L., *Solanum melongena* L., *Solanum torvum* Sw. and *Solanum trilobatum* L. especially *Capsicum annuum* L. was infested with high frequency (Table 4.1). High rate of infest of *B. latifrons* in *C. annuum* was reported in Malaysia (Vijaysegaran and Osman, 1991). Therefore, significant association (χ^2 = 6.368, *P* = 0.012) to host plant species was found for *B. latifrons*.

5.2 COI sequences diversity and DNA barcoding of tephritid fruit flies in northeastern Thailand

A total of 77 mtDNA haplotypes were identified from 140 COI sequences of the nine *Bactrocera* species. Haplotype diversity range from 0.834 in *B. latifrons* to 1.000 in *B. cucurbitae*, *B. dorsalis*, *B. invadens* and *B. philippinensis*. Nucleotide diversity in each species ranged from 0.004 in *B. cucurbitae* to 0.018 in *B. invadens*. The results indicated high genetic diversity of tephritid fruit flies in northeastern Thailand. Species complex often revealed higher nucleotide diversity.

In this study *B. dorsalis* complex (five species), *B. tau* complex revealed higher nucleotide diversity than *B. cucurbitae* and *B. latifrons*. Previous studies reported nucleotide diversity of *B. latifrons* (0.001 – 0.009) and *B. cucurbitae* (0.001 – 0.003) were lower than the *Bactrocera* species complex such as *B. dosalis* (0.007 – 0.02) and *B. tryoni* (0.005 – 0.018) (Hu *et al*., 2008; Blacket *et al*., 2012; Prabhakar *et al*., 2012; Shi *et al*., 2012). *Bactrocera correcta* also showed considerable nucleotide diversity (0.015). This species was divided into two sibling species (Jamnongluk *et al*. 2003b) thus this species should be considered as species complex.

Level of intraspecific genetic divergence of nine fruit fly species in this study ranges from 0.42% in *B. cucurbitae* to 1.84% in *B. invadense* (Table 4.3). High intraspecific genetic divergence was found in *B. dorsalis* complex, *B. correcta* and *B. tau*. Low intraspecific genetic divergence was found in *B. cucurbitae* and *B. latifrons*. Among the nine species included in this study, four species (*B. tau*, *B. cucurbitae*, *B. correcta*, *B. latifrons*) were clearly differentiated based on DNA barcode. These species show clear distinction between intraspecific and interspecific genetic divergence. In addition, these species also received monophyletic supported based on phylogenetic analyses. The remaining five species, all are members of *B. dorsalis* complex (*B. caryeae*, *B. dorsalis*, *B. invadens*, *B. occipitalis* and *B. philippinensis*) were not successful for DNA barcoding. These species show large overlap between intraspecific and interspecific genetic divergences. They are also not received reciprocal monophyletic support in phylogenetic analyses. Limitation of the DNA barcode to differentiate members of the species complex has been reported previously. Armstrong and Ball (2005) found that DNA barcode appear to be limited in their ability to distinguish taxa within the species complexes of *B. dorsalis* and *B. tryoni*.

Given that, DNA barcode relies on the distinction between intraspecific and interspecific sequence divergence. Overlap could lead to errors in species identification (Meyer and Paulay, 2005; Cognato, 2006; Meier *et al*., 2006). Overlapping of intraspecific and interspecific genetic divergence values could due to several factors. Among these, the two major factors are incomplete lineage sorting and imperfect taxonomy. Both of these factors could leading to species non-monophyly

(i.e., paraphyly, polyphyly) (Avise, 2000; Meyer and Paulay, 2005; Pramual *et al*., 2011b).

The reason of unsuccessful identification based on DNA barcode of *Bactrocera dorsalis* complex is most likely due to imperfect taxonomy. Morphological criteria of several fruit fly species have been described based on host plant species and geographic regions (Jamnongluk *et al*., 2003b; Clarke *et al*., 2005). Generally, the different geographic regions compose of both general plant species and native, some as endemic species. *Bactrocera dorsalis* complex is a large group that could infest several plant families (except some species). Therefore, the host plant use patterns depend on host plants availability in each geographic region. Little variations in morphological characters, host use patterns and geographic regions were used to recognized new species (e.g. *B. papayae*, *B. philippinensis*, *B. invadens*) (Drew and Hancock, 1994a; Drew *et al*., 2005). Recent molecular study suggested that *B. dorsalis*, *B. papayae* and *B. philippinensis* should be merged into genetically single species because no genetic differentiation was found among these species (Schutze *et al*., 2012). Consistent with molecular genetic study, their close morphological, genetic, physiological and behavioral similarities have also been reported (Medina *et al*., 1998; Iwahashi, 2001; Smith *et al*., 2003; Tan, 2003).

5.3 Genetic variation at population level: a case study of *Bactrocera latifrons*in Thailand

Phylogenetic analysis revealed that *B. latifrons* was monophyletic species. The MJ mitochondrial haplotype network that included sequences from Thailand and other geographic regions including Malaysia, Japan, Tanzania, Kenya and Hawaii revealed no major divergent lineage. Thus, our data indicates that *B. latifrons* is a single phyletic unit. Genetically closely related between individuals from far geographic regions (e.g. Southeast Asia, Japan, Africa, Hawaii) was consistent with information on the time of the invasion. *Bactrocera latifrons* was suspected to have originated in South and Southeast Asia (White and Elson-Harris, 1992; Liquido *et al*., 1994). This species recently invaded other geographic regions. It was found in Yonaguni Island, Japan, in 1984 where it is now present throughout the island (Shimizu *et al*., 2007).

About the same time (1983), this species was first detected in Hawaii (Vargas and Nishida, 1985). *Bactrocera latifrons* was recently detected in Tanzania. Mwatawala *et al*. (2007) reported that this species was found for the first time in Africa in 2006. Due to the short time span since the invasion, low genetic differentiation would be expected.

Genetic variation of *B. latifrons* was reported previously based on allozyme markers. Yong (1993) found comparable levels of genetic variation in *B. latifrons* in Malaysia with *B. cucurbitae* but these were lower than those of *B. caudata* and *B. dorsalis* complexes. Consistent with the allozyme marker, genetic variation of *B. latifrons* based on COI sequences (0.12% - 0.60%) was lower than the *Bactrocera* species complex such as *B. dorsalis* (0.7% - 2.0%) (Shi *et al*., 2012) and *B. tryoni* (0.5% - 1.8%) (Blacket *et al*., 2012). However, it was found that genetic variation of *B. latifrons* in Thailand based on COI sequences is higher than *B. cucurbitae* (0.1% - 0.3%) (Hu *et al*., 2008; Prabhakar *et al*., 2012) and *B. oleae* (0.09% - 0.48%) (Dogac *et al*., 2013).

