



**Interspecies Interaction of Fig Wasp Species and Their Association with  
*Ficus schwarzi* Koord.**

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**A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of  
Master of Science in Ecology (International Program)**

**Prince of Songkla University**

**2013**

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**Thesis Title** Interspecies Interaction of Fig Wasp Species and Their Association with *Ficus schwarzii* Koord.  
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ชื่อวิทยานิพนธ์	ปฏิสัมพันธ์ระหว่างชนิดของแตนมะเดื่อและความสัมพันธ์ที่มีต่อเตื่อเพาะ
ผู้เขียน	นางสาวจิราพรรณ ยิ้มแก้ว
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ปีการศึกษา	2556

### บทคัดย่อ

การศึกษานี้เป็นการศึกษาองค์ประกอบของชนิดและความสัมพันธ์ระหว่างชนิดของแตนมะเดื่อที่พบในเตื่อเพาะ (*Ficus schwarzii* Koord.) ซึ่งมีระบบการสืบพันธุ์แบบแยกเพศและแยกต้น (Dioecy) บริเวณเขตรักษาพันธุ์สัตว์ป่าโตนาช้าง จังหวัดสงขลา ภาคใต้ของประเทศไทย พบแตนมะเดื่อทั้งหมด 4 ชนิด โดยมี *Ceratosolen vetustus* Wiebes, 1994 เป็นตัวผสมเกสร ส่วนอีก 3 ชนิด ได้แก่ *Apocryptophagus* sp., *Philotrypesis* sp. และ *Apocrypta* sp. เป็นแตนมะเดื่อแบบไม่ผสมเกสรและแสดงความสัมพันธ์เชิงลบกับแตนมะเดื่อผสมเกสร จากการศึกษาความสัมพันธ์ของแตนมะเดื่อแต่ละชนิดด้วยวิธีวิเคราะห์สาเหตุ (Path analysis) โดยพิจารณาจากช่วงเวลาในการวางไข่ของแตนมะเดื่อ พบว่า *Apocryptophagus* sp. เป็นชนิดที่มีสำคัญในการแย่งชิงพื้นที่วางไข่กับแตนมะเดื่อผสมเกสร ในขณะที่ *Philotrypesis* sp. เป็นแมลงเบียนในแตนมะเดื่อผสมเกสร ส่วนแมลงเบียนชนิด *Apocrypta* sp. พบว่าไม่มีความจำเพาะเจาะจงต่อการเลือกแมลงอาศัย ความสัมพันธ์ระหว่างแมลงเบียนทั้ง 2 ชนิดนี้กับแมลงอาศัย (*C. vetustus* และ *Apocryptophagus* sp.) เป็นแบบไม่ขึ้นอยู่กับความหนาแน่น (host density independent) จากการศึกษาอิทธิพลของขนาด จำนวนดอก และความหนาของผนังเตื่อเพาะที่มีต่อจำนวนแตนมะเดื่อ พบว่าจำนวนของแตนมะเดื่อผสมเกสรจะเพิ่มตามขนาดของลูกและตามจำนวนดอก เมื่อลูกมะเดื่อมีขนาดใหญ่ จำนวนดอกที่แตนมะเดื่อผสมเกสรใช้ในการวางไข่ก็เพิ่มขึ้น ส่งผลให้จำนวนแตนมากขึ้น ส่วนความหนาของผนังเตื่อส่งผลทางลบต่อจำนวนแตนมะเดื่อผสมเกสร

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<b>Major Program</b>	Ecology (International Program)
<b>Academic Year</b>	2013

### **ABSTRACT**

The scope of this study was to investigate the species composition of fig wasps and their inter-relationships associated with a dioecious fig. The study focused on *F. schwarzii* Koord. at Ton Nga Chang Wildlife Sanctuary, Songkhla Province, southern Thailand. Four fig wasp species were found; *Ceratosolen vetustus* Wiebes, 1994 as a pollinator species, *Apocryptophagus* sp., *Philotrypesis* sp., and *Apocrypta* sp. as non-pollinator wasps. A total individual of non-pollinator wasps showed a significant negative correlation with the number of pollinators. An actual relationship between fig wasp species was conducted using path analysis based on the oviposition time sequences of each species. The results indicated that *Apocryptophagus* sp. was an important species competing for the same niche as the pollinator. *Philotrypesis* sp. took part as a parasitoid of the pollinator, whereas, *Apocrypta* sp. was considered to be a generalist for host selection. In the host and parasitoid association, the parasitoid (*Philotrypesis* and *Apocrypta*) showed an independent density to the host density (*C. vetustus* and *Apocryptophagus*). The influences of the fig diameter, the number of floret, and the wall thickness were measured and analysed. Increasing pollinator numbers were generally the result of bigger fig sizes and plentiful florets favourable for host utilisation. The wall thickness showed a negative effect to the number of pollinator populations.

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**LIST OF ABBREVIATIONS**

<b>Abbreviation</b>	<b>Term</b>
CFI	Comparative fit index
df	Degrees of freedom
HDD	Host density dependence
HDI	Host density independence
n	Sample size
NFI	Normed fit index
P	Path coefficient
p	Probability value
RMSEA	Root mean squared error of approximation
SD	Standard deviation
TLI	Tuker-Luwis index
TWS	Ton Nga Chang Wildlife Sanctuary

# CHAPTER 1

## INTRODUCTION

*“If you study figs, you will end up studying wasps.”*

--J. T. Wiebes, 1994–1999

*“If you study the wasps, you will end up studying their figs.”*

--Finn Kjellberg

There are more than 800 species of figs (Moraceae, *Ficus* spp.) throughout the tropical and sub-tropical regions (Silvius *et al.*, 2007), around 80–100 species of which are found in Thailand (Tarachai, 2008). Figs play an essential role as keystone plants during periods of insufficient resources (Lambert and Marshall, 1991). They can be divided by their breeding systems into 2 groups; monoecious and dioecious figs. In monoecious figs, male and female flowers are found in the same trees. In contrast, dioecious figs separate male and female trees, and fig wasps can reproduce only in male trees.

Fig wasps are insects in the superfamily Chalcididea associated with figs. Fig wasps play an important role in terrestrial ecosystems as the pollinator, parasitoid and parasite. They can be generally separated into pollinator and non-pollinator wasps. An obligate mutualism between a pollinator and fig, tree allows for reproductive success for both species, and represents a classic example of co-evolution (Weiblen, 2002; Dunn *et al.*, 2008; Cook and Segar, 2010; Yang *et al.*, 2012). From molecular and fossil data, the symbiosis of fig and pollinator wasp diverged at least 60 million years ago to maintain this specialized association (Rønsted *et al.*, 2005). The interaction between a pollinator wasp and its host is closely related along geographical distribution areas (Weiblen, 2002). It demonstrates a strong specificity according to the one-to-one rule, except in some cases when the interaction is broken by hosts switching, geographical isolation and evolutionary change (Kerdelhué *et al.*, 1999; Weiblen, 2002; Cook and Rasplus, 2003; Silvius *et al.*,

2007; Su *et al.*, 2008). Harrison (2003) reported that pollinator wasps in dioecious figs have short dispersal distances and this provides for a lower efficiency for linking wasp populations in isolated areas. In addition, pollinator species have a short lifespan of only up to 3 days (Nason *et al.* 1998; Harrison and Rasplus, 2006). Thus, a short lifespan and limited dispersal distance can have a big indirect effect on genetic exchange between pollinator populations.

Non-pollinator wasps, which are roughly divided into gall makers and parasitoids, are known to be the cause of fig and pollinator mutualistic instability (Dunn *et al.*, 2008). Several studies have shown a negative correlation between the number of pollinators and non-pollinators because of competition for resources which can cause either slight (Bronstein, 1991; Compton *et al.*, 1991) or major impacts on pollinator population (West and Herre, 1994; Kerdelhué and Rasplus, 1996; West *et al.*, 1996; Harrison, 2003). Nevertheless, there are some cases when there is no correlation shown between pollinator and non-pollinator populations (Cook and Power, 1996; Patel, 1998; Karunaratne, 2009). In some studies, a positive impact between them has been reported (Peng *et al.*, 2005b).

In Thailand, although the knowledge of diversity and distribution of figs is well known, the studies on their association with fig wasp species, either pollinator or non-pollinator, are still limited. Moreover, the relationship between fig, pollinators and non-pollinators is still poorly understood. In this study, a dioecious fig, *Ficus schwarzii* Koord. *sensu lato* (Subgenus *Sycomorus*) was chosen for investigating the wasp composition and elucidating population relationships between pollinator and non-pollinator wasps, and whether and/or how they might relate to each other. Although, *F. schwarzii* has recently been redefined into at least five species by using style characteristic and distribution data (Berg, 2010; Berg and Culmsee, 2011), the name, *F. schwarzii*, is still referred to in this study throughout. *F. schwarzii* is a pioneer species (Sinbumroong, 2009) and is usually found in disturbed forests (Clement and Ushihara, 2004). It is a native species in southern Thailand (Chantarasuwan and Thongaree, 2004), and widely distributed from peninsular Thailand to peninsular Malaysia, Sumatra and Borneo (Berg and Corner, 2005). Kameyama *et al.* (1999) and Harrison (2003) reported that *F. schwarzii* in Borneo

Island is pollinated by only one wasp species, *Ceratosolen vetustus* Wiebes, 1994. However, most studies on *F. schwarzii* were carried out only in Borneo and its pollinator species have not been studied elsewhere. Previous surveys have indicated that *F. schwarzii* is abundantly distributed in Ton Nga Chang Wildlife Sanctuary (TWS), in Songkhla Province, southern Thailand. Therefore, TWS was therefore chosen as a study site to examine the association between fig trees and fig wasps.

From the above fig and fig wasp biological information, three main questions are addressed to enhance our knowledge. Firstly, how many fig wasp species are associated with *F. schwarzii*. Secondly, do non-pollinator wasps affect the pollinator wasp population in *F. schwarzii*. And thirdly, how do some measured traits of fig (diameter, wall thickness, and number of florets) influence the number of fig wasps, especially the pollinator population.

### **Objectives**

1. To determine the composition of fig wasp species in *F. schwarzii*.
2. To examine if non-pollinator wasps have any impact on pollinator wasps in *F. schwarzii*.
3. To investigate the effect of some measured traits of fig on the abundance of fig wasps.

### **Hypotheses**

1. There are more than one species each of pollinator and non-pollinator wasps in *F. schwarzii*.
2. Non-pollinator wasps have a negative impact on the pollinator population by reducing the number of pollinators.
3. Certain fig characteristics play a role in influencing the number of fig wasps.

## CHAPTER 2

### LITERATURE REVIEW

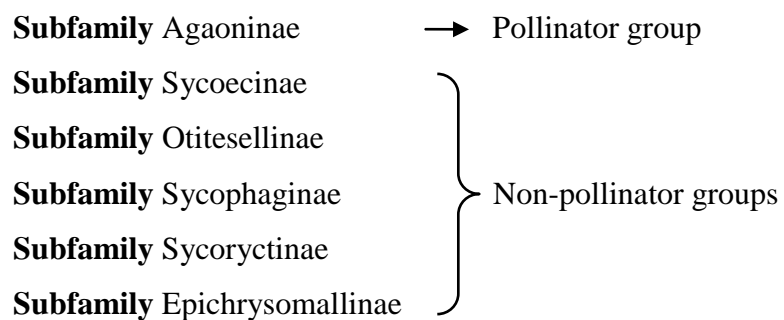
#### 2.1 Fig wasp classification

Fig wasps are tiny insects in the Phylum Arthropoda, Class Hexapoda, Order Hymenoptera as same as ants, bees, sawflies and horntails. A narrow junction between the thorax and the abdomen called “wasp waist” is one characteristic that helps to classify this order into 2 suborders: Symphyta and Apocrita. Sawflies and horntails in the former suborder have an unconstructed wasp waist. However, another group with a constructed wasp waist is in the latter suborder such as ants, bees and wasps. According to Bouček’s classification (1988) based on morphological characteristics, fig wasps are in the family Agaonidae and can be classified into one subfamily of pollinating fig wasp and five subfamilies of non-pollinating fig wasps, including parasitoids and galls as explained below.

#### Suborder Apocrita

##### Superfamily Chalcidoidea

##### Family Agaonidae



Rasplus *et al.* (1998) demonstrated molecular phylogeny from 28S rRNA and morphological evidences from post-occipital structures to show that fig wasps in the family Agaonidae are not monophyletic. This finding was also supported by Campbell *et al.* (2000) who also used 28S rDNA. However, Cruaud *et al.* (2010) showed that this family was a monophyletic group supported by six genes from



parsimony and Bayesian inference, and categorised the family into three subfamilies. Non-pollinators in the subfamily Sycophaginae were also reclassified in the family Agaonidae by using 18S and 28S rDNA (Heraty *et al.*, 2013). For other non-pollinator wasps, they were categorised into 4 families; Pteromalidae, Ormyridae, Eurytomidae, and Torymidae as explained below (Van Noort and Rasplus, 2004).

### **Suborder Apocrita**

#### **Superfamily Chalcidoidea**

##### **Family Agaonidae**

**Subfamily Agaoninae**

**Subfamily Kradibiinae**

**Subfamily Tetrapusiinae**

**Subfamily Sycophaginae**

##### **Family Pteromalidae**

**Subfamily Colotrechinae**

**Subfamily Epichrysomallinae**

**Subfamily Otitesellinae**

**Subfamily Pteromalinae**

**Subfamily Sycoecinae**

**Subfamily Sycoryctinae**

##### **Family Ormyridae**

##### **Family Eurytomidae**

##### **Family Torymidae**

} Pollinator groups

} Non-pollinator groups

## **2.2 The host utilisation**

There are about 800 fig species worldwide (Silvieus *et al.*, 2007). The host utilisation by pollinator wasps depends on the fig breeding system (Weiblen, 2002). About half of the fig species are monoecious figs that have a male flower and both of short and long-styled female flowers on the same tree that develops seeds, pollens and wasps in the same fruit (Weiblen, 2002). The other half is dioecious figs

that have male and female flowers on different trees. Female trees called ‘seed figs’ contain only long-styled female flowers while male trees called ‘gall figs’ have both male and short-styled female flowers. Fig wasps can reproduce in only the gall fig (Fig. 1) because of the consistent relationship between short-styled female flowers and the length of the fig wasp’s ovipositor that allows them access to the oviposition (Nefdt and Compton, 1996; Weiblen, 2004; Shi *et al.*, 2006). However, fig wasps also enter the seed fig because they are unable to distinguish seed and gall figs (Grafen and Godfray, 1991; Weiblen *et al.*, 2001; Cook and Rasplus, 2003).

