CHAPTER III

METHODOLOGY

3.1 TAXONOMIC STUDY

The preliminary survey was taken between 2005-2007 at Chiang Mai province in different kind of ecosystem and elevation. Monthly observation planned was used for investigating. Both fig trees and fig wasps were collected and work on taxonomic study in laboratory.

3.1.1 Fig tree

a. Collection

- Both vegetative and reproductive parts of fig trees were collected such as shoots, leaves, stipule and figs (syconium or inflorescence) throughout all season of the year from June 2005 to May 2007. Surveys base on geographical data in different habits and elevation distributed in Chiang Mai.
- Herbarium specimens were preserved both dry and in 70 % ethyl alcohol, especially fleshy syconium and the voucher specimens were collected at least 3 sets and deposit at Etnobotanical Research Section, Chiang Mai University, Chiang Mai.
- Plant information; habit, color, special characters, bract arrangement, syconium phenology and also date of collection have been recorded.
- Photography was taken.

b. Identification

- Specimens of figs were identified by observing morphological characters using Corner (1965), Berg (2005) and Zhekun and Gilert (2003).
- Plant collections were investigated and rechecked by comparison with the materials at the following herbaria; Forest herbarium, Bangkok (BKF), Chiang

Mai University Herbarium, Chiang Mai, Queen Sirikit Botanic Garden Herbarium, Chiang Mai (QBG), Xishuangbanna Tropical Botanic Garden (XTBG), China

c. Description

- Description of all species represented in site study.
- Taxonomic literatures were included.
- Occurrence in Thailand and their distribution taken from literature were cited.
- Ecological and phonological information of each species were described.
- Photographs and line drawing of each species were illustrated.

3.1.2 Fig wasp

a. Collection

- All of wasp immerged from syconium in each fig were collected.
- Insect specimens were preserved both dry and in 70 % ethyl alcohol.
- Insect information; host, color, size, dominant characters, and also date of collection have been recorded.
- Number of wasps in each species per syconium was measured.

b. Identification

- Morphological characters were used for verifying wasp to species level by using Bouček (1988), Weibes (1994) and Noort and Rasplus (2005b).
- Fig wasps specimens were rechecked by expert entomologist in fig wasps (Rasplus J.Y′s, INRA-Centre de Biologie et de Gestion des Populations, France).

c. Description

- Description of all species of fig wasps pollinators which is represented in the study area.
- Occurrence in Thailand and their distribution taken from literature were cited.
- Photographs under the stereo microscope were taken.