Levels of genetic variations across the host plant species were similar. The exception to this is low genetic variation in *Solanum melongena*. This could be due to limitations of the sample size and sampling area as only six individuals from a single location were examined. In addition to the similar levels of genetic variation, several haplotype were also shared by flies from different host plants. AMOVA result revealed no genetic differentiation among *B. latifrons* from different host species. These results indicated that *B. latifrons* move freely across these host plants. This is not unexpected given that all four host plants belong to the same family, Solanaceae, and are commonly found in the same area. However, many other host plants were reported for *B. latifrons* (Allwood *et al*., 1999), but not included in the present study, such as the families Cucurbitae (Liquido *et al*., 1994) and Combretaceae (Somta *et al*., 2010). Thus, it would be interesting for further study to investigate genetic differentiation between *B. latifrons* from different host plants, particularly plants from different families.

Population pairwise F_{ST} values indicated overall low level of genetic structure in *B. latifrons* in Thailand. Most (65%) of the population pairwise F_{ST} comparisons were not significant. This suggests considerable gene flow between populations. Since the host plants for *B. latifrons* (*C.annuum*, *S. torvum*, *S. trilobatum* and *S. melongena*) are very common in Thailand thus continuous habitats of this species would be

expected. This could promote gene flow between populations. The exception to the overall low level of genetic structure is the location NN2 where all comparisons with other sites were significantly different. This is due to highly divergent haplotypes found in this site. Among the four haplotype, three were divergent haplotypes unique to this location. Another haplotype was shared with location NN1, the geographically close sampling site. The NN2 site was geographically adjacent to the large natural tropical forest. Thus, it could be possible that the private, divergent haplotypes found in this site were derived from the wild host plants. Many host plant species reported for *B. latifrons* (Allwood *et al*., 1999) are native plant species commonly found in the natural forest in Thailand. Therefore, the private, divergent haplotypes found in the NN2 could be derived from adjacent natural forest. Shared haplotype between NN1 and NN2 indicated that they were gene flow among these locations. Geographically, these two sites were approximately 72 km apart and were isolated by large mountain ranges. Because the geographic distance between these sites was far greater than the dispersal ability of *B. latifrons* (200 m, Peck and McQuate, 2004) and the mountain ranges could also be the effective barrier for gene flow as has been reported in other fruit flies (Shi *et al*., 2005). Thus, haplotype that shared between these locations most likely due to long distance migration. This could be a result of either the historical population expansion or human-mediate gene flow. In both cases, it must happen not often otherwise no genetic differentiation between these populations would be expected.

The overall low level of genetic differentiation between populations could also due to recent history of this species. Demographic history analyses indicated that *B. latifrons* in Thailand has experienced recent population expansion dating back to be at the end of the last glaciations (16,000 years ago). Previous studies in several insects have revealed the significant role of the Pleistocene climatic change on genetic structure and diversity in the Southeast Asian mainland, including Thailand (Pramual *et al*., 2005; Morgan *et al*., 2011; Pramual and Kuvangkadilok, 2012). Similar historical demographic patterns among geographically co-distributed species indicated that they were experiencing the same historical event, the Pleistocene climatic change. The results thus support previous views on the importance of this historical event in shaping genetic structure and diversity of the Southeast Asian mainland faunas.

Chapter 6

Conclusion

The present study examined species diversity, host plant association and DNA barcode of tephritid fruit flies in northeastern Thailand. Nine fruit fly species in the genus *Bactrocera* were detected from 19 host plants that almost all plants are economically important species. Four fruit fly species including *B. caryeae*, *B.invadens*, *B. occipitalis* and *B. philippinensis* are first record in Thailand. However, they are closely related with *B. dorsalis*and ambiguous identification based on morphological criteria was found. The association between fruit fly species with family of host plants were found in *B. cucurbitae*, *B. latifrons* and *B. tau*.

DNA barcode is useful for species identification of fruit flies. Although the ambiguous identification of the members of *B. dorsalis* complex were found, however, at the wider sense (i.e. to differentiate *B. dorsalis* complex from other species) DNA barcode is of great efficiency. The COI barcoding sequences report in this study will be very useful for species identification of the immature stage where morphological identification is problemetic. In addition, the results revealed COI barcoding sequences can use to infer population genetic structure and population history of fruit fly species. In this case, both ongoing (i.e. ongoing gene flow) and historical factor (i.e. Pleistocene climatic change) played significant roles in determining genetic structure and diversity of fruit fly species in Thailand. Given that 87 species of *Bactrocera* fruit flies were reported in Thailand, the results of this study represent small fraction of such diversity. Further study should emphasize on remaining species that will enable us to fully understanding of biodiversity of these economically important pests. It is also equally important to study other aspects such as biology and ecology. These studies will provide significant information for effective management program for these economically important insects.

References

References

วิสุทธ์ ใบไม้ (2553) *ทำไมต้องทำวิจัยพื้นฐาน*. กรุงเทพฯ: โรงพิมพ์กรุงเทพ (1984) จำกัด.

วิสุทธ์ ใบไม้, เฉลียว กุวังคะดิลก, ปัทมาภรณ์ กฤตยพงษ์, สังวรณ์ กิจทวี, จอหน์มิลน์, ปรีชา ประเทพา และ ถาวร สุภาพรม (2545) *พันธุศาสตร์เชิงประชากรของ (1) แมลงที่มีความสำคัญทางเศรษฐกิจ ที่สัมพันธ์กับ Wolbachia และ (2) สัตว์สะเทินน้ำสะเทินบก*. รายงานการวิจัยฉบับสมบูรณ์ โครงการ “พันธุศาสตร์เชิงประชากรของ (1) แมลงที่มีความสำคัญทางเศรษฐกิจ ที่สัมพันธ์กับ *Wolbachia* และ (2) สัตว์สะเทินน้ำสะเทินบก”. กรุงเทพฯ, สำนักงานกองทุนสนับสนุนการวิจัย.

Aemprapa S (2007) Entomopathogenic fungi screening against fruit fly in Thailand. *KMITL Sci. Tech. J.*, 7: 122-126.

Aketarawong N, Bonizzoni M, Malacrida AR, Gasperi G and Thanaphum S (2006) Seventeen novel microsatellite markers from an enriched library of the pest species Bactrocera dorsalis sensu stricto. *Journal Molecular Ecology Notes*, 6(4): 1138–1140.

Aketarawong N, Chinvinijkul S, Orankanok W, Guglielmino CR, Franz G, Malacrida AR, Thanaphum S (2011) The utility of microsatellite DNA markers for the evaluation of area-wide integrated pest management using SIT for the fruit fly, *Bactrocera dorsalis* (Hendel), control programs in Thailand. *Genetica* 139(1): 129-140.