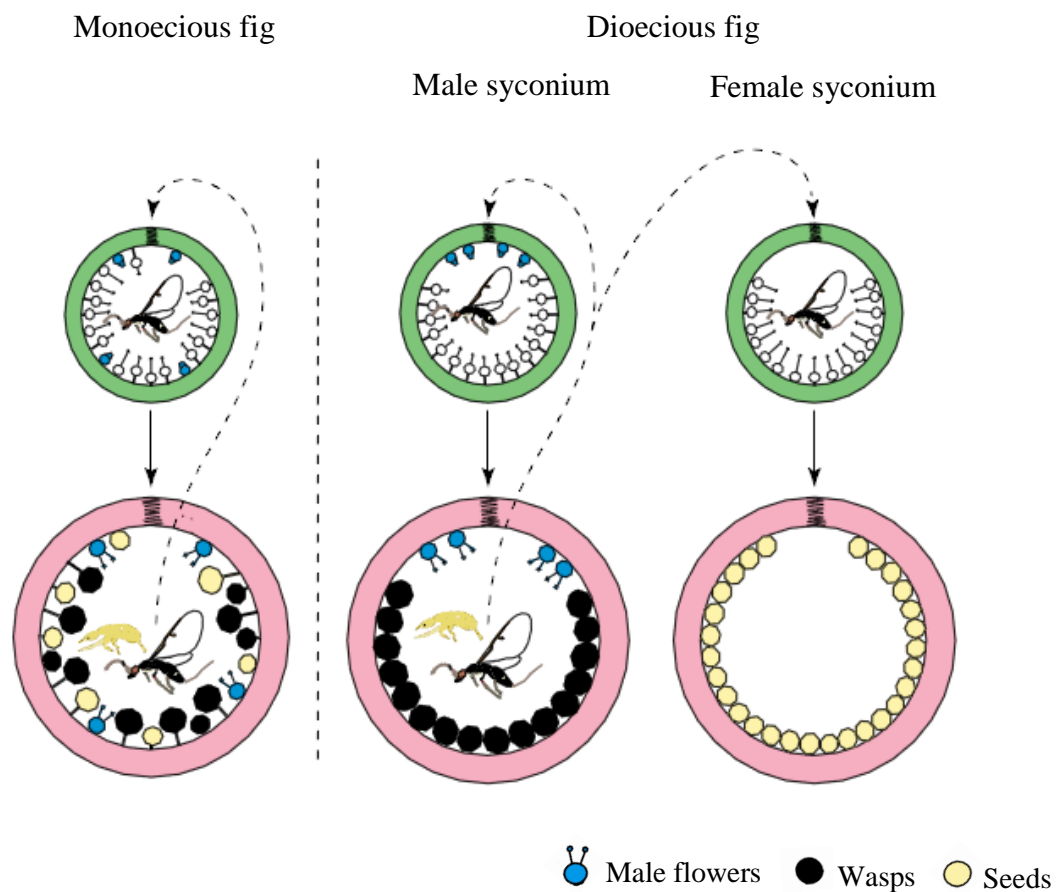


Figure 1. Growth stages from young (green) to ripe (pink) in a monoecious fig produce both seeds and wasps, while in dioecious fig only the male syconium can produce wasps (Cook and Rasplus, 2003).

### 2.3 The fig wasp life cycle

The fig-fig wasp interaction covers the range from mutualism to parasitism (Weiblen *et al.*, 2001). Pollinator wasps play an important role and also are a classic example of obligate mutualism (Weiblen, 2002; Cook and Rasplus, 2003; Dunn *et al.*, 2008). The life cycle of fig-fig wasps begins with female pollinators being attracted by pheromones released from the young fig's stigmas when they are in the receptive phase. A female fig wasp that enters the ostiole called a foundress. The wings and antennae of the foundress are lost during entrance through the ostiole (tiny inflorescence bracts at the apex of the fig). Foundresses then lay their eggs in a syconium (fruit of the fig with urn-shaped inflorescence concealed by a receptacle) by using their ovipositors to penetrate into the style of the fig flowers and deposit their eggs between the integument and the nucellus (Fig. 2). They can also pollinate by depositing pollen from their thoracic pockets (active pollinator) or haphazardly transfer pollen (passive pollinator) into the fig stigmas. Then, the foundresses die and the larvae develop to pupae in the flower concurrently with the developing fig.

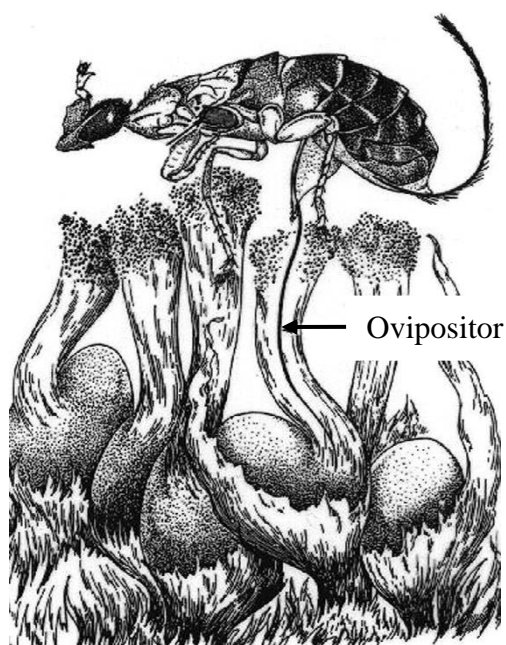


Figure 2. Ovipositing point of *Ceratosolen arabicus* Mayr, pollinator of *F. sycomorus* (Galil and Eisikowitch, 1968).

During the fig development, non-pollinators also find a fig to lay their eggs outside fig wall through long ovipositor for example in Fig. 3, a galler induces gall inside a syconium by penetrating with its long ovipositor. There are three groups of non-pollinating fig wasps: gallers that can induce galls by inserting their ovipositor outside the fig, gallers that can act like pollinator, and their parasitoids or inquilines (Weiblen, 2002). An inquiline is a phytophagous in the induced gall used to eliminate host larvae (Elias *et al.*, 2008) by starving it to death (Kerdelhué and Rasplus, 1996) This differs from the parasitoid by directly feeding on its host in its immature state (Roberts and Janovy, 2009). Non-pollinators in the subfamily Sycoecinae can compete with pollinator wasps by internal oviposition as a consequence of a convergence after selection of a head shape between the Sycoecinae and Agaoninae (Van Noort and Compton, 1996). Kerdelhué and Rasplus (1996) demonstrated the diet of non-pollinators in *Ficus* within the subgenus *Sycomorus* as shown in Fig.4 and can be divided into 4 phases along the fig diameter: gallers arrive before, at the same time and after the pollinator for a few days and their parasitoids (feed directly on the host larva) or inquilines (as parasite that makes galler larva starve).

When the figs are ripe, male offspring emerge from their galls before females. Most of them do not have wings, so male wasps cannot fly to find a young fig in the way that females can. Males have two functions, namely mating with females within a gall and chewing a hole for the female to escape to find a young fig. Pollen are also spread by attachment to the female's legs or are kept in the pollen pockets during breakout by the female pollinators. Female offspring search for a young fig by olfactory attraction to the source of the pheromone emitted by a young stigma. Pollination and reproduction of fig wasps will occur successfully when female pollinators enter a young fig via its ostiole to begin fig-fig wasp life cycles in the next generation. For female non-pollinators, laying their eggs throughout the fig surface and lacking the pollen-loading adaptation in gallers that enter to fig cavity are the cause of pollination failure (Kerdelhué and Rasplus, 1996).

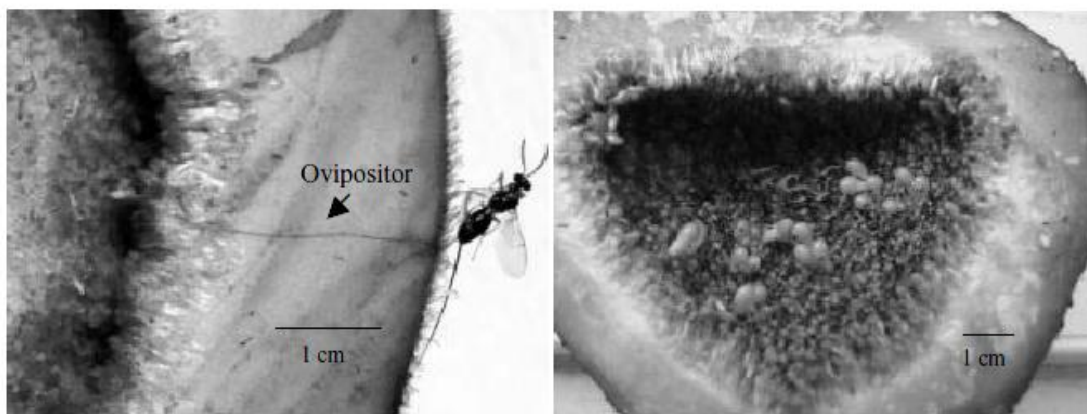


Figure 3. Egg laying of *Apocryptophagus* sp. through long ovipositor outside fig wall (left) and inducing gall inside a syconium of *Ficus auriculata* (right) (Peng *et al.*, 2005a).

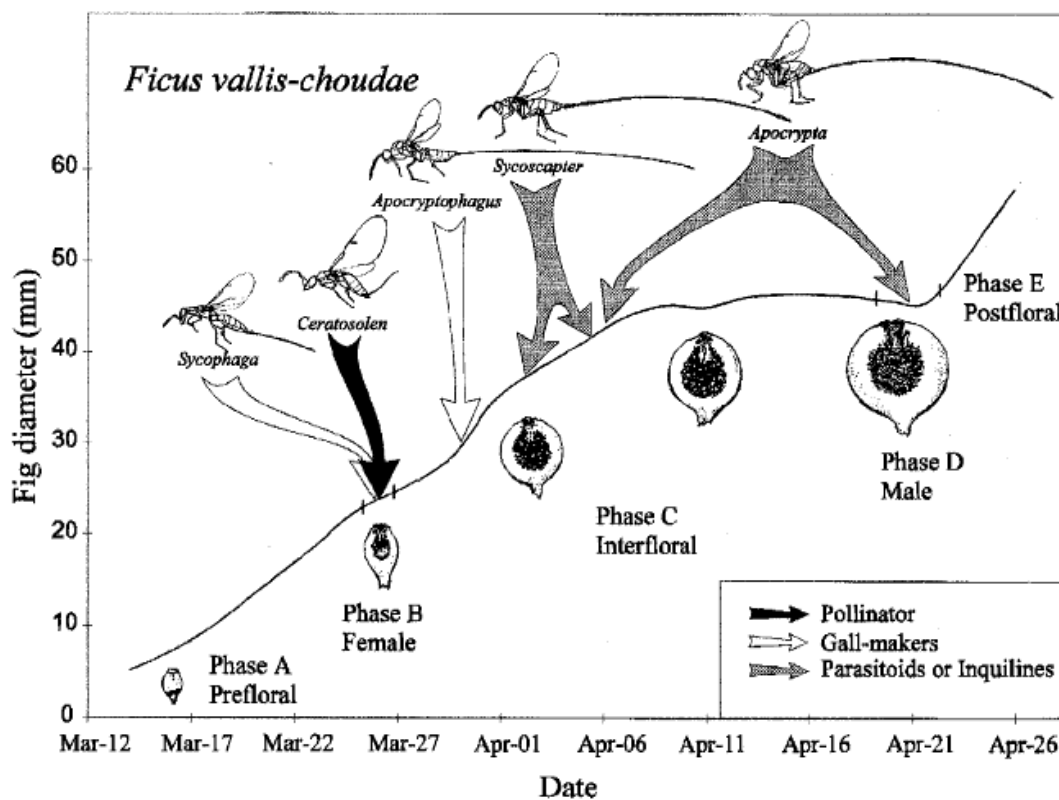


Figure 4. Growth curve of *Ficus vallis-chouae* Delile found fig wasps in each phase from early receptive phase to the full mature fig (Kerdelhué and Rasplus, 1996).

## 2.4 Host specificity in a fig wasp

Distribution patterns show a closely related spatial distribution between pollinators and their host (Weiblen, 2002). Many species of pollinator wasps are specific to one species of fig according to the one-to-one rule. Nowadays, some publications have revealed that wasps lose host specificity by the processes of host switching (Su *et al.*, 2008), geographic isolation, host dependence (Weiblen, 2002; Silvieus *et al.*, 2007) or by different divergence times between fig-wasps (Machado *et al.*, 2005). For example, there are two pollinators in *Ficus sur* Forsskål.; *Ceratosolen flabellatus* Grandi, 1916 and *C. silvestrianus* Grandi, 1916 that resulted from allopatry of *C. flabellatus* now speciated into *C. silvestrianus* without fig diversification within an ecological barrier or shifts in habitat (Michaloud *et al.*, 1996; Kerdelhué *et al.*, 1999). Nonetheless, Kerdelhué *et al.* (1997) reported *C. silvestrianus* and *C. flabellatus* as sympatric active pollinators both found in *F. sur* due to niche partitions within a syconium. In addition, *Ceratosolen galili* Wiebes, 1964 and *C. arabicus* Mayr, 1906 are two pollinators associated with *Ficus sycomorus* L. because their host is not dependent on their pollinators and areas into which the host shifts. *C. galili* changes its host from one species to other species in different geographic areas, while the old host gradually becomes extinct (Kerdelhué *et al.*, 1999).

