CHAPTER I

INTRODUCTION

Stingless bees, the highly eusocial hymenopteran insects belonging to subfamily Meliponinae (Wille, 1983), are sometimes called stingless honey bees (Michener, 1974). Stingless bees have no sting which honey bees use for defensive purposes. Workers of stingless bee species use other means of defense very aggressive. In some species, they ejected burning liquid while they were biting their predators (Radovic, 1981).

Stingless bees are easily distinguished from honey bees which belonging to subfamily Apinae by the reduced wing venation, presence of a penicillum (a brush of long setae on the outer, apical surface of the hind tibia), and a vestigial sting (Michener, 1990, 2000; Ruttner, 1988; Will, 1979, 1983). Honey bees and stingless bees are the only highly social bees, but stingless bees lead more diverse life styles, including obligate necrophagy (Camargo and Roubik, 1991; Roubik, 1982), and can recruit for resources such as dead animal, pollen, nectar, mud, resin, water, and nests (Roubik, 1989). Only some members of the stingless bees families store honey in quantities that can be harvested by man (Ruttner, 1988).

These bees are of great importance for the pollination of many wild plants (Augspurger, 1980; Nogueira-Neto, 1970) in addition to tropical crops (Roubik, 1990).

Some species swarm out from the nest to attack a large intruder more aggressively than the workers of *Apis*. However, instead of stinging, they bite, and can be very effective because they crawl into the eyes, nose, ears, or hair; their mere presence is a terrible nuisance and their ability to nip with the jaws makes almost any enemy retreat (Michener, 1974). Species of one subgenus, *Trigona* (*Oxytrigona*), drop the caustic secretion from the enlarged mandibular glands (Kerr and Costa, 1961) on the skin of the intruder and then bite it into the flesh with the mandibles. The result is very painful and causes lesions that last for many days and may cause almost permanent scar.

Stingless bees are a monophyletic group principally found in tropical and subtropical areas of America, Africa, Australia, and parts of Asia (Roubik, 1989). Stingless bees consist of several hundred species distributed through more than 36 genera (Michener, 2000). At present, more than 500 species of Meliponinae have been described (Roubik, 1987; Ruttner, 1988). Most stingless bees exist in the tropics of the America continent (185 species), another 35 species exist in Africa, 42 exist in Asia, and 20 exist in Australia (Kerr and Maule, 1964).

The tribe of Meliponini is generally divided into five different genera; (1) *Melipona* lives only in tropical America; (2) *Meliponula* of which only a single species is found in Africa; (3) *Trigona* is spread in all the tropics and occupies 16 subgenera having a large variation in morphology and biology; (4) *Dactylurina* has only a single species in Africa; (5) *Lestrimelitta* is found in Africa and tropical America (Crane, 1999; Michener, 1974; Rinderer, 1986).

In Thailand, all species of *Trigona* are commonly known as "Channarong" (local Thai name). They are found throughout Thailand and are importance pollinators of natural plants. Because of their visit a wide range of crops (polylecty), tolerant of high temperatures, active throughout the year (Amano, 1999) and absconding of entire colonies as a response to disturbance is extremely rare in stingless bees (Eltz *et al.*, 2003). Individual colonies are generally perennial and reported maximum life spans range from 10 to 26 years (Roubik, 1989; Wille, 1983). Two species of stingless bees, *T. laeviceps* and *T. pagdeni*, they are kept by farmers in hive for agricultural pollination.

Klagasikorn *et al.* (2005) reported that there were 32 species of *Trigona* in Thailand as shown in Table 1.

Table 1 Species list of *Trigona* in Thailand.