Allwood AJ (2000) Regional approaches to the management of fruit flies in the Pacific. In: Keng-Hong Tan (ed.) *Area Wide Control of Fruit Flies and Other Insect Pests.*, Sinaran Bros., Sdn. Bhd., Penang, Malaysia. pp. 439-448.

Allwood, A.J., Chinajariyawong, A., Drew, R.A.I., Hamacek, E.L. and Hancock, D.L. (1999). Host plant records for fruit flies (Diptera: Tephritidae) in South East Asia. *Raffles Bull. Zool.* Supplement No. 7: 1-92.

Allwood AJ and Leblanc L (1997) Losses caused by fruit flies (Tephritidae) in seven Pacific Island countries and territories (Diptera). In: Allwood AJ and Drew RAI (eds.) *Management of Fruit Flies in the Pacific. ACIAR Proceedings* No 76 pp. 208-211.

Aluja, M., and R. L. Mangan. (2008) Fruit fly (Diptera: Tephritidae) host status determination: critical conceptual, methodological and regulatory considerations. *Annu. Rev. Entomol.,* 53: 473–502.

Armstrong KF and Ball SL (2005) DNA barcodes for biosecurity: invasive species identification. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*. 360: 1813–1823.

Armstrong KF, Cameron CM and Frampton ER (1997) Fruit fly (Diptera: Tephritidae) species identification: a rapid molecular diagnostic technique for quarantine application. *Bull. Entomol.Res.*,87: 111-8.

Armstrong JW and Jang EB (1996) An Overview of Present and Future Fruit Fly Research in Hawaii and the US Mainland. In: Allwood AJ and Drew RAI (eds.) *Management of Fruit Flies in the Pacific: A regional symposium, 28-31 October 1996*, Nadi, Fiji, pp. 30-42.

Arita LH and Kaneshiro KY (1988) Body size and differential mating success between males of two populations of the Mediterranean fruit fly. *Pacific Science*, 42: 173–177.

Asoka R, Rebijith KB, Singh SK, Sidhu AS, Siddharthan S, Karanth PK, Ellango R and Ramamuathy VV (2011) Molecular Identification and Phylogeny of *Bactrocera* species (Diptera: Tephritidae). *Florida Entomologist*, 94(4): 1026-1035.

Avise JC (2000) *Phylogeography: the history and formation of species*. Harvard University Press, Cambridge, Mass.

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA and Saunders NC, (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Ann. Rev. Ecol. Syst.*, 18: 489–522.

Barcode of Life Database. available from: http://www.barcodinglife.com/ [Cited 10 April 2013].

Bateman MA (1972) The ecology of fruit flies. *Annu. Rev. Entomol.*,17, 493–518.

Bailey P and Cartwright D (1994) *Status of fruit flies in South Australia, SCARM fruit fly workshop: a national strategy for fruit fly research and development*. *Fruit* Fly Workshop Report, Greenmount.

Bandelt HJ, Forster P, Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16, 37–48.

Barrett RDH and Hebert PDN (2005) Identifying spiders through DNA barcodes. *Canadian Journal of Zoology*, 83, 481–491.

Ball SL, Hebert PDN, Burian SK and Webb JM (2005) Biological identifications of mayflies (Ephemeroptera) using DNA barcodes. *Journal of the North American Benthological Society*, 24(3), 508-524.

Baimai V, Phinchongsakuldit J and Trinachartvanit W (1999a) Mataphase karyotypes of fruit flies of Thailand. III. Six members of the *Bactrocera dorsalis* complex. *Zool. Stud.*, 38(1), 110-118.

Baimai V, Phinchongsakuldit J, Sumrandee C and Tigvattananont S (1999b) Mataphase karyotypes of fruit flies of Thailand. IV. Evidence for six new species of the *Bactrocera dorsalis* complex. *Cytologia*, 64, 371-377.

Baimai V, Phinchongsakuldit J, Sumrandee C and Tigvattananont S (2000a) Cytological evidence for a complex of species within the taxon *Bactrocera tau* (Diptera: Tephritidae) in Thailand. *Biological Journal of the Linnean Society*, 69, 399-409.

Baimai V, Phinchongsakuldit J, Sumrandee C, Tigvattananont S and Trinachartvanit W (2000b) Mataphase karyotypes of fruit flies of Thailand. V. Cytotaxonomy of ten additional new species of the *Bactrocera dorsalis* complex. *Zool. Cytologia,* 65, 409-417.

Baimai V, Trinachartvanit W, Tigvattananont S, Grote PJ, Poramarcom R and Kijchalao U (1995) Metaphase karyotypes of fruit flies of Thailand. I. Five sibling species of the *Bactrocera dorsalis* complex (Diptera: Tephritidae). *Genome*, 38, 1015–1022.

Baimai V, Trinachartvanit W, Tigvattananont S and Grote PJ (1996d) Metaphase karyotype of fruit flies of Thailand. II. Five species of four subgenera of *Bactrocera*. *J. Sci. Soc. Thailand*, 22, 97-104.

Bedo DG (1975) Polytene chromosomes of three species of blackflies in the Simulium pictipes group (Diptera: Simuliidae). *Can. J. Zool*, 53, 1147-1164.

Bedo DG (1986) Polytene and mitotic chromosome analysis in *Ceratitis capitata* (Diptera, Tephritidae). *Genet.Cylol*, 28, 180-188.

Bedo DG (1987) Polytene chromosome mapping in *Ceratitis capitata* (Diptera: Tephritidae).*Genome*, 29(4), 598-611.

Blaxter ML (2004) The promise of a DNA taxonomy. *Philosophical Transactions ofthe Royal Society of London.Series B, Biological Sciences*, 359(1444), 669-679.

Blacket MJ, Semeraro L and Malipatia MB (2012) Barcoding Queensland Fruit Flies (Bactroceratryoni): impediments and improvements. *Molecular Ecology Resources*, 12, 428–436.

Bohonak AJ (2002) IBD (Isolation by Distance): a programme for analysis of isolation by distance. *Journal of Heredity*, 93, 153–154.

Boore JL (1999) Animal mitochondrial genomes. *Nucleic Acids Research*, 27(8), 1767-1780.

Brower AVZ (1994) Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato*, inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences, USA*, 91, 6491–6495.

Brncic D, Nair PS and Wheeler MR (1971) Cytotaxonomic relationships within the *mesophragmatica* species group of *Drosophila*. *Univ Tex Publs*, 7103, 1-16.