## 2.5 Biology of *Ficus schwarzii* Koord. *sensu lato* and their wasps

During a period of insufficient food, figs (Moraceae, *Ficus* spp.) are keystone plants for maintaining the population of frugivores because they provide fruits that are carbohydrate-rich, especially in Southeast Asia and the Neotropics (Lambert and Marshall, 1991). *F. schwarzii* Koord. *sensu lato* (a dioecious fig in subgenus *Sycomorus*, section *Sycocarpus*, subsection *Sycocarpus*) is a pioneer species in lower canopy vegetation at a maximum height of 16 meters height (Sinbumroong, 2009). It is usually found in disturbed forests (Clement and Ushihara, 2004). Berg and Corner (2005) reported that this species is distributed from lower Myanmar,

Thailand, Malay Peninsula, Sumatra and Borneo including Anambas and Natoena (Natuna) Islands as shown in Fig.5 and are also found among streams at altitudes of up to 1,200 meter. In Thailand, Chantarasuwan and Thongaree (2004) documented that this species is commonly found in southern Thailand, and is being used as a vegetable by local people (Chantarasuwan, 2009). Moreover, young leaf and young fruit of this species are usually used as an ingredient in curry with coconut milk or Thai spicy mixed vegetable soup (Thungsong Municipal Public Library, 2009).

Although, Berg (2010) reported this species is heterogeneous and split it up at least into Sulawesi and another distribution. In this study, *F. schwarzii* was the term used in a broad sense to scope only on fig wasp composition.



Figure 5. Distribution of *F. schwarzii* (dot-dash line) from lower Myanmar, Thailand, Malay Peninsula and Sumatra to Borneo including Anambas and Natoena Islands (Berg and Corner, 2005).

The phenology of *F. schwarzii* has been documented by Kameyama *et al.* (1999) into 4 phases;

1. Receptive phase is the phase when wasps enter the syconia (3-21 days).
2. Interfloral phase is the phase of growth of the syconia (39 days).
3. Emergence phase is the phase when wasps emerge from the gall within the syconia (12 days).
4. Interval phase is the phase when the wasps emerge to find a new fig in the next receptive phase (49days).

Thus, a complete life cycle totals approximately 103 days. This result is similar to the study of Harrison (2003) that showed the duration of the interfloral phase in male and female figs of this species to be  $39.0 \pm 5.2$  and  $85.1 \pm 14.7$ , and the emergence phases for the wasps of both are  $13.9 \pm 3.6$  and  $32.0 \pm 12.7$  days, respectively.

Kameyama *et al.* (1999) used a computer simulation model to explain how wasp populations can be maintained in the small number of *F. schwarzii* in Borneo. The result showed that differences between the number of fig trees and timings of the fig's receptive phase could account for the wasp survival rate. Increasing the number of fig trees maintained a higher wasp population than when the number reduced. Also, in this case, as the number of fig trees decreased the pollination and wasp survival rates were increased by an expanded duration of the fig's receptive phase by up to 21 days.

Furthermore, Harrison (2000) reported that *F. schwarzii* still flowers but less frequently in the dry conditions at Lambir Hills National Park in Borneo. A graph showing the relationship between the month and the number of pollinated syconia implied a frequent flowering of *F. schwarzii* except during the drought period (Harrison, 2000). This finding is similar to that of Kameyama *et al.* (1999) at the same site that *F. schwarzii* flowered continuously. This is another reason, why fig pollinating wasp populations can survive in their habitat.



Harrison (2003) documented the genera of pollinating fig wasps in dioecious figs as; *Blastophaga*, *Ceratosolen*, *Kradibia*, *Lipporhopalum* and *Wiebesia*. In Borneo, many studies have shown *Ceratosolen vetustus* Weibes, 1994 is the pollinator of *F. schwarzii* (Kameyama *et al.*, 1999; Harrison, 2003; Moore *et al.*, 2003; Harrison and Shanahan, 2005). Moreover, Harrison and Shanahan (2005) also reported another fig wasp species in a non-pollinator group that was found in this fig; *Apocryptophagus* sp. is a galler, *Apocrypta* sp. is a parasitoid, and *Philotrypesis* sp. is an inquiline.

The short lifespan and limitation of dispersal seem to be restrictive factors that control the reproduction of fig-pollinating wasps. A wasp lifespan varies depending on the species and natural conditions such as climatic changes and predation. For example, *Blastophaga psenes* L. can survive at least 2 days in natural conditions (Kjellberg *et al.*, 1998), but female *Blastophaga* sp. may survive for only up to 4 or 5 hours in a dry period or during natural catastrophes (Janzen, 1979).

In addition, although the long-distance dispersal of fig wasps, is believed to be mainly by passive dispersal (e.g. by wind) this has not been confirmed experimentally. However, it could be examined by analysing fig paternity. By this technique, for example, Nason *et al.* (1998) reported the distance over which fig wasps can disperse can be from 5.8–14.2 km. Harrison (2003) proposed that the dispersal distance of a pollinator was influenced by physical factors such as dispersal behaviour (active or passive), diurnal activity (day or night), flight heights of wasp and density of hosts. In addition, dioecious fig pollinators have more restricted dispersal ranges than monoecious fig pollinators (Harrison and Rasplus, 2006).

## CHAPTER 3

### MATERIALS AND METHODS

#### 3.1 Study sites

Ton Nga Chang Wildlife Sanctuary (TWS) is a part of Ban-tad Mountain Range, Southern Thailand. Its geological structure is mostly composed of granite stone (Kaew-on, 1996). The vegetation in TWS was first described as a lower tropical rain forest (Smitinand, 1977). Later, in 1984, Whitmore studied the relationship between vegetational structure and the environment and described the forest as a tropical semi-evergreen rain forest. This study was initially surveyed for the occurrence of *F. schwarzii* depending upon the accessibility of routes around TWS in Songkhla and Satun provinces. A total of eight survey sites were chosen (Fig. 6). A list and survey position of all eight sites are as follow.

- Wang Pa Guard Station (S1) in Hat Yai District, Songkhla Province (6° 53.739'N, 100° 14.048'E)
- Pha Dum Guard Station (S2) in Sa Dao District, Songkhla Province (6° 47.164'N, 100° 13.648'E)
- Pa Nan Guard Station (S3) in Khuan Kalong District, Satun Province (6° 51.444'N, 100° 9.730'E)
- Ka Ming Guard Station (S4) in Khuan Kalong District, Satun Province (6° 49.161'N, 100° 9.055'E)
- Boriphat Waterfall (S5) in Rattaphum District, Songkhla Province (6° 59.696'N, 100° 8.791'E)
- Ton Plew Waterfall (S6) in Khuan Don District, Satun Province (7° 0.170'N, 100° 14.107'E)
- Klong Jum Lai Guard Station (S7) in Khlong Hoi Khong District, Songkhla Province (6° 51.568'N, 100° 15.176'E)
- Ton Nga Chang Waterfall (S8) in Hat Yai District, Songkhla Province (6° 57.173'N, 100° 14.113'E)

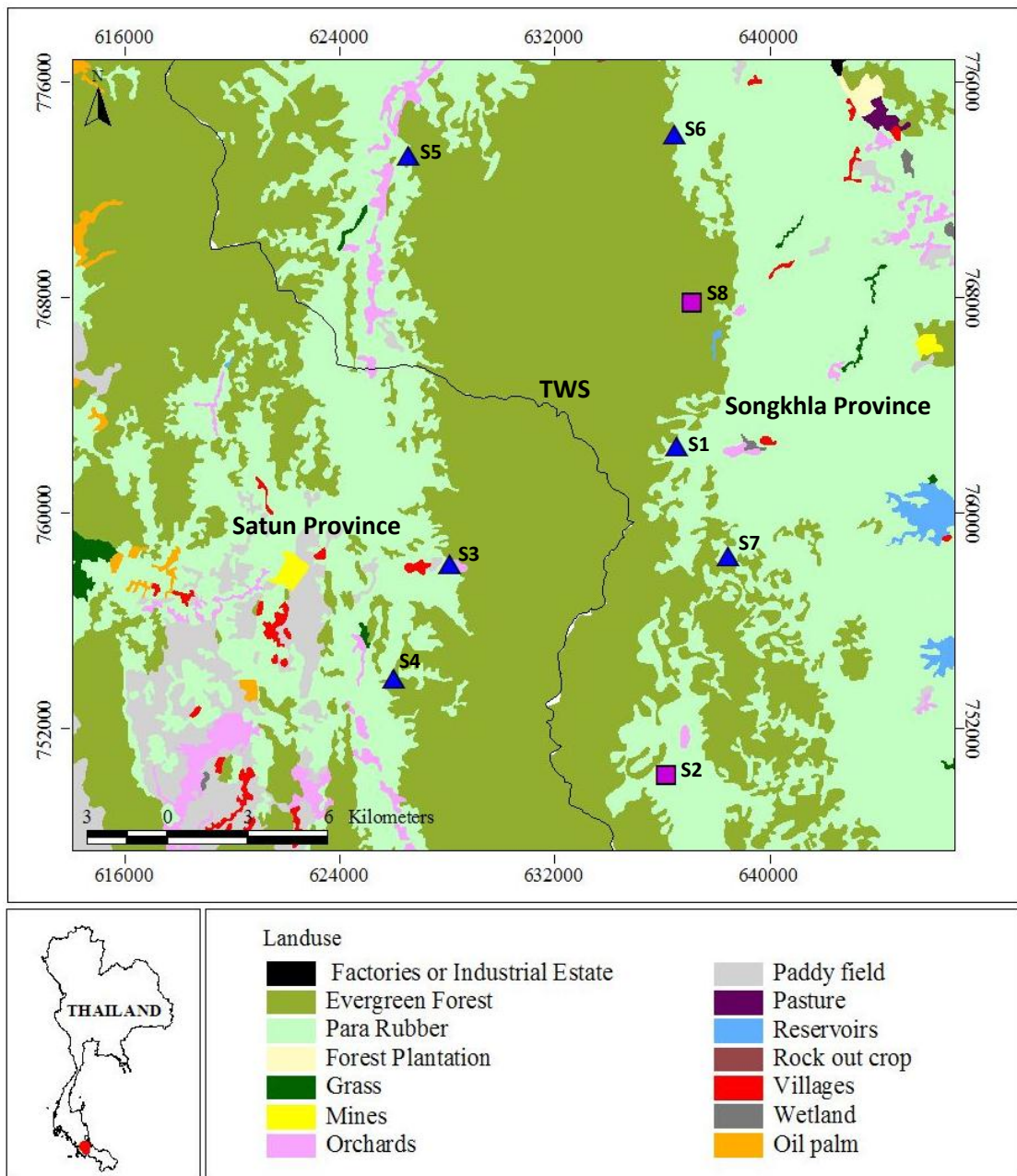


Figure 6. Locality of study sites in TWS; ■ represents sites that *F. schwarzii* were found, ▲ represents sites that *F. schwarzii* were not found. (Land-used source: Royal Thai Survey Department, 2002 and Landsat MT5, 2009. Printed by: GEO-Informatics Research Center for Natural Research and Environment, Prince of Songkla University, Thailand.)

From the initial study, *F. schwarzii* was found at only two sites at Pha Dum Guard Station (S2) and Ton Nga Chang Waterfall (S8). The distance between the two sites is approximately 15.5 km. These two sites had been disturbed by logging and tin mining since 1969 and the activities stopped in 1972 (personal communication). Therefore, two sites were disturbed and the general characteristics match the habitat description of a disturbed forest (Clement and Ushihara, 2004). General information including collecting trail of these two sites is described below.

**Pha Dum Guard Station (S2)** is located near the Thai-Malaysia border. The vegetation in this site is mostly composed of upper and lower canopy i.e., Annonaceae, Dipterocarpaceae, Fabaceae, Malvaceae, Moraceae, Sapindaceae and understory shrub and seedling in the families Dilleniaceae and Zingiberaceae (Fig. 7). An average temperature and relative humidity are around 27.5 °C and 76.8% respectively. Collecting sites have an elevation range of 23 to 220 meters above sea level. In the forest, there are two trails running along a stream from a waterfall. The first trail is the Kai Chon trail with a distance of 1,675 meters along a stream to Kai Chon Waterfall. The second, called the Chang Phuak trail located at the edge of the forest connected to a rubber plantation with a distance of 1,700 meters along a stream to Chang Phuak Waterfall. From the preliminary study, 34 and 31 gall trees of *F. schwarzii* were found in Kai Chon and Chang Phuak trails, respectively.

**Ton Nga Chang Waterfall (S8)**, including its natural trails, situated in TWS's main office area and is also a favourite place for tourism. This waterfall can be divided by different layers into seven cascades. The vegetation at this site is the same as at the Pha Dum Guard Station (Fig. 8). The average temperature and relative humidity are around 28.6 °C and 68.2% respectively. The elevation of the collecting sites ranges from 40 to 226 meters above sea level. This study was carried out along the two trails: the first trail is a man-made natural trail that starts from the second layer of the waterfall and ends at the camping center zone, approximately 2,200 meters long; the second trail is in the forest patch nearby the camp houses and it is a long natural trail of about 3,300 meters in length. The stream of this second trail is a branch of the major stream that runs from Ton Nga Chang Waterfall. From the preliminary study, 9

and 28 gall trees of *F. schwarzii* was found in the first and the second trail respectively.





Figure 7. Vegetation in Pha Dum Guard Station.



Figure 8. Vegetation in Ton Nga Chang Waterfall.

## 3.2 Collecting data

### 3.2.1 Species composition of fig wasps in *F. schwarzii*

#### Field work

To study the species composition of the fig wasps in *F. schwarzii*, field collection was carried out every week from May to October 2009. The gall trees bearing figs in an early emergence phase (the phase when wasps emerge from the gall with the fig still closed, Kameyama *et al.* 1999) with perfect skin condition were chosen for collecting. There were 18 and 12 gall fig trees in such conditions at Pha Dum Guard Station and Ton Nga Chang Waterfall, respectively. One to three ripe gall figs in an emergence phase were randomly collected from each tree in each study site. A total of thirty figs from each study site was collected. In addition, the foundresses from two seed figs in a receptive phase (the phase when wasps enter the syconia, Kameyama *et al.* 1999) were collected to ensure if the pollinator species in *F. schwarzii* is the same species as in a gall fig.

Each collected fig was placed into a 6x8 inches zip-locked plastic bag (one fruit per bag) until the emergence of fig wasps. The date of collection, GPS coordinates, collecting code and collector name were recorded.