No.	Species
1	*T. aliceae* Cockerell, 1929
2	*T. apicalis* Smith, 1857
3	*T. atripes* Smith, 1857
4	*T. binghami* Schwarz, 1937
5	*T. canifrons* Smith, 1857
6	*T. collina* Smith, 1857
7	*T. doipaensis* Schwarz, 1939
8	*T. ferrea* Cockerell, 1929
9	*T. fimbriata* Smith, 1857
10	*T. flavibasis* Cockerell, 1929
11	*T. fuscibasis* Cockerell, 1920
12	*T. fuscobalteata* Cameron, 1908
13	*T. geissleri* Cockerell, 1918
14	*T. hirashimai* Schwarz, 1939
15	*T. iridipennis* Smith, 1854

Table 1 Species list of *Trigona* in Thailand (cont.).

No.	Species
16	*T. itama* Cockerell, 1918
17	*T. laeviceps* Smith, 1857
18	*T. latigenalis* Cockerell, 1969
19	*T. melanoleuca* Cockerell, 1929
20	*T. melina* Gribodo, 1893
21	*T. mimor* Sakagami, 1978
22	*T. nitidiventris* Smith, 1857
23	*T. pagdeni* Schwarz, 1939
24	*T. pagdeniformis* Sakagami, 1978
25	*T. peninsularis* Cockerell, 1927
26	*T. sarawakensis* Schwarz, 1937
27	*T. scintillans* Cockerell, 1920
28	*T. sirindhornae* Michener and Boongird, 2004
29	*T. terminata* Smith, 1878
30	*T. thoracica* Smith, 1857
31	*T. valdezi* Cockerell, 1918
32	*T. ventalis* Smith, 1857

Studies on variation in honey bees were firstly done using morphological characters or morphometric (Ruttner, 1988; Smith, 1999). Morphometric characters are quantitative characters with generally high heritability (Cornuet and Garnery, 1991). The second method, protein markers (allozyme) was used to study genetic variation in honey bees (Smith and Brown, 1988; Sylvester, 1986; Yik-Yuen *et al.*, 1991). Finally, studies of the genetic variation of *Apis* at the DNA level have been developed (Cornuet and Garnery, 1991). Various molecular biology techniques, such as

RAPD (Random Amplified Polymorphic DNA) (Hunt and Page, 1995; Suazo *et al.*, 1998), RFLP (Restriction Fragment Length Polymorphism) (Deowanish *et al.*, 1996; Garnery, *et al.*, 1993; Meusel and Moritz, 1992; Sheppard and McPheron, 1993; Smith and Brown, 1990), Microsatellite (Estoup *et al.*, 1995; Frank *et al.*, 1998; Oldroyd *et al.*, 1996, 1998; Rowe *et al.*, 1997), and DNA sequencing (Cameron, 1993; Cornuet *et al.*, 1991; Crozier and Crozier, 1993; Estoup, 1994; Flook *et al.*, 1995; Garnery *et al.*, 1991, 1995; Meixner *et al.*, 2000; Sheppard and McPheron, 1993; Smith *et al.*, 1991; Willis *et al.*, 1992). However, very few studies have reported genetic variation of stingless bees using the DNA markers of Microsatellites (Green *et al.*, 2001; Peter *et al.*, 1998) and DNA sequencing (Costa *et al.*, 2003). Recently, Francisco *et al.*, (2001) characterized the mitochondrial DNA of five *Plebeia* species by RFLP. They detected a high level of interspecific variability and estimated a total mitochondrial DNA length of 18,500 bp.

In Thailand, species diversity and ecology of stingless bees have been reported by Rajitparinya (2000). However, the study of genetic variation has not been done.

The objectives of this study are to determine diversity of five species of *Trigona* (*T. collina* Smith, 1857; *T. fuscobalteata* Cameron, 1908; *T. laeviceps* Smith, 1857; *T. terminata* Smith, 1878; and *T. thoracica* Smith, 1857) from different regions of Thailand by using PCR-RFLP techniques of 16S rRNA gene of mtDNA. In more detail, development of PCR conditions for studying genetic variation of stingless bees belonging to *Trigona* will be performed. The result of this study will provide basic information in biology; biodiversity, geographic variation and genetic relationship among *Trigona* populations in Thailand, which may be applied for conservation biology.