Bush GL (1962) Thecytotaxonomy of the larvae of some Mexican fruit flies in the genus*Anastrepha* (Tephritidae, Diptera). *Psyche*, 69 (3), 87-100.

Christenson LD and Foote FH, (1960) Biology of fruit flies. *Annual Review of Entomology*.,5, 171-192.

Clarke AR, Allwood AJ, Chinajariyawong A, Drew RAI, Hengsawad C, Jirasurat M, Krong CK, Kritsaneepaiboon S and Vijaysegaran S (2001) Seasonal abundance and host use patterns of seven *Bactrocera* Macquart species (Diptera: Tephritidae) in Thailand and Peninsular Malaysia. *Raffles Bulletin of Zoology,* 49, 207–220.

Clarke AR, Armstrong KF, Carmichael AE, Milne JR, Raghu S, Roderick GK and Yeates DK (2005) Invasive phytophagous pests arising through a recent tropical evolutionary radiation: the *Bactrocera dorsalis* complex of fruit flies. *Annual Review of Entomology*, 50, 293–319.

Cognato AI (2006) Standard percent DNA sequence difference for insects does not predict species boundaries. *J. Econ. Entomol.*, 99(4), 1037–1045.

Courtice, AC and Drew RAI (1984) Bacterial regulation of abundance in tropical fruit flies (Diptera: Tephritidae). *Aust. Zool.,* 21, 251–268.

Cugala DR and Mangana S (1999) *Establishment and Maintenance of Fruit Flies Pest Free Area and/or Pest Free Places of Production in Mozambique*, Eduardo Mondlane University.

Cywinska A, Hunter FF and Hebert PD (2006) Identifying Canadian mosquito species through DNA barcodes. *Medical and Veterinary Entomology,* 20, 413-424.

Dogaç E, Kandemir İ and Taskin V (2013) The Genetic Polymorphisms and Colonization Process of Olive Fly Populations in Turkey. *Public Library of Science One*, 8(2), e56067.

Drew RAI (2004) Biogeography and speciation in the Dacini (Diptera: Tephritidae: Dacinae).*Bishop Mus. Bull. Entomol.*, 12, 165-178.

Drew RAI (1989) The tropical fruit flies (Diptera: Tephritidae: Dacinae) of the Australasian and Oceanian regions. *Memoirs of the Queensland Museum*, 26, 1–521.

Drew RAI, Courtice AC and Teakle DS (1983) Bacteria as a natural source of food for adult fruit flies (Diptera: Tephritidae). *Oecologia* (Berlin), 60,279–284.

Drew RAI and Hancock DL (1994a) The *Bactrocera dorsalis* complex of fruit flies (Diptera: Tephritidae: Dacinae) in Asia. *Bulletin of Entomolical Research, Suppelment No. 2. International Institute of Entomology,* 1-68.

Drew RAI and Hancock DL (2000) Phylogeny of the Tribe Dacini (Dacinae) based on morphological, distributional, and biological data. In: Aluja M, Norrbom AL (eds.) *Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior*. CRC Press, Boca Raton, pp. 491–504.

Drew RAI and Hancock DL (2001a) Phylogeny of the Tribe Dacini (Dacinae) Based on Morphological, Distributional, and Biological Data. In: Aluja A, Norrbom AL (eds.) *Fruit flies (Tephritidae): phylogeny and evolution of behavior*. CRC, Boca Ratón, FL, pp. 491–503.

Drew RAI and Lloyd AC (1987) Relationship of fruit flies (Diptera: Tephritidae) and their bacteria to host plants. *Ann. Entomol. Soc. Am.*,80, 629–636.

Drew RAI and Romig M (1996) Overview – Tephritidae in the Pacific and Southeast Asia. In: Allwood AJ and Drew RAI (eds.) *ACIAR Proceeding of Regional Symposium: Management of Fruit Flies in the Pacific*, Brown, Prior and Anderson, Melbourne, pp. 46-53.

Drew RAI and Romig M (2001b) The Biology and Behavior of Flies in the Tribe Dacini (Dacinae). In: Aluja A and Norrbom AL (eds.) *Fruit flies (Tephritidae): phylogeny and evolution of behavior*. CRC, Boca Ratón, FL, pp. 491–503.

Drew RAI, Tsuruta K and White IM (2005) A new species of pest fruit fly (Diptera: Tephritidae: Daciniae) from Sri Lanka and Africa. *Afr. Entomol*., 13(1), 149-154.

Dujardin JP and Kitthawee S (2013) Phenetic structure of two *Bactrocera tau* cryptic species (Diptera: Tephritidae) infesting *Momordica cochinchinensis* (Cucurbitaceae) in Thailand and Laos. *Zoology*, 116 (2), 129-38.

Ekesi S, Billah MK, Nderitu PW, Lux SA, and Rwomushana I (2009) Evidence for Competitive Displacement of *Ceratitis cosyra*by the Invasive Fruit Fly *Bactrocera invadens* (Diptera: Tephritidae) on Mango and Mechanisms Contributing to Displacement. *Entomological Society of America*, 102(3), 981-991.

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

Ferrar P (2010) *FRUIT FLIES IN ASIA (especially Southeast Asia) Species, biology and management. ACIAR***,** Available from: http://ipm.ait.asia [Cited 10 September 2012].

Feraday RM and Leonhardt KG (1989) Absence of population structure in black flies as revealed by enzyme electrophoresis. *Genome*, 32, 531-537.

Fitt GP (1990) Variation in ovariole number and egg size of species of *Dacus* (Diptera; Tephritidae) and their relation to host specialization. *Ecol. Entomol.* 15, 255–264.

Floyd R, Lima J, deWaard J, Humble L and Hanner R (2010) Common goals: policy implications of DNA barcoding as a protocol for identification of arthropod pests. *Biological Invasions*, 12 (9), 2947-2954.

Folmer O, Black M, Hoeh W, Lutz R and Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I form diverse metazoan invertebrates. *Molecular Marine Biology Biotechnology*, 3, 294–299.

Fu YX (1997) Statistical test of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, 147, 915–925.

Gariou-Papalexiou A, Gourzi P, Delprat A, Kritikou D, Rapti K, Chrysanthakopoulou B, Mintzas A and Zacharopoulou A (2002) Polytene chromosomes as tools in the genetic analysis of the Mediterranean fruit fly*, Ceratitis capitata. Genetica*, 116, 59-71.

Godwin ID, Mace ES, and Nurzuhairawaty (2001) Genotyping Pacific Island Taro (Colocasia esculenta (L.)Schott) germplasm. In: Henry RJ (ed.) *Plant genotyping: DNA fingerprinting,* Canada, CAB International. pp. 109-128.