#### Laboratory work

Fig wasps that emerged from the figs were collected from the zip-locked bags. In addition, the figs were dissected for collecting fig wasps that were still inside the figs. Both pollinator and non-pollinator fig wasps were identified to species following Wiebes (1994), Bouček (1988), Cruaud *et al.* (2010), and Heraty *et al.* (2013). The number of each species was counted using a stereomicroscope. Fig wasp specimens in each species were finally preserved in 95% ethanol in a microtube and deposited at Princess Maha Chakri Sirindhorn Natural History Museum, Prince of Songkla University.

### **3.2.2 The inter-relationship of fig wasp species and their association with some measured traits of *F. schwarzii***

#### **Field work**

In order to identify the oviposition sequence of fig wasp species, field observation was monitored. A gall tree in an early receptive phase was selected to determine the oviposition time sequences of each fig wasp species. The oviposition sequence can be defined as the order and period of egg-laying of each fig wasp species that were related to fig development based on the release of different volatile compounds at each fig state (Grison-Pigé *et al.*, 2002; Proffit *et al.*, 2007; Elias *et al.*, 2008). The timing of the oviposition sequence refers to the niche partition among the fig wasps and the stability of the species coexistence (Proffit *et al.*, 2007). The figs were observed for every branch from the height at the level of the observer's head down to the ground. The fig diameter which had been oviposited by fig wasps was measured (Fig.9). This observation was undertaken about two hours per day in the morning for 30 days until the fig became ripe.

#### **Laboratory work**

The fig diameter in an early emergence phase that represents the fig size was measured at the widest dimension of the fruit. The wall thickness of both the left and right sides of the fruit were also measured (Fig.9). Measurements were taken using a dial vernier caliper to the nearest 0.01 mm. Then, the number of florets inside the fig was counted to compare the host using capability of the fig wasp. Finally, figs were preserved in 95% ethanol in glass bottles with code labels and then put into a plastic box with each site separated.



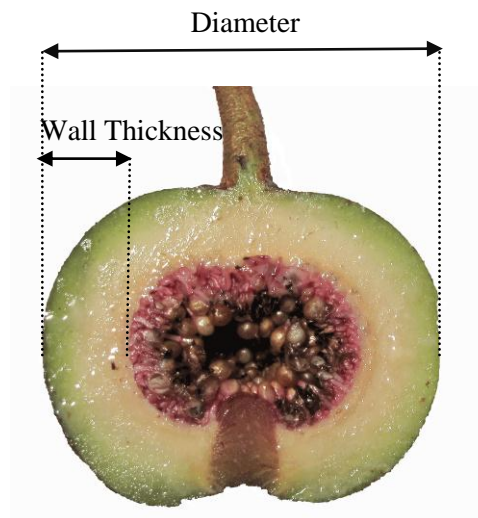


Figure 9. Vertical dissection of *F. schwarzii* showing measurements of diameter and wall thickness.

### 3.3 Data analysis

#### 3.3.1 Preparing data for analysis

Understanding the nature of the data is a first requirement for data analysis. Most of the collected data had to be used to answer the questions posed in this thesis and is counting data which is a discrete data (O'Hara and Kotze, 2010), although, there was some information obtained from the measuring data including the diameter and wall thickness. Thus, the normality of the number of pollinators and non-pollinators, the number of fig wasps in each species, the number of florets including the fig diameter and the wall thickness were tested to determine if these data would fit into either a parametric or a non-parametric test assumption. The test was made by using the Anderson-Darling test in Minitab14 (Minitab Inc., State College, PA., USA).

Subsequently, in the case of a non-normal distribution of any of the parameters, improving the normality of the data was made by using the Cox Box transformation method represented by the  $\lambda$  parameter (Sakia, 1992). The number of pollinators and non-pollinators, the total number of fig wasps and the number of each

fig wasps species were tested by this method. The aim of this analysis was to define the optimal traditional transformations (e.g. square root, log and arcsine transformations). This is a common tool to approximate a range of power transformation and is also important for both parametric and non-parametric test data (Osborne, 2010).

### **3.3.2 Transformation data to fit a statistical assumption**

The number of pollinators and non-pollinators, and the number of fig wasps of each species were transformed by using a square root transformation by adding a 0.5 constant to the raw data to ensure the minimum value was above 0 (Osborne, 2010) in the transformed processes. Thus, all normal distributed data can be analysed by parametric statistics such as the t-test and Pearson's Correlation, however, the number of fig wasps in each species was analysed by using non-parametric statistics.

### **3.3.3 Species composition of fig wasps in *F. schwarzii***

To demonstrate the real number of fig wasp species, the transformation method was not including in this part for more ease of understanding. Thus, two non-parametric statistics were computed by Minitab 14 were used. First, Mann-Whitney U test was used to establish if there any difference of mean numbers of fig wasp species between the Pha Dum Guard Station and the Ton Nga Chang Waterfall. This statistic is an alternative to and an extension of the t-test (Hawkins, 2005). In the second, the Kruskal-Wallis H-test was used to test the differences between the mean numbers of each species at each site. Then, if the result showed a difference within all pairs of means, the Mann-Whitney U test as post-hoc analysis was calculated to compare each pairwise of mean number.

In addition the range of the numbers of individuals was recorded to show a minimum and maximum number of each fig wasp species at each site.

Finally, a percentage of each fig wasp species was calculated to demonstrate the proportion of each fig wasp at each site.

$$\text{Percentage of fig wasp} = \left( \frac{\text{Number of Sp. A}}{\text{Number of total wasps}} \right) \times 100$$

### 3.3.4 The inter-relationship of fig wasp species and their association with some measured traits of *F. schwarzii*

A correlation between the number of pollinators and non-pollinators in both sites was determined by using the Pearson's Correlation in Minitab 14. The rate of parasitism was calculated using the following equation (Weiblen, 1999, Dunn *et al.*, 2008). Then, the relationship between the rate of parasitism and the host density was demonstrated by using regression analysis (Price, 1988) to reveal host-parasitoid aggregation, and this can be divided into host density dependence (HDD) and host density independence (HDI) (Hassell and May, 1973).

$$\text{Rate of parasitism} = \frac{\text{Num. of parasitoids}}{\text{Total num. of fig wasp (Pollinator+ Galler+ Parasitoids)}}$$

A path analysis was conducted to determine the actual relationships between and within fig wasp species and with some measured traits in *F. schwarzii* such as fig diameter, the number of florets and wall thickness, by using AMOS 5 (student version). This statistical analysis has been used to examine the causal relationships between variables represented by an over identified model (Lleras, 2005). The first model, 'just identified model' was modified from an original model (Kerdelhué *et al.*, 2000, Fig.10) by adding the number of floret and wall thickness variables, and deleting seed variable because there is no seed in selected gall figs in figure11. In the just identified model, all pathways were tested to eliminate non-causal pathways with zero coefficients or non-significance (Fig. 11). After the pathways, were reduced all possible variables were built in an 'over identified model'. A standardized path coefficient (P) indicated a direct effect and the values were

defined into three levels (Suhr, 2008); a small effect ( $P < 0.10$ ), a medium effect ( $P = 0.30$ ), and a large effect ( $P > 0.05$ ). The criterion for indicating a good model fit in the over identified model was described by using chi-square ( $\chi^2$ ) and fit indices (Hu and Bentler, 1995; Lleras, 2005; Ullman, 2006).

- Comparative Fit Index (CFI) is greater than 0.9
- Tucker-Lewis Index (TLI) is greater than 1.0
- Bentler-Bonett Index or Normed Fit Index (NFI) is greater than 0.95
- Root Mean Squared Error of Approximation (RMSEA) is less than 0.06

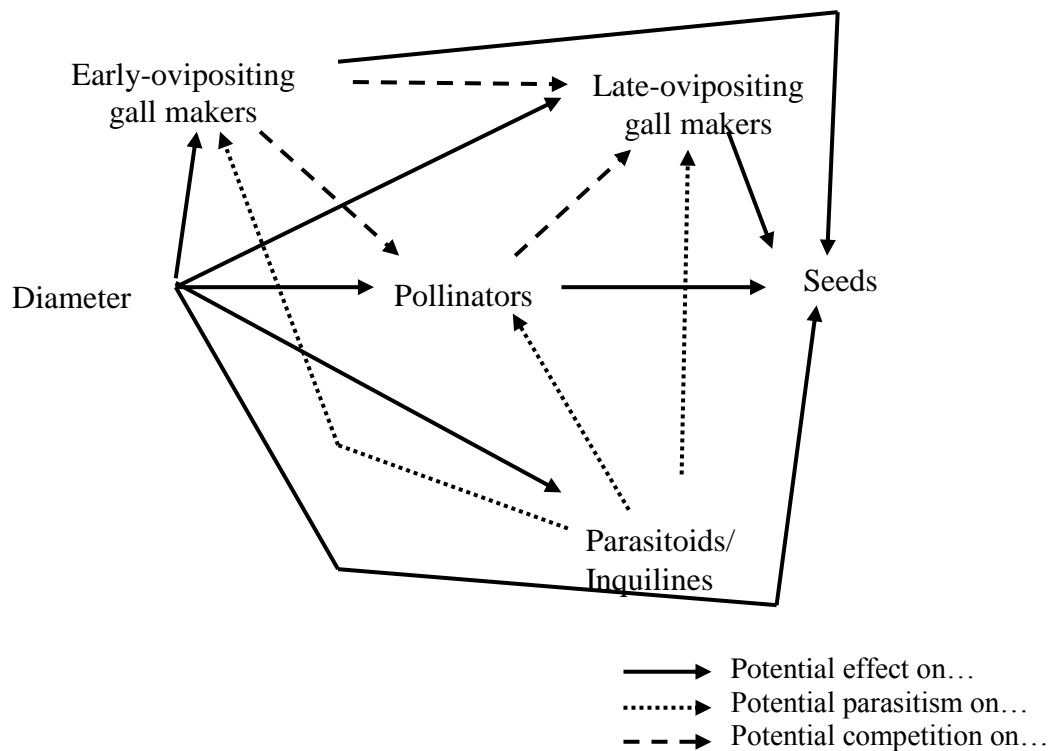


Figure 10. An original path model for the fig wasp community and their host plants in monoecious fig (Kerdelhué *et. al.*, 2000).

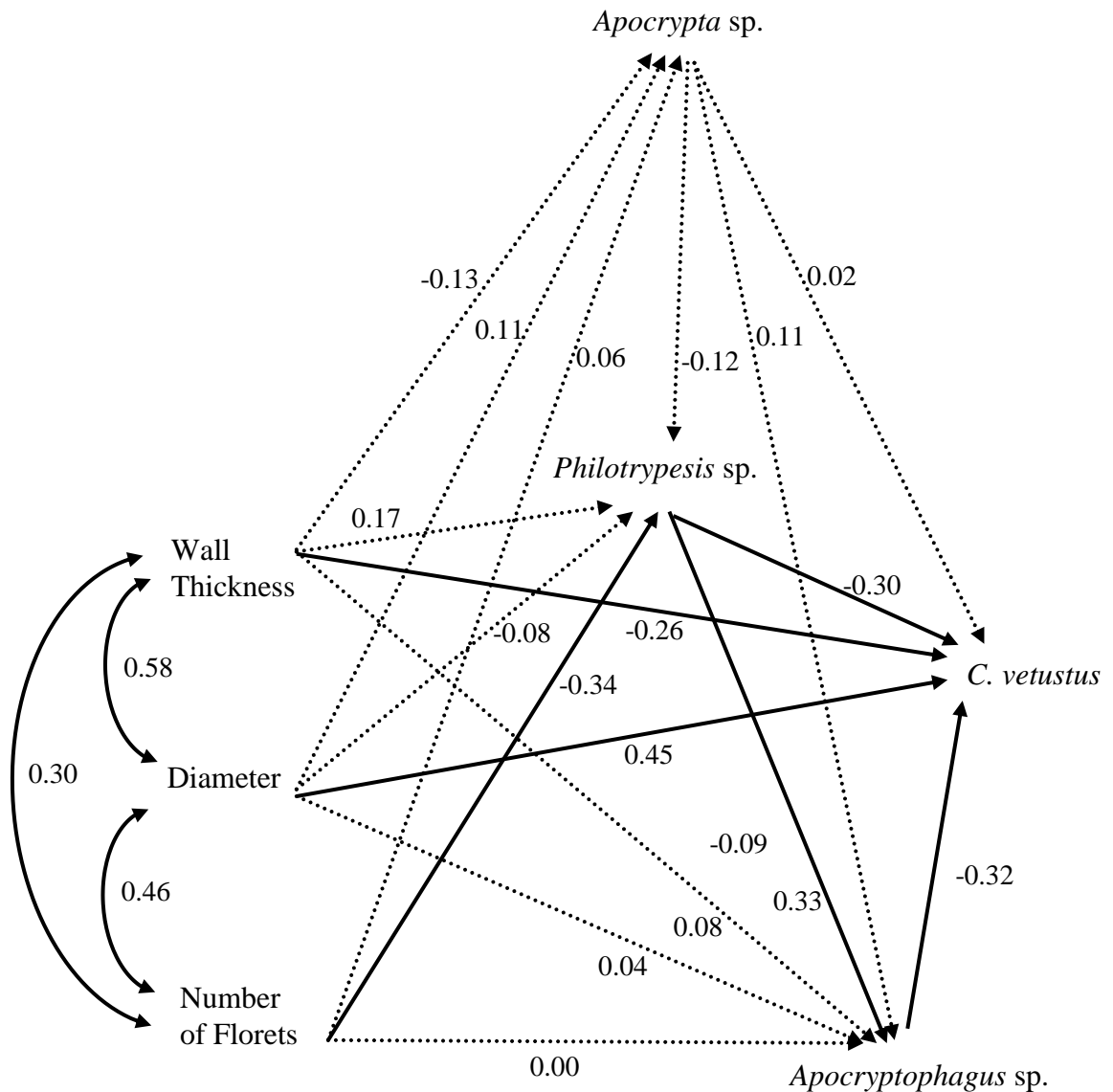


Figure 11. The 'Just identified model' presented as a path diagram and that illustrates all causal relationships between variables. Four fig wasp species were set as endogenous (effect) variables where other variables are exogenous (causal) variables. Path coefficients (direct effects) and correlation coefficients are shown as single-headed arrows and double-headed arrows respectively. The solid lines represent significant levels whereas a non-significant level is shown in dashed lines.