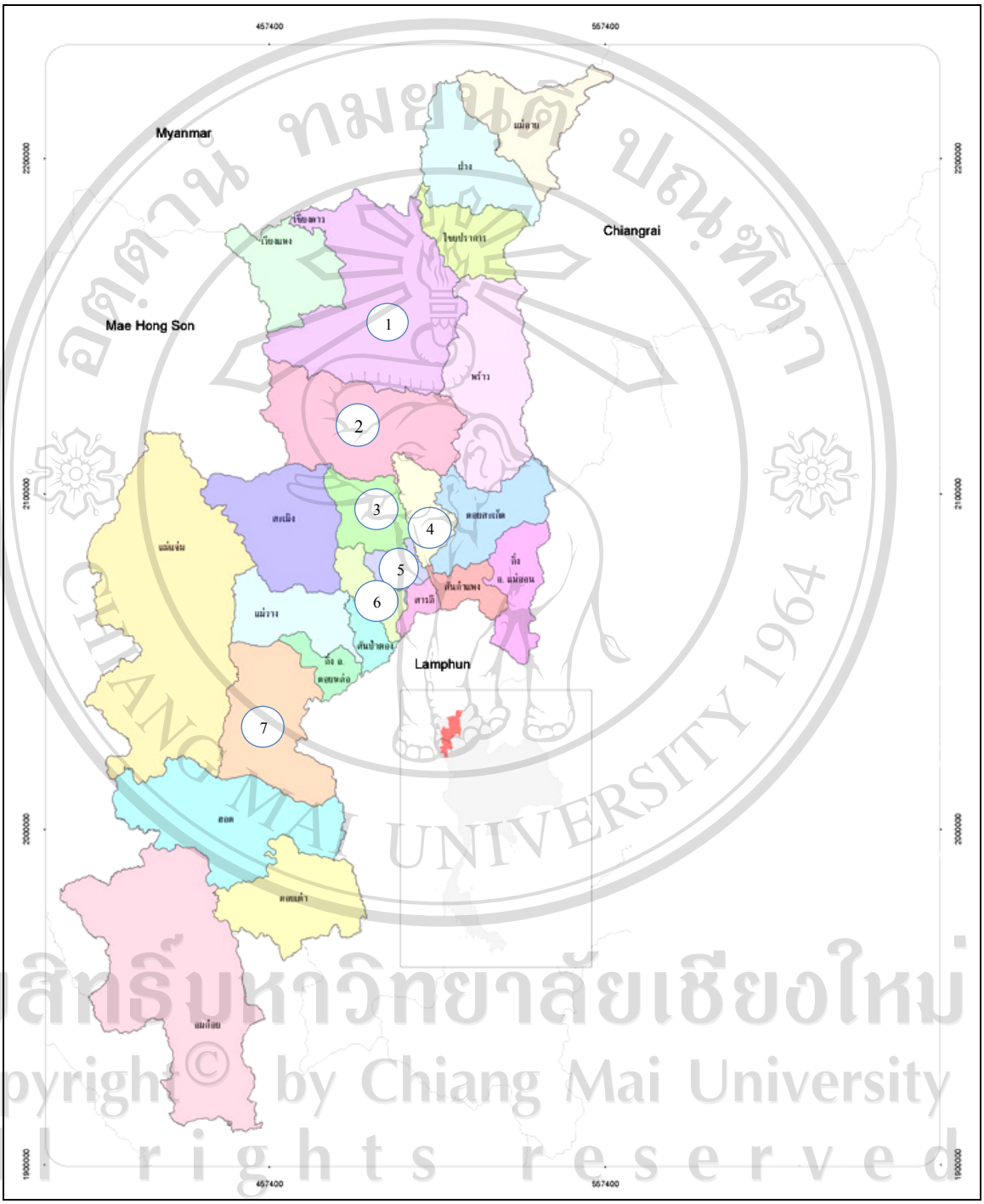

Figure 3.1 *Ficus* and fig wasps diversity's studies site, Chiang Mai, Thailand: 1. Chiang Dao, 2. Mae Tang, 3. Mae Rim, 4. Sansai, 5. Muang, 6. Hang Dong, 7. Chom Tong (http://www.chiangmai.go.th, 18 September 2007)

3.2 POLLINATION STUDY

The pollination study was examinated with a monoecious and a dioecious fig. For the monoecious species, *F. racemosa* were selected under the condition of easy to find and observe. Whereas the dioecious fig is rarely represented and male and female plants are separate in nature. Therefore the population of lithophytes fig species, *F. montana* Burm.f., in the glasshouse of Leeds University were investigated.

3.2.1 Pollination study of dioecious fig: *F. montana* Burm. f.

3.2.1.1 Species and study site

F. montana Burm.f. (section *Sycidium*) is a dioecious fig tree distributed in South East Asia (Berg, 2003b). It is a small shrub that is sometimes a climber, reaching 1–3 m tall. Its specific, active, pollinator is *Liporrhopalum tentacularis* (Grandi). The University of Leeds's glasshouse populations of *L. tentacularis* and *F. montana* were used for two experiments that were carried out between November 2005 and June 2006.

There are two aspects of the mutualistic relationship study between *F. montana* and its pollinator; the first is 'can pollinator success to produce offspring if without pollination? And the second is 'can male produce seeds if without egg laying?' The experiments on 'pollen-free' and 'oviposition prevention' were conducted.

3.2.1.2 Statistic analysis

The chi-square test was used to test the difference number of pollinator progeny, seeds, bladders and flowers (Nefdt, 1989; Hampton, 1994; Triola, 2004).

3.2.2 Pollination study of momoecious fig: *F. racemosa* L.

3.2.2.1 Species and study site

F. racemosa is a common fig tree with widely distribution in Southeast Asia (Zhang *et al*., 2006). It is the one of few monoecious figs of subgenus *Sycomorus*

(Berg, 1989). The habit is a tree, reaching 25-40 m in height. It is a dominant species along the riverside of several rivers in Chiang Mai. Flowering and fruiting year-round represent. Its specific pollinator is *Ceratosolen fusciceps* Mayr. The population of ***F. racemosa*** in urban area of Chiang Mai were used for investigating that carried out from September 2006 to September 2007.

3.2.2.2 Field observation

Phenological censuses of ***F. racemosa*** were made around Chiang Mai, 10 figs tree were monitored during September 2006-September 2007. Censuses were made from each crop of all sampling tree as following issues;

1. Figs bearing in each crop
2. Leave covering in each crop
3. Fig size and flower number in each syconium
4. Style length in the B-C phase
5. The proportion of syconium containing pollinators, non pollinators and seeds

3.2.2.3 Laboratory study

Some figs were sampling to observe their six developing phase by picking 5 figs in each phase and study. The fig developing phase was described as follow (Yang *et al*., 2002);

1. Early-floral phase, the syconium is seen as the size of a soybean seed, when the florets in the fig cavity are not visible.
2. Pre-female floral phase, when the syconium has just developed for 3-5 days, the miniature florets can be seen.
3. Female-floral phase, the female flower inside the syconium is in blossom and the fig wasp penetrates into the female syconium for pollination and the male ones for oviposition.
4. Interfloral phase, after fertilization took place in the female syconium, the fig embryo begin to develop and in the male syconium the gall flower are formed after oviposition by the foundress wasps and the larvae fully developed.

5. Male-floral phase, the flowers in the male syconium are open and become mature, and then carried by wasp.
6. Post-floral phase, seeds inside the female syconium ripened and soon female and male syconia both fell.