Goergen G, Vayssières JF, Gnanvossou D and Tindo M (2011) *Bactrocera invadens* (Diptera: Tephritidae), a new invasive fruit fly pest for the Afrotropical region : host plant range and distribution in West and Central Africa. *Environmental Entomology*, 40, 844-854.

Hagen KS (1953) Influence of adult nutrition upon the reproduction of three fruit fly species. In *Third Special Report on the Control of the Oriental Fruit Fly (*Dacus dorsalis*) in the Hawaiian Islands*. The Senate of the State of California. pp. 72–76.

Hardy DE (1973) The fruit flies (Tephritidae - Diptera) of Thailand and bordering countries. *Pacific Insects Monograph*, 31, 1-353.

Harpending HC (1994) Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Human Biology*, 66, 591–600.

Hamada N and Adler PH (1999) Cytotaxonomy of four species in the *Simulium perflavum* Species-Group (Diptera: Simuliidae) from Brazilian Amazonia. *SystEntomol*, 24,273-288.

Hancock DL, Hamacek EL, Lloyd AC and Elson-Harris MM (2000) *The Distribution and Host Plants of Fruit Flies (Diptera: Tephritidae) in Australia*. Department of Primary Industries, Queensland, Brisbane.

Harris EJ, Liquido N and Spencer JP (2001) Distribution and host utilization of *Bactrocera latifrons* (Diptera: Tephritidae) on the island of Kauai. *Proceedings of the Hawaiian Entomological Society***,** 35, 55–56.

Hebert PDN (2007) BOLD: the Barcode of Life Data System (http://www.barcodinglife. org). *Mol Ecol Notes*, 7, 355–364.

Hebert PDN, Cywinska A, Ball SL and Dewaard JR (2003a) Biological identifications through DNA barcodes. *Philosophical Transactions of the Royal Society of London*. *Series B, Biological Sciences*, 270(1512), 313-321.

Houdt JKJ, Breman FC, Virgilio M and de Meyer M (2010) Recovering full DNA barcodes from natural history collections of Tephritid fruitflies (Tephritidae, Diptera) using mini barcodes. *Molecular Ecology Resources*, 10, 459–465.

Hogg ID and Hebert PDN (2004) Biological identifications of springtails (Collembola: Hexapoda) from the Canadian arctic, using mitochondrial DNA barcodes. *Canadian Journal of Zoology,* 82, 1–6.

Hunwattanakul N and Baimai V (1994) Mitotic karyotype of four species of fruit flies (*Bactrocera*) in Thailand. *Kasetsart J Nat Sci*., 28,142–148.

Huelsenbeck JP and Ronquist FR (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17(8), 754–755.

Hu J, Zhang LJ, Nardi F and Zhang RJ (2008) Population genetic structure of the melon fly, Bactrocera cucurbitae (Diptera: Tephritidae), from China and Southeast Asia. *Genetica*, 134, 319–324.

Iwahashi O (2001) Aedeagal length of the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), and its sympatric species in Thailand and the evolution of a longer and shorter aedeagus in the parapatric species of *B. dorsalis*. *Appl Entomol Zool*., 36(3), 289–297.

Jang EB (2007) Fruit flies and their impact on agriculture in Hawaii. *Hawaiian Entomological Society*, 39, 117-119.

Jaturat K (2007) *Relationship between Morphological Characteristics of Chilli fruits and infestation of fruit fly (Bactrocera latifrons (Hendel))*. M. Sc. thesis. Kasetsart University, Bangkok.

Jamnongluk W, Baimai V and Kittayapong P (2003a) Molecular phylogeny of tephritid fruit flies in the *Bactrocera tau* complex using the mitochondrial COI sequences. *Genome*, 46, 112-118.

Jamnongluk W, Baimai V and Kittayapong P (2003b) Molecular evolution of tephritid fruit flies in the genus *Bactrocera* based on the cytochrome oxidase I gene. *Genetica*, 119, 19-25.

Jitklang S, Kuvangkadilok C, Baimai V, Takaoka H, and Adler PH (2008) Cytogenetics andmorphotaxonomy of the *Simulium (Gomphostilbia) ceylonicum*species group (Diptera: Simuliidae) in Thailand. *Zootaxa*, 1917, 1–28.

Kapoor VC (2005) Taxonomy and Biology of Economically Important Fruit Flies of India. *Israel Journal of Entomology*, 35-36, 459-475.

Kaneta M, Sugimoto S and Ishihara H (1985) Discovery of *Dacuslatifrons*on Yonaguni Island.*Naha Plant ProtectionNews*,59, 294.

Kerr KCR, Stoeckle MY, Dove CJ, Wegt LA, Frances CM and Hebert PDN (2007) Comprehensive DNA barcode coverage of North American birds.*Molecular Ecology Notes*, 7, 535–543.

Kitthawee S (2000) Seasonal Occurrence of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), a Parasitoid of *Bactrocera correcta* (Bezzi) (Diptera: Tephritidae) in a Guava Orchard in Central Thailand. *ScienceAsia,* 26, 87-92.

Kitthawee S and Dujardin JP (2010) The geometric approach to explore the *Bactrocera tau*complex (Diptera: Tephritidae) in Thailand. *Zoology*, 113, 243-249.

Kittayapong P, Milne JR, Tigvattananont S and Baimai V (2000) Distribution of the Reproduction-modifying Bacteria, Wolbachia, in Natural Populations of Tephritid Fruit Flies in Thailand. *ScienceAsia*,26, 93-103.

Korneyev VA (1999) Phylogeny relationships among the families of the superfamily tephritoidea. In: Aluja A, Norrbom AL (eds.) *Fruit flies (Tephritidae): phylogeny and evolution of behavior*. CRC, Boca Ratón, FL, pp. 3–37.

Koveos DS and Tzanakakis ME (1993) Diapause aversion in the adult olive fruit fly through effects of the host fruit, bacteria, and adult diet. *Ann. Entomol. Soc. Am.*,86, 668–673.

Kumar P, AlmaLinda A, Ketelaar J and Shanmugam V (2011) *FIELD EXERCISE GUIDE ON FRUIT FLY INTEGRATED PEST MANAGEMENT: Area-Wide Integrated Pest Management of Fruit flies in South and Southeast Asian Countries*. Bangkok, Asian Fruit Fly IPM Project.

Kuvangkadilok C, Lualon U and Baimai V (2008) Cytotaxonomy of *Simulium siamense* Takaoka and Suzuki (Diptera: Simuliidae) in Thailand. *Genome*, 51(12), 972–987.

Leh TY (2004) *Identification of Bactrocera Genus (Diptera: Tephritidae) via Molecular Marker*. MSc. thesis. Malaysia University of Science and Technology.