## CHAPTER 4

### RESULTS

#### 4.1 Species composition of fig wasps in *F. schwarzii*

In 30 collected gall figs of *F. schwarzii* from 19 trees in Pha Dum and 10 trees in Ton Nga Chang Waterfall, four fig wasp species within two families were identified. The number of individuals of each species from both sites are summarised in Table 1.

-*Ceratosolen vetustus* Wiebes, 1994 (Figs.12–13); Family Agaonidae, Subfamily Kradibinae. *C. vetustus* is defined as a pollinator on *F. schwarzii* based on the presence this species in both collected gall and seed figs. This species was found in every gall fig collected from both sites. The species exhibited a highest mean number per fig ( $\pm$ SD) and also a widest individual range in these two sites;  $391.90 \pm 219.07$  (range 70–1,092) at Pha Dum, and  $495.93 \pm 238.05$  (range 96–1,106) at Ton Nga Chang Waterfall.

-*Apocryptophagus* sp. (Figs.14–15); Family Agaonidae, Subfamily Sycophaginae. This Subfamily is a non-pollinator and is defined as a galler group (Weiblen *et al.*, 2001; Cruaud *et al.*, 2011). *Apocryptophagus* sp. was found in 20 and 15 gall figs of the total collected figs from Pha Dum and Ton Nga Chang Waterfall respectively. It had a lowest mean number per fig ( $\pm$ SD) and a narrowest individual range in these two sites;  $18.03 \pm 28.23$  (range 0–117) at Pha Dum, and  $7.50 \pm 11.72$  (range 0–53) at Ton Nga Chang Waterfall

-*Philotrypesis* sp. (Figs.16-17); Family Pteromalidae, Subfamily Sycoryctinae. This Subfamily is a non-pollinator group. This species was found in 13 and 18 gall figs of the total collected figs from Pha Dum and Ton Nga Chang Waterfall respectively. The mean wasp number per fig ( $\pm$ SD) was  $27.77 \pm 63.99$  (range 0–251) at Pha Dum and less than that at Ton Nga Chang Waterfall, ( $36.77 \pm 69.09$ , range 0–229).

-*Apocrypta* sp. (Fig.18-19); Family Pteromalidae, Subfamily Sycoryctinae. This Subfamily is also part of a non-pollinator group. It was found in 26 and 24 gall figs of the total collected figs from Pha Dum and Ton Nga Chang Waterfall respectively. The mean wasp number per fig ( $\pm$ SD) was  $31.83\pm 37.82$  (range 0–125) at Pha Dum and higher than that at Ton Nga Chang Waterfall, ( $23.70\pm 33.41$ , range 0–157).

The mean number of each fig wasp species between the two sites calculated with Mann-Whitney U test revealed no significant difference at a 95% confidence interval ( $p > 0.05$ , Table 1). However, a comparison between the mean number of fig wasp species in each site calculated using Kruskal-Wallis test with a post-hoc Mann-Whitney U test showed a significant difference between pollinator and three non-pollinators species in both Pha Dum ( $H = 69.59$ ,  $df = 3$ ,  $p < 0.001$ ) and Ton Nga Chang Waterfall sites ( $H = 71.14$ ,  $df = 3$ ,  $p < 0.001$ ).

In addition, the abundance of each fig wasp species was calculated at both Pha Dum and Ton Nga Chang Waterfall, TWS. *C. vetustus* was the most abundant species comprising 83.47% and 87.95% of the total individual number, respectively.

Table 1. Fig wasps composition collected from 30 gall figs (*F. schwarzii*)

Species	Sites						p-value (Mann-Whitney U test)
	Pha Dum (n= 30)			Ton Nga Chang (n= 30)			
	Mean±SD	Range	Percentage Presence (n)	Mean±SD	Range	Percentage Presence (n)	
<b>Family Agaonidae</b>							
Subfam. Kradibinae							
<i>Ceratosolen vetustus</i>	391.90±219.07 <sup>a</sup>	70-1092	83.47 (30)	495.93±238.05 <sup>a</sup>	96-1106	87.95 (30)	0.061 NS
Subfam. Sycophaginae							
<i>Apocryptophagus</i> sp.	18.03±28.23 <sup>b</sup>	0-117	3.84 (21)	7.50±11.72 <sup>b</sup>	0-53	1.33 (15)	0.100 NS
<b>Family Pteromalidae</b>							
Subfam. Sycoryctinae							
<i>Philotrypesis</i> sp.	27.77±63.99 <sup>b</sup>	0-251	5.91 (16)	36.77±69.09 <sup>b</sup>	0-229	6.52 (19)	0.344 NS
Subfam. Sycoryctinae							
<i>Apocrypta</i> sp.	31.83±37.82 <sup>b</sup>	0-125	6.78 (27)	23.70±33.41 <sup>b</sup>	0-157	4.20 (26)	0.496 NS

NS = not significant

<sup>ab</sup>: Mean values with different superscript letters in the same column were significantly different by the Mann-Whitney U test at p <0.001



(a.)



(b.)



Figure 12. Female *Ceratosolen vetustus* (a.) at 40X and (b.) dorsal view at 90X.

(a.)

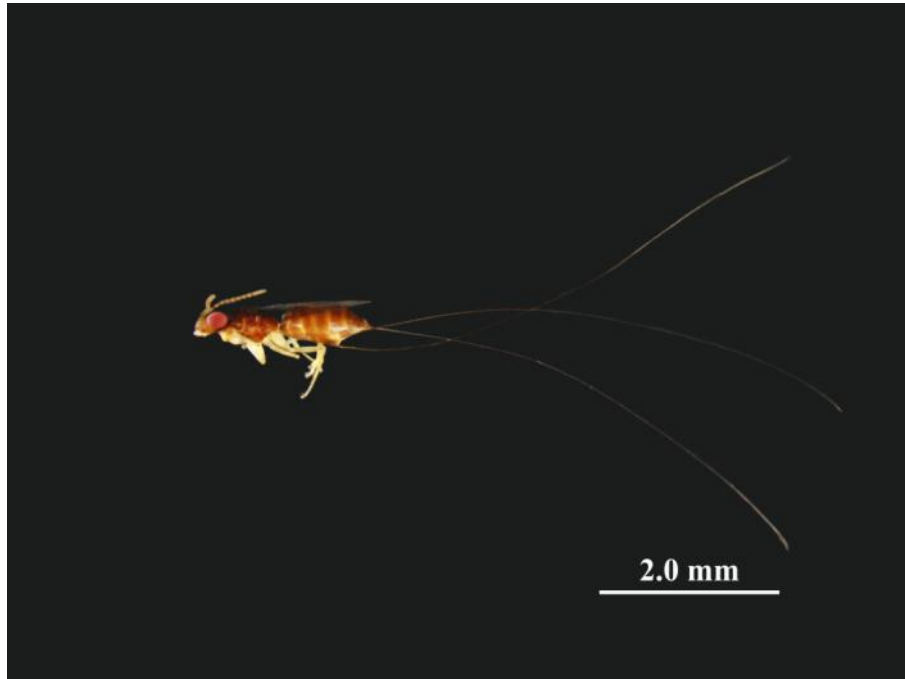


(b.)



Figure 13. Male *Ceratosolen vetustus* (a.) at 40X and (b.) dorsal view at 90X.

(a.)



(b.)

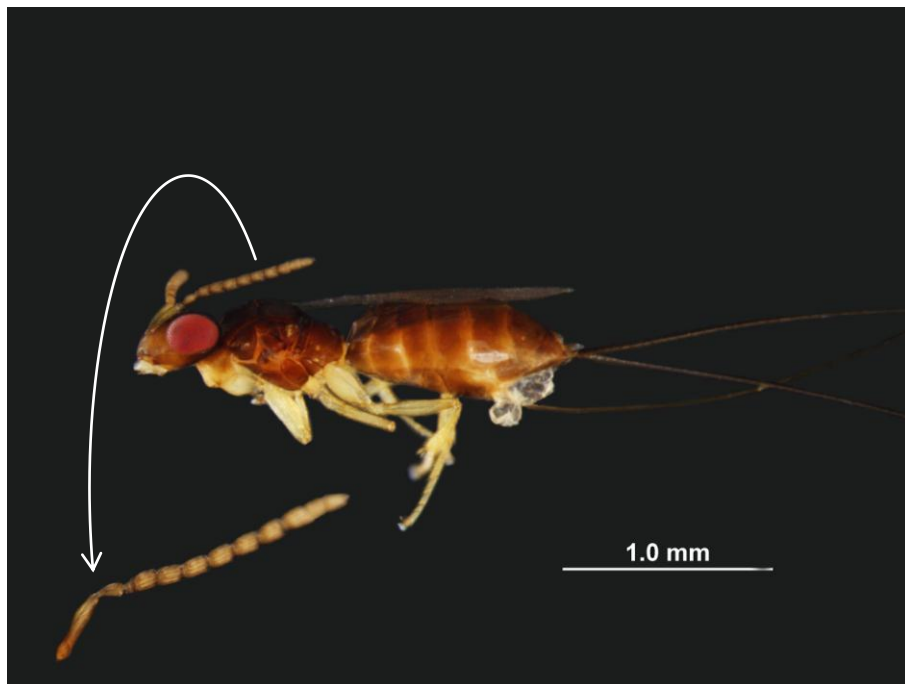
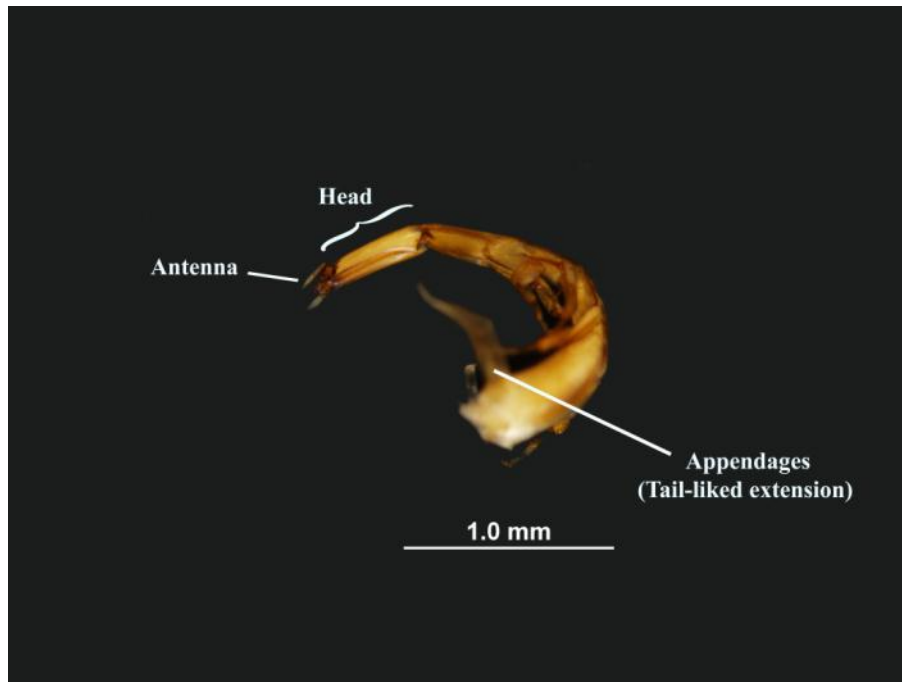


Figure 14. Female *Apocryptophagus* sp. (a.) at 25X and (b.) at 40X and a zoom of an antenna at 90X.

(a.)



(b.)



Figure 15. Male *Apocryptophagus* sp. (a.) at 40X and (b.) dorsal view at 40X.

(a.)



(b.)

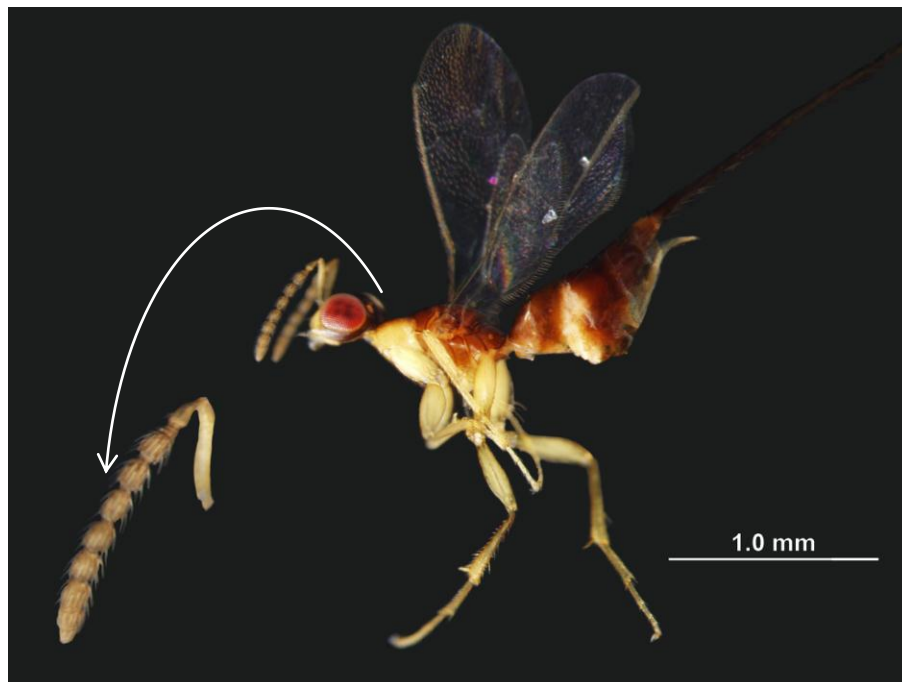
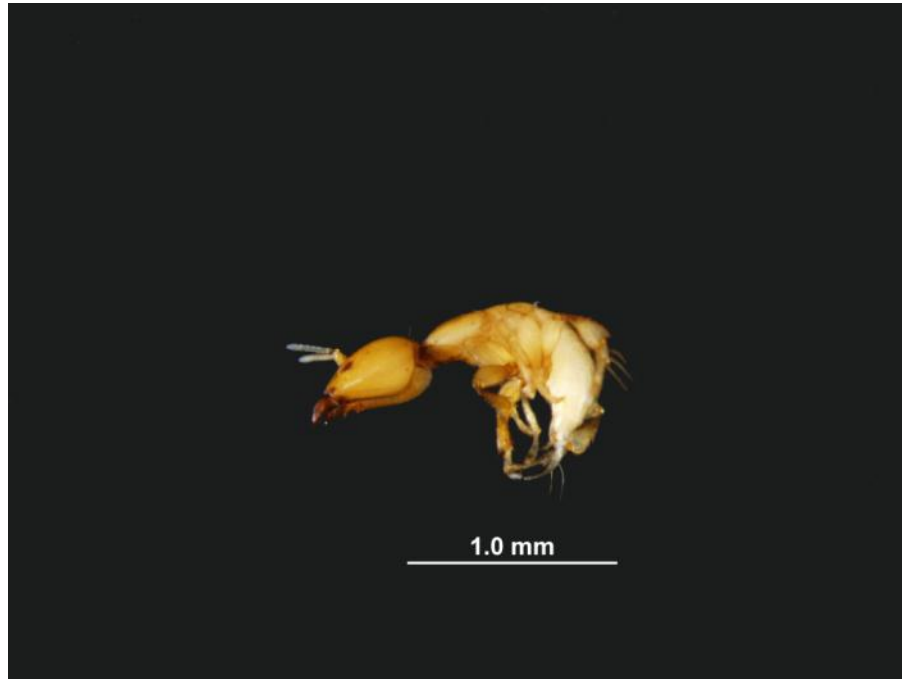


Figure 16. Female *Philotrypesis* sp. (a.) at 25X and (b.) at 40X and a zoom of an antenna at 90X.