Fig specimens would be collected from the study areas during flowering stage. Plant specimens such as leaves shoots and syconium were taken to the laboratory, also their fig wasps. Twenty figs in early D phase were collected and kept in small containers that covered by fine cloth. All of wasps emerged were then dried for counting. Figs were taken to dissect in search of some remain wasps and to sample their seed production.

Both plant and insect specimens would be preserved in 70 % alcohol solution for future study. Morphological study of figs and their pollinators will be described by description and photography.

The fig wasps' specimens were classified by Bouček (1988) and Wiebes (1994).

3.2.2.4 Statistic analysis

The t-test was used to analyze quantitative measurements such as number of figs/tree, male pollinators/fig, female pollinators/fig, seeds/fig, total flowers/fig etc. (Nefdt, R.J.C. 1989; Hampton, 1994; Triola, 2004).

Figure 3.2 The species study of dioecious fig, *F. montana* in glasshouse

Figure 3.3 The species study of dioecious fig, *F. montana* in nature

Figure 3.4 *F. montana, a.* the syconium female fig, *b.* male fig

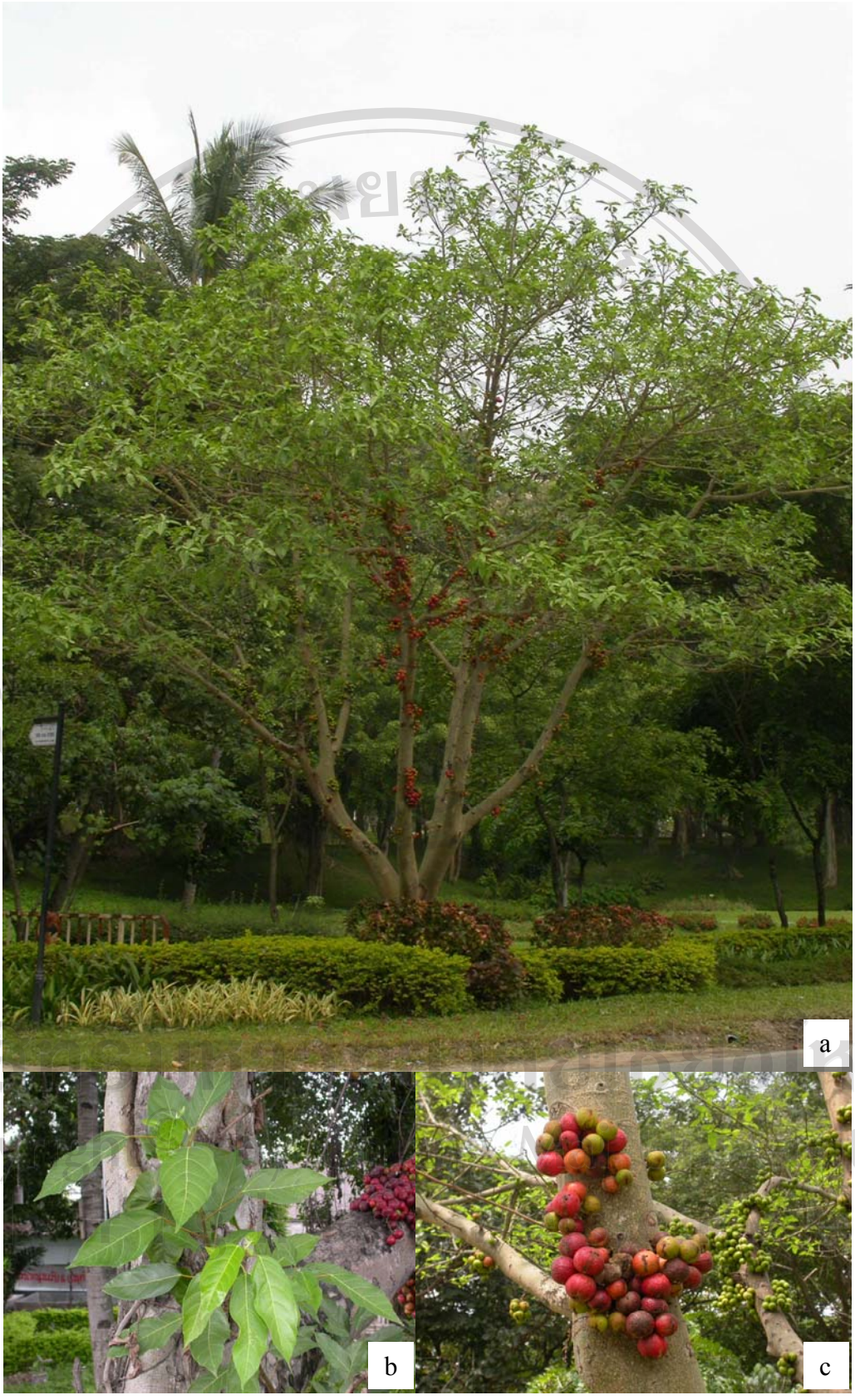

Figure 3.5 *F. racemosa* L., *a.* Tree with figs bearing, *b.* leaves, *c.* ripen figs