Leblanc L, Balagawi S, Mararuai A, Putulan D, Tenakanai D and Clarke A (2001) Fruit flies in Papua New Guinea. *Plant Protection Service Secretariat of the Pacific Community Pest advisory leaflet*, 37, 12p.

Loxdale HD and Lushai G (1998) Molecular markers in entomology. Bulletin of *Entomological Research*, 88, 577-600.

Liquido NJ, Harris EJ and Dekker LA (1994) Ecology of *Bactrocera latifrons* (Diptera: Tephritidae) populations: Host plants, natural enemies, distribution, and abundance. *Annals of the Entomological Society of America*, 87, 71-84.

Liu L, Liu J, Wang Q, Ndayiragije P, Ntahimpera A, Nkubaye E, Yang Q and Li Z (2011) Identification of *Bactrocera invadens* (Diptera: Tephritidae) from Burundi, based on morphological characteristics and DNA barcode. *African Journal of Biotechnology*, 10(62), 13623-13630.

Mahmood K (2004) Identification of Pest Species in Oriental Fruit Fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) Species Complex. *Pakistan J. Zool*., 36(3), 219-230.

Mantel, N. (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, 27, 209–220.

Mavragani-Tsipidou P, Karamanlidou G, Zacharopou-lou A, Koliais S, and Kastritsis C (1992) Mitotic and polytene chromosome analysis in *Dacusoleae* (Diptera: Te-phritidae). *Genome*, 35, 373-378.

McLeod R (2005) *Management of fruit flies in the Pacific*, Impact Assessment Series Report No. 37

McQuate GT, Bokonon-Ganta AH and Peck SL (2007) Population Biology and Prospetcs for Suppression of the Solanaceous Fruit Fly, *Bactrocer alatifrons* (Diptera: Tephritidae). Prospetcs for *Bactrocera*. *Proceedings of the Hawaiian Entomological Society*,39, 111–115.

McKenzie C, Gillespie P and Hailstones D (2004) *Draft molecular diagnostic standard for fruit flies of economic significance to Australia*. NSW Agricultural Institute, Orange. 41 pp.

Medina FIS, Carillo PAV, Gregorio JS amd Aguilar CP (1998) The mating compatibility between *Bactrocera philippinensis* and *Bactrocera dorsalis*. In: Tan KH Penang (ed.) *5th International Symposium on Fruit Flies of Economic Importance*. 1–5 June., Malaysia, pp.155.

Meier R, Kwong S, Vaidya G, Ng Peter K L (2006) DNA Barcoding and Taxonomy in Diptera: a Tale of High Intraspecific Variability and Low Identification Success. *Systematic Biology*, 55, 715-728.

Meyer CP and Paulay G (2005) DNA barcoding: error rates based on comprehensive sampling. *PLoS Biol*., 3(12), e422.

Michailova P (1996) Cytotaxonomy of Chironomidae (Diptera) from Lake Shabla (Bulgaria): cytogenetic evidence for introgressive hybridization. *Hydrobiologia*, 318, 25-42.

Michailova P, Petrovan SG, Bovero S, Ramella L, Regoli F and Zelano V (2001) Genotoxic effects of chromium on polytene chromosomes of *Chironomus riparius* Meigen 1804 (Diptera Chironomidae). *Caryologia*, 54, 59-71.

Miller DR and Rossman AY (1995) Systematics, biodiversity, and agriculture. *BioScience,* 45*,* 680-686.

Morgan K, O'Loughlin SM, Chen B, Linton Y, Thongwat D, Somboon P, Fong MY, Butlin R, Verity R, Prakash A, Htun PT, Hlaing T, Nambanya S, Socheat D, Dinh TH and Walton C (2011) Comparative phylogeography reveals a shared impact of Pleistocene environmental change in shaping genetic diversity within nine *Anopheles* mosquito species across the Indo–Burma biodiversity hotspot. *Molecular Ecology*, 20, 4533–4549.

Muraji M and Nakahara S (2001) Phylogenetic relationships among fruit flies, *Bactrocera* (Diptera, Tephritidae), based on the mitochondrial rDNA sequences. *Insect. Mol.Biol.*,10, 549–559.

Muraji M and Nakahara S (2002) Discrimination among pest species of *Bactrocera* (Diptera; Tephritidae) based on PCR-RFLP of the mitochondrial DNA. *Appl. Entomol.Zool.*,37, 437–446.

Mwatawala M, Meyer MD, Makundi RH, Maerere AP (2006) Seasonality and host utilization of the invasive fruit fly, *Bactrocera invadens* (Diptera, Tephritidae) in central Tanzania. *J. Appl. Entomol.*, 130(9-10), 530-537.

Mwatawala M, Meyer MD, White IM, Maerere A and Makundi RH (2007) Detection of the solanum fruit fly, *Bactroceralatifrons* (Hendel) in Tanzania (Dipt., Tephritidae). *Journal of Applied Entomology*, 131(7), 501-503.

Nakahara LM (1977) *A Re-appraisal of the importance of fruit flies to Hawaii's Agricultural economy*. Hawaii Department of Agriculture, Agricultural Report Nov 21 1977.

Nakahara S, Masaki M, Kaneda M, Sugimoto T and Muraji M (2000) Identification of *Bactrocera dorsalis* complex species (Diptera: Tephritidae: Dacinae) by PCRRFLP analysis. I. A study of variation in mitochondrial DNA D-loop region. *Res. Bull. Pl. Prot. Japan*,36, 37–41 (in Japanese with English summary).

Nakahara S, Kato H, Kaneda M, Sugimoto T and Muraji M (2001) Identification of *Bactrocera dorsalis* complex species (Diptera: Tephritidae: Dacinae) by PCRRFLP analysis. II. A study of genetic variation in *B. dorsalis* complex (Philippines population) and *B. dorsalis* (Taiwan population). *Res. Bull. Pl. Prot. Japan*,37,69–73 (in Japanese with English summary).

Nei M (1987) *Molecular Evolution Genetics*. Columbia University Press, NY.

Norrbom AL, Carroll LE and Freidberg A (1998) Status of knowledge, In: Thompson FC (ed.) *Fruit Fly Expert Identification System and Systematic Information Database*. Myia, 9, pp. 9-47.

Norrbom AL, Carroll LE, Thompson FC, White IM and Freidberg A (1999) Systematic database of name; in Fruit Fly Expert System and Systematic Information Database. In: Thompson FC (ed.) Diptera Data Dissemination Disk1 & Myia. pp. 65-251.

Nopparat B, Zhihong L, Jiajiao W and Jiaqi L (2011)*Molecular identification of fruit fly larvae from Thailand based on DNA barcoding*. Plant Quarantine, 2011.Available from: http:// en.cnki.com.cn [Cited 20 September 2012].