(a.)



(b.)

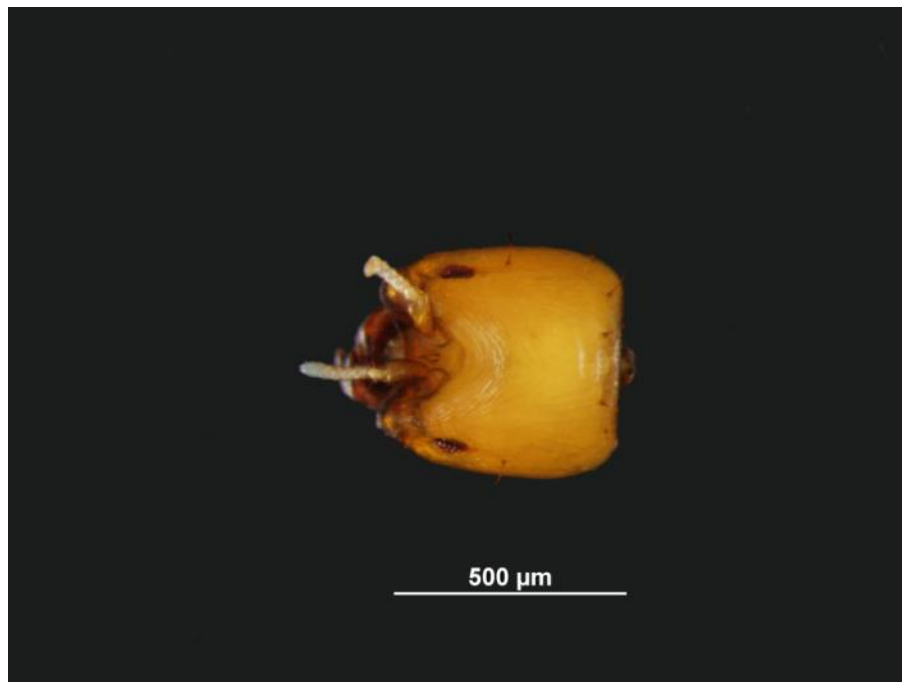


Figure 17. Male *Philotrypesis* sp. (a.) at 40X and (b.) head at 90X

(a.)



(b.)

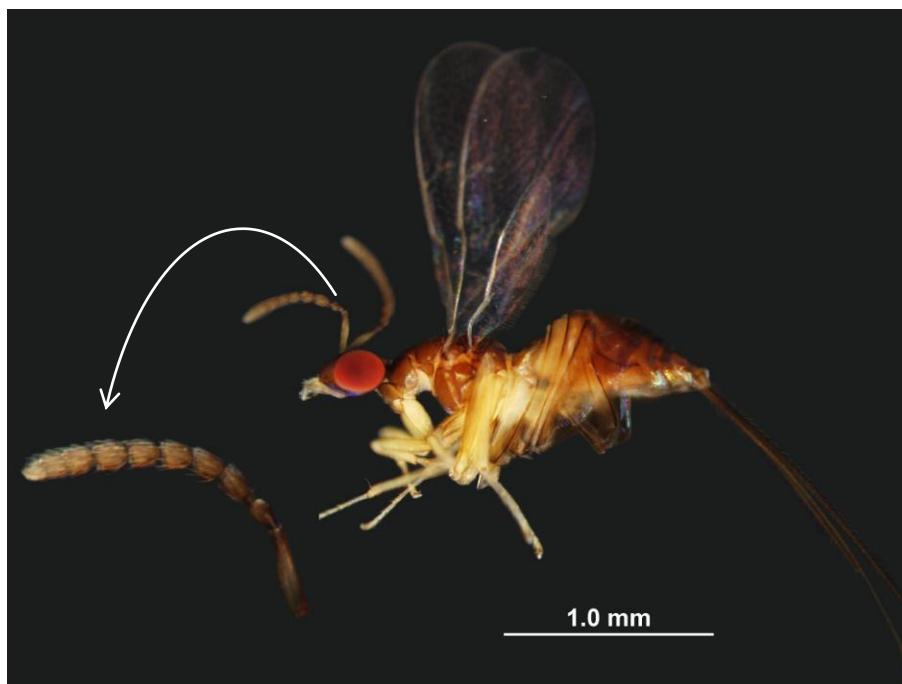
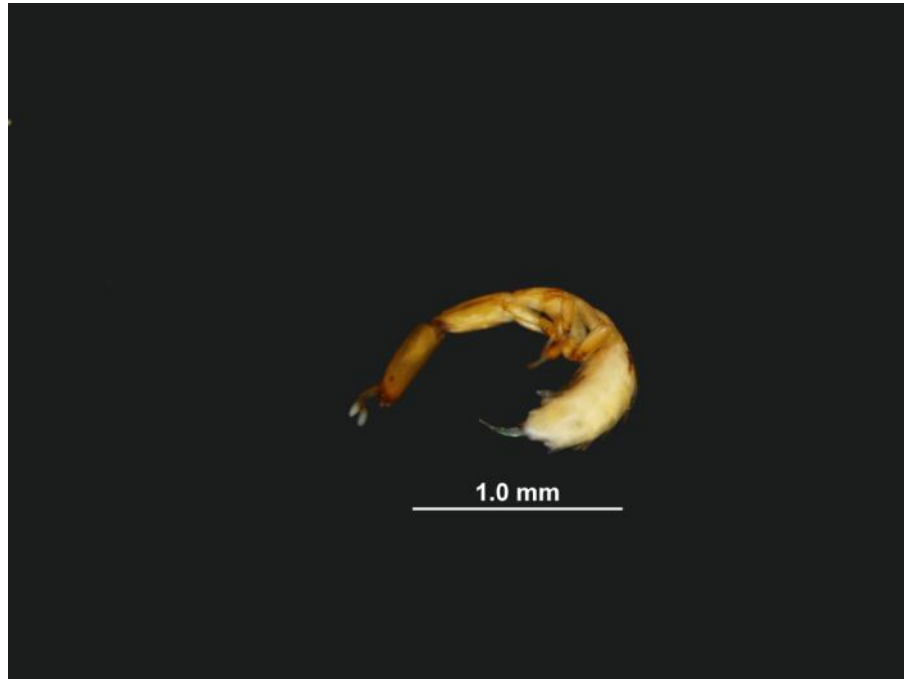


Figure 18. Female *Apocrypta* sp. (a.) at 25X and (b.) at 40X and a zoom of an antenna at 90X.

(a.)



(b.)

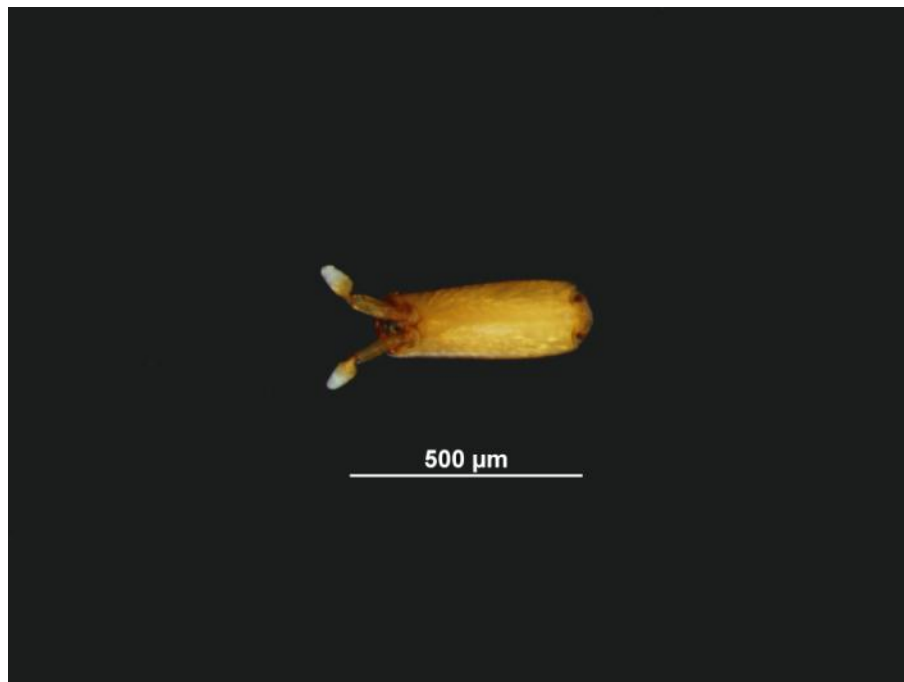


Figure 19. Male *Apocrypta* sp. (a.) at 40X and (b.) head at 90X.



## 4.2 The inter-relationship of fig wasp species and their association with some measured traits of *F. schwarzii*

### 4.2.1 The inter-relationship of fig wasp species

The correlation between a transformed number of pollinator and non-pollinator calculated by Pearson's Correlation were significantly negatively correlated ( $r = -0.440$ ,  $n = 60$ ,  $p < 0.001$ , Fig. 20). An increasing in pollinator individuals led to a decrease in the non-pollinator number.

In this study, *C. vetustus* and *Apocryptophagus* sp. were considered to be a host density. Figure 21 showed the relationship between the host density (X) and its rate of parasitism (Y). This demonstrated a significant negative relationship ( $r^2 = 0.36$ ,  $n = 60$ ,  $p < 0.001$ , Fig. 21). The rate of parasitism decreased with increasing host density.

### 4.2.2 Association of fig wasp species with some measured traits of *F. schwarzii*

#### 4.2.2.1 Association of fig wasps species with fig development

Figure 22 represents the pattern of an ovipositing time sequence of each fig wasp species. In the diagram *Apocryptophagus* sp. was an early galler oviposited on small figs with an average diameter of 20.93 mm ( $\pm 1.07$ ,  $n = 169$ ). Any increase in the fig diameter reduced the number of *Apocryptophagus*'s ovipositions and allowed the second ovipositor to invade. An average diameter of 22.10 mm ( $\pm 0.72$ ,  $n = 92$ ) favoured the oviposition of *C. vetustus*. *Philotrypesis* sp. and *Apocrypta* sp. were the last two species oviposited in the gall fig at an average diameter of 23.95 mm ( $\pm 1.28$ ,  $n = 196$ ) and 24.13 mm ( $\pm 0.95$ ,  $n = 236$ ), respectively.

#### 4.2.2.2 Association of fig wasps species with fig diameter, number of florets, and wall thickness

Path analysis using AMOS 5 indicated an elimination of *Apocrypta* sp. from all pathways in a ‘just identified model’. Figure 23 demonstrates a well fitted ‘over identified model’ ( $\chi^2 = 2.854$ ,  $df = 6$ ,  $n = 59$ ,  $p = 0.827$ ,  $CFI = 1.000$ ,  $TLI = 1.116$ ,  $NFI = 0.965$ ,  $RMSEA = 0.000$ ). All pathways between variables in this model were significantly different at 95% confidence interval. The effect of variables on the pollinator was represented by path coefficient values (P) (Table 2.). *Apocryptophagus* sp. ( $P = -0.32$ ) and *Philotrypesis* sp. ( $P = -0.28$ ) had a negative direct effect on the pollinator (*C. vetustus*). However, *Philotrypesis* sp. also exhibited an indirect effect on *C. vetustus* through the *Apocryptophagus* sp. with the path coefficient of *Philotrypesis* sp. on *Apocryptophagus* sp. at 0.31, and *Apocryptophagus* sp. on *C. vetustus* at  $P = -0.32$  (Fig. 23). Therefore, a total effect of *Philotrypesis* sp. on *C. vetustus* was at the path coefficient of -0.38, (Table 2).

In addition, the diameter, the number of florets and the wall thickness had a significantly positive correlation on each other in path analysis (Fig. 23). The correlation estimated from a Pearson analysis showed the same trend in that the diameter had a positive correlation to the wall thickness ( $r = 0.68$ ,  $n = 59$ ,  $p < 0.001$ ) and to the number of florets per fig ( $r = 0.40$ ,  $n = 59$ ,  $p < 0.001$ ) (Appendix 1). Moreover, the fig diameter and wall thickness had a direct effect on the pollinator at path coefficients of 0.42, and -0.27 respectively. The number of florets showed two distinct pathways on the pollinator. The first pathway was a positive indirect effect via *Philotrypesis* sp. ( $P = -0.10$ ).

$$\begin{array}{c} -0.34 \qquad \qquad \qquad -0.28 \\ \text{Number of florets} \longrightarrow \textit{Philotrypesis} \text{ sp.} \longrightarrow \textit{C. vetustus} \end{array}$$

$$\text{Indirect effect} = 0.10 \quad [(-0.34) \times (-0.28)]$$

And, the second pathway also showed the same effect via *Philotrypesis* sp. and *Apocryptophagus* sp. ( $P = 0.03$ ).

Number of florets  $\xrightarrow{-0.34}$  *Philotrypesis* sp.  $\xrightarrow{0.31}$  *Apocryptophagus* sp.  $\xrightarrow{-0.32}$  *C. vetustus*

Indirect effect = 0.03 [(-0.34) x (0.31) x (-0.32)]

Therefore, a total effect of the number of florets to the pollinator is 0.13 (Table 2).