Orankanok W, Chinvinijkul S, Sittilob P, Thanaphum S, Somboonprasert P and Enkerlin WR (2006) Integrating the sterile insect technique (SIT) for fruit fly control in Thailand. *In: Proceeding 7th economic fruit fly symposium, 10–15 September 2006*, Salvador, Bahia.

Orankanok W, Chinvinijkul S, Thanaphum S, Sittilob P, Enkerlin WR (2007) Area-wide integrated control of oriental fruit fly *Bactrocera dorsalis* and guava fruit fly Bactrocera correcta in Thailand. In: Vreysen MJB, Hendrichs J, Robinson AS (eds.) *Area-wide control of insect pests: from research to field implementation.* Springer, Dordrecht, pp. 517–526.

Pearson K (1990) On the criterion that a given system of deviations from the probable in the case of correlated system of variables is such that it can be reasonably supposed to have arisen from random sampling. *Philosophical Magazine Ser.*, 5(50), 157-172.

Peck SL and McQuate GT (2004) Ecological aspects of *Bactrocera latifrons* (Diptera: Tephritidae) on Maui, Hawaii: movement and host preference. *Environmental Entomology*, 33, 1722–1731.

Pinero JC, Jacome I, Vargas RI and Prokopy RJ (2006) Behavioral response of female melon fly, *Bactrocera cucurbitae* to host associated visual and olfactory stimuli, *Entomoligia Experimental Applicata*. pp. 261-269.

Plant Health Australia (2011) *The Australian Handbook for the Identification of Fruit Flies*.Version 1.0.Plant Health Australia. Canberra, ACT.

Poramakom R, Baimai V and Tigvattananont S (1994) Sexual behavior and signals used for attraction and mating of *Bactrocera correcta*. *4 th International Symposium on fruit flies of economic importance, 5-10 June 1994*, Sand Key, Florida, U.S.A. (Abst.) II-7.

Poramakom R and Baimai V (1996) Sexual behavior and signals used for mating of *Bactrocera coretca* In: Mcpheron BA and Steck GJ (eds.) *Fruit fly pest: A world assessment of their biology and management*. St. Lucia Press, pp. 51-58.

Pramual P (2006) *Population Genetic Structure of Black Flies (Diptera: Simuliidae) From Thailand Inferred From Mitochondrial DNA Sequences*. Ph.D. thesis. Mahidol University, Bangkok.

Pramual P, Gomontean B, Buasay V, Srikhamwiang N, Suebkar P, Niamlek C, Donsinphoem Y and Chalat-Chieo K (2008) Population cytogenetics of *Chironomus circumdatus* Kieffer, 1921 (Diptera, Chironomidae) from Thailand. *Genetica*, 135, 51-57.

Pramual P and Kuvangkadilok C (2012) Integrated cytogenetic, ecological, and DNA barcode study reveals cryptic diversity in *Simulium* (*Gomphostilbia*) *angulistylum* (Diptera: Simuliidae). *Genome*,55, 447–458.

Pramual P, Kuvangkadilok C, Baimai V and Walton C (2005) Phylogeography of the black fly *Simuliumtani* (Diptera: Simuliidae) from Thailand as inferred from mtDNA sequences. *Molecular Ecology*, 14(13), 3989–4001.

Prabhakar CS, Mehta PK, Sood P, Singh SK, Sharma P and Sharma PN (2012) Population genetic structure of the melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) based on mitochondrial cytochrome oxidase (COI) gene sequences. *Genetica*, 140, 83-91.

Pramual P, Wongpakam K and Adler PH (2011b) Cryptic biodiversity and phylogenetic relationships revealed by DNA barcoding of Oriental black flies in the subgenus *Gomphostilbia* (Diptera: Simuliidae). *Genome*, 54 (1), 1-9.

Raghu S (2003) *The autecology of Bactrocera cacuminata* (Hering) (Diptera: Tephritidae: Dacinae): *Functional significance of resources*. Ph.D. thesis. Griffith University, Brisbane. 240 pp.

Rattanapun W, Amornsak W and Clarke AR (2009) *Bactrocera dorsalis* preference for and performance on two mango varieties at three stages of ripeness. *Entomol. Exp. Appl*., 131, 243-253.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, 43, 223-225.

Rivera J and Currie DC (2009) Identification of Nearctic black flies using DNA Barcodes (Diptera: Simuliidae). *Molecular Ecology Resources*, 9, 224-236.

Roderick GK (1996) Population genetics studies of Tephritid flies of economic importance. In: BA McPheron and JS Gary (eds.) *Fruit fly pests: a world assessment of their biology and management*. St. Lucie Press, Florida, pp. 267 – 271.

Roger AR and Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, 9, 552–569.

Samie EMA and Fiky ZAE (2011) Molecular phylogeny and identification of the peach fruit fly, *Bactrocera zonata*, established in Egypt. *Journal of Insect Science*, 11, 1-11.

Sambrook J, Fritsch EF and Maniatis T (1987) *Molecular cloning: a laboratory manual*. 2nd ed. Cold spring Harbor Laboratory Press, USA.

Saelee A, TigvattananontS and Baimai V (2006) Allozyme electrophoretic evidence for a complex of species within the *Bactrocera tau* group (Diptera: Tephritidae) in Thailand. *Songklanakarin J. Sci. Technol*., 28(2), 249-259.

Schutze MK, Krosch MN, Armstrong KF, Chapman TA, Englezou A, Chomic A, Cameron SL, Hailstones D and Clarke AR (2012) Population structure of *Bactrocera dorsalis* s.s., *B. papayae* and *B. philippinensis* (Diptera: Tephritidae) in southeast Asia: evidence for a single species hypothesis using mitochondrial DNA and wingshape data. *BMC Evolutionary Biology*, 12, 130.

Shi W, Kerdelhué C and Ye H (2012) Genetic structure and inferences on potential source areas for *Bactrocera dorsalis* (Hendel) based on mitochondrial and microsatellite markers. *Public Library of Science One*, 7(5), e37083.

Shi W, Kerdelhue C and Ye H (2005) Population genetics of the oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae), in Yunnan (China) Based on Mitochondrial DNA sequences. *Environmental Entomology*, 34, 977–983.

Shimizu Y, Kohama T, Uesato T, Matsuyama T and Yamagishi M (2007) Invasion of solanum fruit fly *Bactrocera latifrons* (Diptera: Tephritidae) to Yonaguni Island, Okinawa Prefetcure, Japan. *Applied Entomology and Zoology*, 42, 269–275.