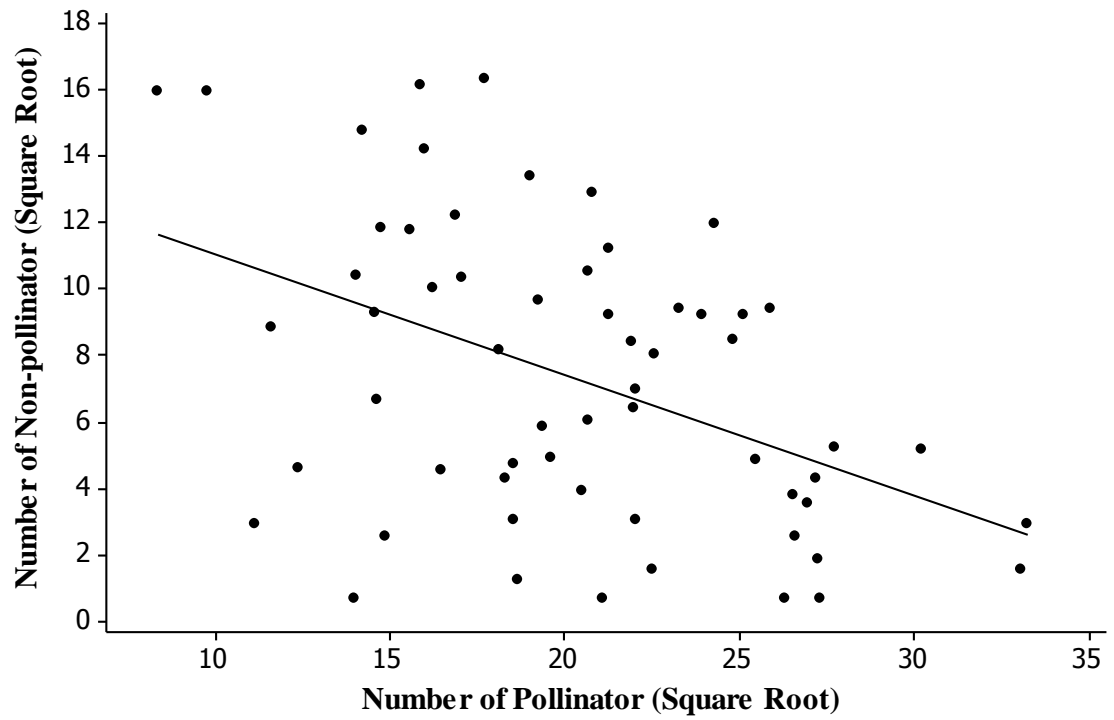


Figure 20. Negative correlation between pollinator and non-pollinator fig wasps.

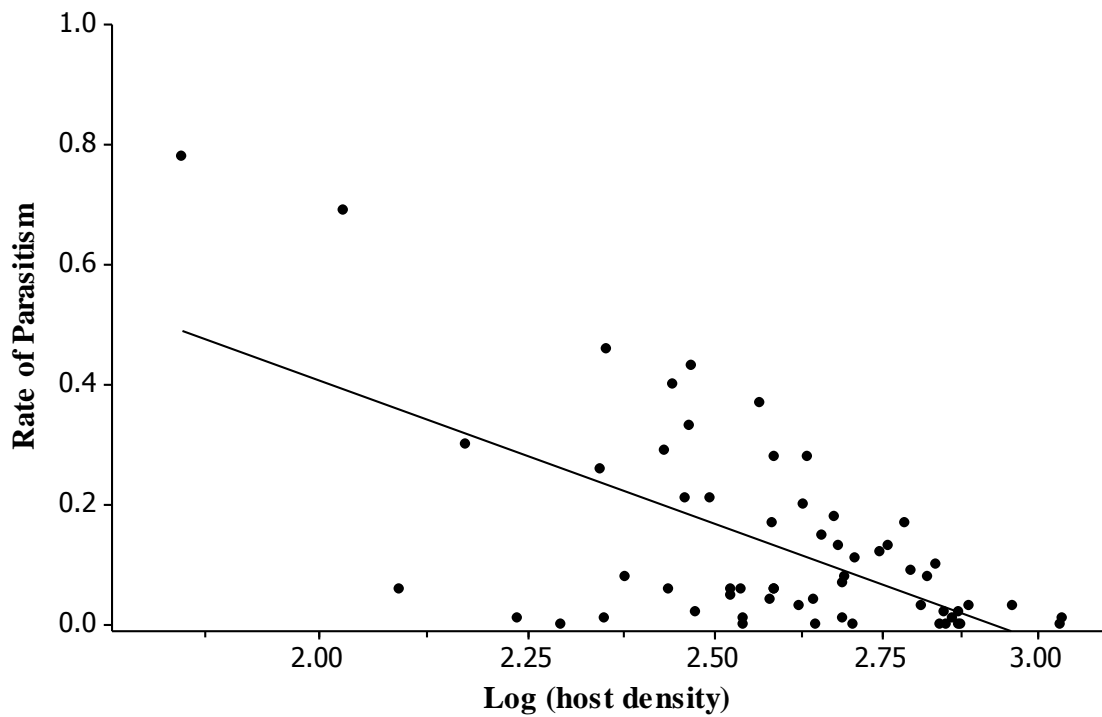


Figure 21. The relationship between host density (X) and rate of parasitism (Y) in *F. schwarzii*.

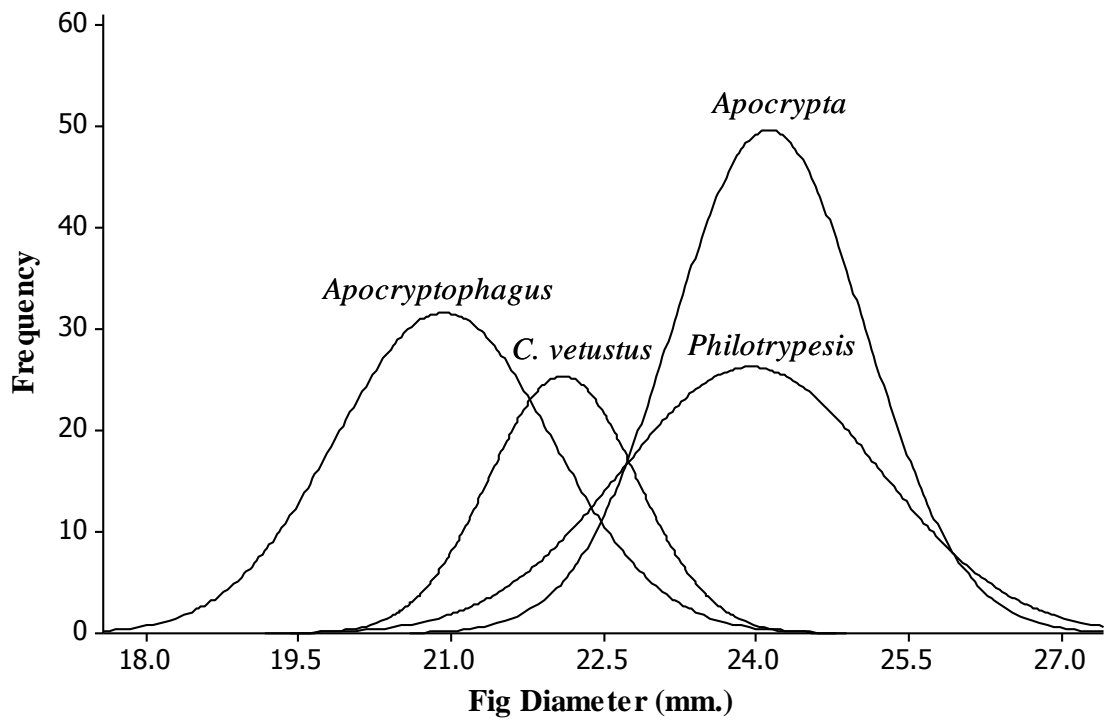


Figure 22. An ovipositing time sequences of four fig wasp species related with fig development represented by the fig diameter.

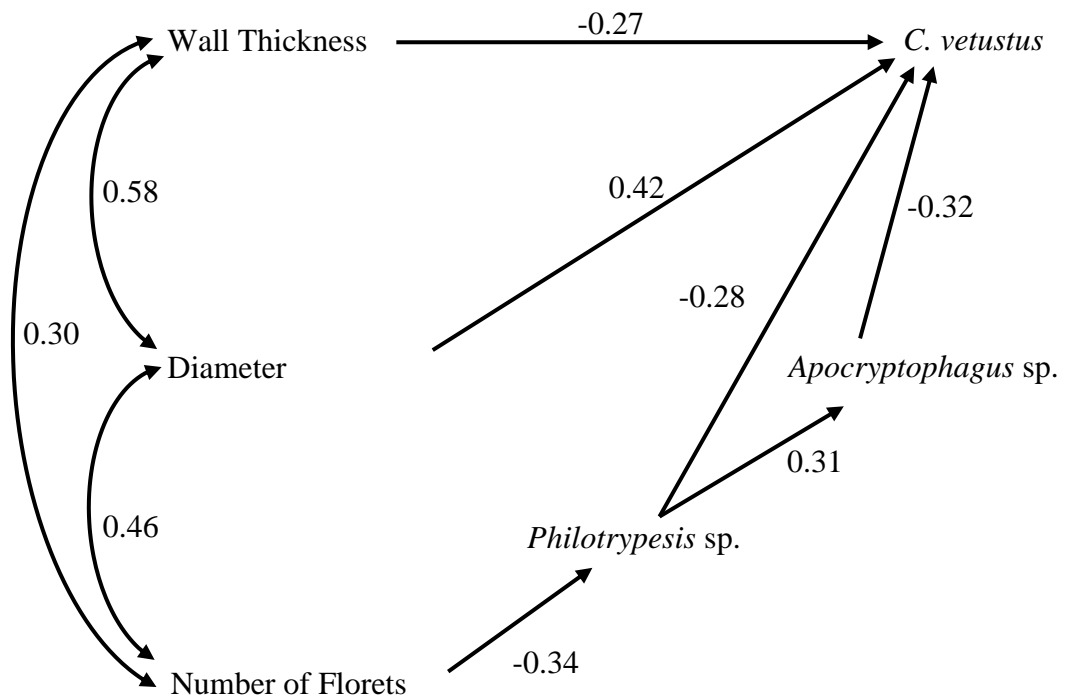


Figure 23. The 'Over identified model' (n= 59) in path diagram of the significant direct and indirect effects of causal variables on *C. vetustus*. Path and correlation coefficients are shown in single-headed arrows and double-headed arrows respectively. The solid lines represent a significant effect on the variables.

Table 2. Summary of the effect of variables in the ‘over identified model’ with 95% confidence intervals

<b>Variables1</b>	<b>Variables2</b>	<b>Direct Effect</b>	<b>Indirect Effect</b>	<b>Total Effect</b> (direct + indirect effects)
<i>C. vetustus</i>	<i>Apocryptophagus</i> sp.	-0.32	-	-0.32
	<i>Philotrypesis</i> sp.	-0.28	$(0.31 \times -0.32) = -0.10$	-0.38
	Wall thickness	-0.27	-	-0.27
	Diameter	0.42	-	0.42
	Number of florets	-	$(-0.34 \times -0.28) + (-0.34 \times 0.31 \times -0.32) = 0.13$	0.13
<i>Apocryptophagus</i> sp.	<i>Philotrypesis</i> sp.	0.31	-	0.31
	Number of florets	-	$(-0.34 \times 0.31) = -0.11$	-0.11
<i>Philotrypesis</i> sp.	Number of florets	-0.34	-	-0.34

## CHAPTER 5

### DISCUSSION AND CONCLUSION

#### 5.1 Species composition of fig wasps in *F. schwarzii*

In this study, four fig wasps species; *C. vetustus*, *Philotrypesis* sp., *Apocryptophagus* sp., and *Apocrypta* sp. were found in *F. schwarzii* at Pha Dum and Ton Nga Chang Waterfall, TWS, Southern Thailand. Of those, *C. vetustus* was the only pollinator species whereas the rest were from non-pollinator groups. The pollinator, *C. vetustus*, was the dominant species in the fig with over 80% of the total fig wasps population. The result agreed with Kameyama *et al.* (1999), Machado *et al.* (2001), and Harrison (2003) who observed *F. schwarzii* in Borneo and also found the same pollinator species. According to the one to one rule, each *Ficus* species showed host specificity with one pollinator wasp (Kerdelhué *et al.*, 1997; Cook and Rasplus, 2003; Rønsted *et al.*, 2005). Since the distribution of *F. schwarzii* ranges from peninsular Thailand across Sumatra to Borneo, therefore, this study suggested that *F. schwarzii* has probably demonstrated a one to one rule along its distribution. This suggestion is in agreement with Silvieus (2006) who reported that pollinators in fourteen fig species in the subgenus *Sycomorus* are mostly specific with their hosts following the one-to-one rule. It indicated that, at present, there was no evidence for any breakdown of host specificity on *F. schwarzii*. In nature, host specificity can be broken down in time by several factors such as geographic variation, host switching, host dependence (Weiblen, 2002; Silvieus *et al.*, 2007) or different divergence times between fig and wasps (Machado *et al.*, 2005). However, Berg (2010) and Berg and Culmsee, (2011) recently separated *F. schwarzii* into at least five species along its distribution based on style characteristic. This indicated that the speciation process of the host (*F. schwarzii*) might be currently ongoing and this might lead to host specificity switching over a period of time. For future study it might be worth focusing on the pollinator species and to examine if it still follows the one to one rule according to its separated host.



*Philotrypesis* sp., *Apocryptophagus* sp., and *Apocrypta* sp., as non-pollinators, were found in *F. schwarzii* at both Pha Dum and Ton Nga Chang Waterfall. They were also recorded in *F. schwarzii* in Borneo (Harrison and Shanahan, 2005). These species assemblages belong to two subfamilies; Sycoryctinae and Sycophaginae, the subfamilies that are found in monoecious figs from the old world (Cook and Segar, 2010). These three species were not significantly different in the mean number of individuals and were counted as a minority in the total population (less than 7% of total fig wasp for each species). This is in contrast with Segar and Cook (2012), who reported that the number of non-pollinator, such as Sycoryctinae, was found up to 50% of the total number of fig wasps.

It was observed that the non-pollinators at both sites were not found in every collected fig. There are several factors that may explain species limitations and individual ranges of non-pollinator. Cushman *et al.*, (1998), Weiblen *et al.* (2001) and Schatz *et al.* (2006) reported that ants are important predators of non-pollinator wasps during oviposition time on figs. In this study, *Polyrhachis bihamata* (Drury, 1773) and *Anoplolepis gracilipes* (Smith, 1857) were found on *F. schwarzii* preying on non-pollinator fig wasps (J. Yimkaew, personal observation). Another factor proposed by Weiblen *et al.* (2001) is an incompatibility during the stages of development between host and non-pollinator, as well as a limitation in searching time for oviposition. It has also been suggested that the level of host specificity of non-pollinator is less than that in the case of the pollinator (Marussich and Machado, 2007; Cook and Segar, 2010; McLeish *et al.*, 2012). According to Cook and Segar (2010), *Apocrypta* may be a generalist for host selection, while no evidence was found to support a possibility of host switching of the non-pollinators in the genus *Philotrypesis*. Although the level of host specificity in non-pollinator is less than in the pollinator, host specificity in the non-pollinators is still indispensable and constrained by four factors; a consistency between fig and fig wasp reproduction, the fit or compatibility between ovipositor length and wall thickness, recognition of volatile cues, and niche exclusion by their host (Marussich and Machado, 2007).

## 5.2 The inter-relationship of fig wasp species and their association with some measured traits of *F. schwarzii*

### 5.2.1 The inter-relationship of fig wasp species

The results from this study indicated that there was a negative correlation between pollinator and non-pollinator individuals. The finding coincided with several studies that concentrated in both monoecious and dioecious figs (Bronstein, 1991; Compton *et al.*, 1991; West and Herre, 1994; Kerdelhué and Rasplus, 1996; West *et al.*, 1996; Harrison, 2003). However, a positive (Peng *et al.*, 2005b) and no correlations (Cook and Power, 1996; Patel, 1998; Karunaratne, 2009) between the number of non-pollinators and pollinators were also reported. Peng *et al.* (2005b) observed that the non-pollinators found in the dioecious fig species (*F. hispida* L.) are all parasitoids or inquilines that can exploit after pollinator's oviposition, therefore the number of non-pollinators increases with the number of pollinators. In this study, however, not only non-pollinators (*Philotrypesis* sp. and *Apocrypta* sp.) oviposited after the presence of pollinator species (*C. vetustus*), another important non-pollinator species (*Apocryptophagus* sp.) was recorded as an early ovipositing galler which oviposited before the pollinator (Fig. 22). In this case, the competition between non-pollinator, *Apocryptophagus* sp. and the pollinator, *C. vetustus* for oviposition sites (flower) was occurred. Thus, this non-pollinator species may has greater negative effect to pollinator although it has a lowest mean number than another non-pollinator species.

Bronstein (1991) suggested that the presence of non-pollinators may affect mutualism between the fig and its pollinator. Dunn *et al.* (2008) proposed the role of parasites in maintaining the stability of mutualism between the fig and its pollinator via the mechanism called 'enemy free space'. This mechanism explained that the length of the fig pedicel played an important role in parasite oviposition. A long pedicel is considered to be an obstacle for the parasite to insert their ovipositors into the syconium, hence there is less effect on the pollinator and the stability of mutualism is maintained. It also indicated that long pedicel favoured the presence of the pollinators and, therefore, had a negative influence on the number of

parasites. Although the length of the pedicel was not measured in this study, it could explain the negative correlation result.

In this study, the association between host density and rate of parasitism was negatively related. The result indicated host density independence (HDI) on host-parasitoid aggregation. The finding was in agreement with Weiblen *et al.* (2001) who reported a negative trend of a host parasitoid relationship in *F. trachypison*. The Parasitoids-Sink Hypothesis could be one of the explanations for HDI in the study. In this hypothesis, it was stated that ‘seed figs’ play important roles in reproductive failure in the parasitoid and this leads to a smaller number of parasites that would attack the ‘gall fig’ (Weiblen *et al.*, 2001; Cook and Rasplus, 2003). *F. schwarzii* in this study was also composed of both ‘seed’ and ‘gall’ fig. Moreover, several studies on host-parasitoid interactions indicated potential limiting factors in aggregation of parasites in each fig including searching time, predator problem, host finding efficiency, failure to detect a fig with a full wasp host (Weiblen *et al.*, 2001), or spatial and temporal asynchrony occurred (Hassell and May, 1973). A detailed study of these integrating factors, and long term monitoring of wasp’s ecology in both gall and seed fig in *F. schwarzii* and another dioecious fig, is recommended.

## **5.2.2 Association of fig wasp species with some measured traits of *F. schwarzii***

### **5.2.2.1 Association of fig wasps species with fig development**

The pattern of the ovipositing time sequences of fig wasps in *F. schwarzii* indicated that *Apocryptophagus* sp. was the first species oviposited in fig at the receptive phase. From field observation, an oviposition time of *Apocryptophagus* sp. was two days prior to the oviposition by the pollinator (*C. vetustus*). This agrees with the result reported by Cruaud *et al.* (2011) who found that the pollinator was the second species ovipositing after the galler group. The *Apocryptophagus* species was classified as a small galler group with the characteristics of

a thin and long ovipositor. The length of the ovipositor was longer than its body Cruaud *et al.* (2011). Many studies in other *Ficus* species reported similar results of an early ovipositing time by gallers (Peng *et al.*, 2005a; Profitt *et al.*, 2007; Ghara and Borges, 2010; Cruaud *et al.*, 2011). However, some gallers showed an overlapping oviposition time with pollinators (Ranganathan, *et al.*, 2010). Profitt *et al.* (2007) proposed that the sequences of oviposition by different fig wasp species were dependent on volatile signals releases at different stages of fig development. Moreover, they reported that three species of *Apocryptophagus* were the first group ovipositing in *Ficus racemosa*, and they were attracted by a chemical signal produced from the plant tissue. Hossaert-McKey *et al.* (2010) suggested that pollinators were subjected to specific floral scents.

*Philotrypesis* sp., and *Apocrypta* sp. were found to oviposit after the pollinator (Fig. 22) at the interfloral phase (personal observation). Elias *et al.* (2008) stated that non-pollinators oviposited later after the pollinators were assumed to be parasitoids or inquilines that probably respond to scent from another wasp larvae (Profitt *et al.*, 2007). Although the function of *Philotrypesis* sp. is still unclear, either as an inquiline or a parasitoid (Weiblen *et al.*, 2001; Zhen *et al.*, 2005; Karunaratne, 2009; Kuaraksa, 2012), Harrison and Shanahan (2005) suggested *Philotrypesis* sp. may be an inquiline of a pollinator while *Apocrypta* is probably a parasitoid of *Apocryptophagus* sp. on *F. schwarzii*. However, there was no evidence to support their actual functions such as any experiment on feeding behaviour, and histological studies (Elias *et al.*, 2008) which are necessary to understand the biology of *Philotrypesis* sp. and *Apocrypta* sp. in this fig. Therefore, in this study, these species were referred to parasitoids which are not specific to their host.

#### **5.2.2.2 Association of fig wasps species with fig diameter, number of florets, and wall thickness**

As in the above discussion, there was a negative correlation between the non-pollinator and pollinator as explained in a broad sense. An in depth analysis on the interactions of fig wasp species with *F. schwarzii* was carried out in order to

provide detailed explanations on their relationship by including main factors associated with fig such as fig diameter, wall thickness, and the number of florets. The result from ‘the over identified model’ suggested to withdraw *Apocrypta* sp. from all pathways because of the insignificant statistical results. It could be implied that this parasitoid species was much less specific with *Ficus* species. Cook and Segar (2010) also suggested that *Apocrypta* species is a generalist in host selection. Only three variables; *Apocryptophagus* sp., *Philotrypesis* sp., and wall thickness showed direct negative effects, while the diameter demonstrated a positive effect on a pollinator. The results indicated that *Apocryptophagus* sp., as a small galler, may compete with a pollinator for the same niche (flowers), while only *Philotrypesis* sp. was expected to be a parasitoid on a pollinator. The presence of *Philotrypesis* sp. reduced the number of pollinators through parasitism. The analysis showed that this parasitic wasp had a medium effect on increasing the number of galls (*Apocryptophagus* sp., Fig. 23). A possible explanation may concern the energy allocation to each flower. A decrease of pollinator larvae resulting from *Philotrypesis* sp. might increase the energy allocation to a flower containing *Apocryptophagus* larva, and then increase the survival rate of this species.

Figure 23 illustrates that wall thickness and diameter had a direct negative effect and a direct positive effect on pollinator, respectively. Wall thickness and diameter were probably associated with ostiole characteristic which, in turn, play an important role as a selective filter to the pollinator (Liu *et al.*, 2011). The thickness of the ostiole is represented by the wall thickness that may have a direct effect on the ability of the pollinator to enter a fig’s cavity. On the other hand, the diameter may reflect the width and the compact of the ostiole (personal observation). The wider the ostiole, the less compact it is, and it is probably easier for the foundress to enter into the cavity. Although there was a positive correlation between fig diameter and wall thickness, the effect of the diameter was stronger than latter variables on the pollinator (Fig. 23).

The number of florets showed a positive indirect effect on pollinator (*C. vetustus*) through two distinct pathways that were associated with non-pollinators (*Philotrypesis* sp. and *Apocryptophagus* sp., Fig. 23). This variable also had an

indirect effect on the *Apocryptophagus* sp. However, these effects were slight with path coefficients of 0.13 for pollinator, and -0.11 for *Apocryptophagus*.

In conclusion, this study is the first to report of the species composition of fig wasp in *F. schwarzii* in southern Thailand. Host specificity between fig and pollinator fitted the one to one rule. It is also a first report on a detailed set of investigation on the effect of each non-pollinator species on the pollinator population. The functional model demonstrated interspecies interaction in the fig wasp community on *F. schwarzii*. The pollinator was affected by *Apocryptophagus* sp. and *Philotrypesis* sp. through a competitive niche and parasitism, respectively. Three measured traits of *F. schwarzii* (diameter, wall thickness, and number of florets) were proposed as factors that maintained a stability of mutualism between fig and pollinator. Further studies were suggested in each section.

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**APPENDIX 1**

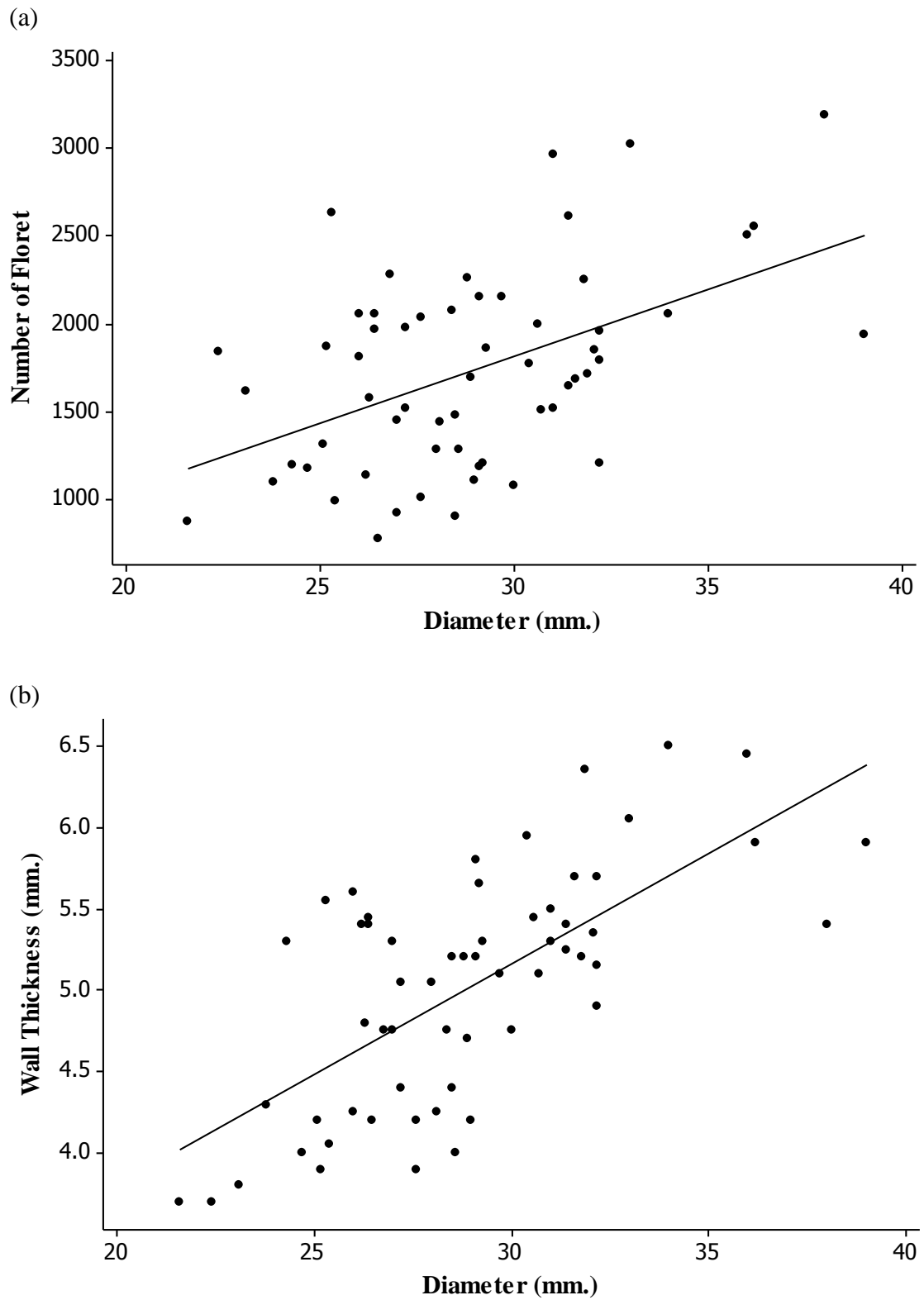


Figure 1. Positive correlation of fig diameter with (a) number of florets and (b) wall thickness.