Singh RB (1991) Significance of fruit flies in fruit and vegetable production in the Asia-Pacific region. In: Vijaysegaran S and Ibrahim AG (eds.) *Proceedings First International Symposium on Fruit flies inthe Tropics*. *Kuala Lumpur, 1988*. Malaysian Agricultural Research and Development Institute, Kuala Lumpur. pp. 11- 29.

Sivinski JM (1993) Longevity and fecundity in the Caribbean Fruit flies (Diptera: Tephritidae) effects of mating, strain and body size. *Florida Entomologist*, 76(4), 635-644.

Slatkin M and Hudson RR (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics*, 129, 555–562.

Smith PT, Kambhampati S and Armstrong KA (2003) Phylogenetic relationships Among Bactrocera species (Diptera: Tephritidae) inferred from mitochondrial DNA sequences. *Mol Phylogenet Evol*., 26, 8–17.

Somta C, Winotai A and Ooi PAC (2010) Fruit flies reared from *Terminalia catappa*in Thailand. *Journal of Asia-Pacific Entomology*, 13, 27-30.

Stur E and Ekrem T (2011) Exploring unknown life stages of Arctic Tanytarsini (Diptera: Chironomidae) with DNA barcoding. *Zootaxa*, 2743, 27-39.

Swan DC (1949) *Fruit Flies*. Bulletin South Australia, Dept. of Agriculture.

Swofford DL (2002) PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4.b.10.Sinauer, Sunderland.

Tajima F (1989) Statistical methods for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123, 585–595.

Tajima F (1993) Simple methods for testing the molecular evolutionary clock hypothesis. *Genetics*, 135, 599-607.

Tan KH (2003) Interbreeding and DNA analysis of sibling species within the *Bactrocera dorsalis* complex. In Recent trends on sterile insect technique and area-wide integrated pest management - economic feasibility, control projects, farmer organization and *Bactrocera dorsalis* complex control study, pp. 113–122.

Tangkawanit U, Kuvangkadilok C, Baimai V and Adler PH (2009) Cytosystematics of the *Simulium tuberosum* group (Diptera: Simuliidae) in Thailand. *Zool. J. Linn. Soc*., 155(2), 289–315.

Tamura K., Peterson D, Peterson N, Stecher G, Nei M. and Kumar S (2011) MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular biology and evolution*, 28(10), 2731–2739.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F and Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.*, 25(24), 4876–4882.

Timm AE, Warnich L and Geertsema H (2007) Morphological and molecular identification of economically important Tortricidae (Lepidoptera) on tropical and subtropical fruit in South Africa. *African Entomology*, 15(2), 269-286.

Timm AE, Warnich L and Geertsema H (2008) Morphological and molecular identification of economically important Tortricidae (Lepidoptera) on deciduous fruit tree crops in South Africa. *African Entomology*, 16(2), 209-219.

Tsitsipis JA (1989) Nutrition In Fruit Flies: Their Biology, Natural Enemies and Control. In: Robinson AS and Hooper G (eds.) *World Crop Pests Vol. 3A. Elsevier Science Publishers*, Amsterdam. pp. 103–119.

Vargas RI and Nishida T (1985) Survey for *Dacus latifrons* (Diptera: Tephritidae). *Journal of Economic Entomology*,78,1311–1314.

Verheij EWM and Coronel RE (1992) Introduction Edible Fruits and Nuts. In: Verheij EWM, Coronel RE (eds.) *Plant Resources of South-East Asia. No. 2.*Bogor, Indonesia: PROSEA Foundation.

Vijaysegaran S and Osman MS (1991) Fruit flies in Peninsular Malaysia: their economic importance and control strategies. In: K Kawasaki, O Iwahashi and K Kaneshiro (eds.) *Biology and control of fruit flies (Proc. Int. Symp. Okinawa, Japan 1991)*. Ginowan, Okinawa, Japan, pp.105-115.

Wang X J (ed.) (1996) *The Fruit Flies (Diptera: Tephritidae) of the East Asian Region.* Science Press, Beijing, China.

Ward RD, Zemlak TS, Innes BH, Last PR and Hebert PDN (2005) DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society of London*. *Series B, Biological Sciences*, 360, 1847–1857.

Whitmore TC (1986) *Tropical Rainforests of the Far East*, 2nd ed. Oxford University Press, Oxford.352 pp.

White IM and Elson-Harris MM (1992) *Fruit flies of Economic Significance*. *Their Identification and Bionomics*, CAB International, Wallingford, UK.

Waugh WJ (2011) *DNA Barcoding the Birds of New Zealand*. Ph.D. thesis. Massey University, New Zealand.

Yong H S (1993) Allozyme variation in the solanaceous fruit fly *Bactrocera latifrons* (Insecta: Diptera: Tephritidae) from peninsular Malaysia. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*,106(4), 933-935.

Zacharopoulou A, Bourtzia K and Kerremans P (1990) A comparison of polytene chromosomes in salivary glands and orbital bristle trichogen cells in *Ceratitis capitata. Genome*, 34, 215-219.

Zacharopoulou A, Frisardic M, Savakisa C, Robinson S, Tolias P, Konsolaki M, Komitopoulou K and Kafatos FC(1992) The genome of the Mediterranean fruitfly *Ceratitis capitata:* localization of molecular markers by *in situ* hybridization to salivary gland polytene chromosomes. *Chromosoma*, 101, 448-455.

Zhimulev IF, Belyaeva ES, Semeshin VF, Koryakov DE, Demakov SA, Demakova OV, Pokholkova GV and Andreyeva EN (2004) Polytene chromosomes: 70 years of genetic research. *Int Rev Cytol*, 241, 203–275.

Zambetaki A, Kleanthous K and Mavragani-Tsipidou P (1995) Cytogenetic analysis of Malpighian tubule and salivary gland polytene chromosomes of *Bactrocera oleae* (Dacusoleae) (Diptera: Tephritidae). *Genome*, 38, 1070-1081.

Zhao JT, Frommer M, Sved J and Zacharopoulou A (1998) Mitotic and polytene chromosome analyses in the Queensland fruit fly, *Bactrocera tryoni* (Diptera : Tephriti-dae). *Genome*, 41, 510-526.

Biography

Biography

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Research output

1. **Kowit Meeyen**, Piyamas Nanork Sopaladawan, Pairot Pramual (2013) Population structure, population history and DNA barcoding of fruit fly *Bactrocera latifrons* (Hendel) (Diptera: Tephritidae). *Entomological Science* (in press). (Impact Factor = 0.981)
2. Pairot Pramual, **Kowit Meeyen**, Komgrit Wongpakam, Usa Klinhom (2013) Genetic diversity of Thai native chicken inferred from mitochondrial DNA sequences. *Tropical Natural History* (in press).