CHAPTER III

MATERIAL AND METHOD

1. Materials

1.1 Specimen collecting equipments

- a plant press, 30 x 45 cm.
- sheets of newspaper
- corrugated cardboard
- hand pruner
- spade
- plastic bage
- field note
- hand lens
- camera
- films (colour print and transparency slide)
- altimeter
- tags

1.2 Herbarium specimen preparing equipments

- Deep freezer (-40 c)
- Hot air oven
- mounting paper, 30 x 42 cm.
- species covers, 30 x 42 cm.
- genus covers, 30 x 42 cm.
- latex mixed with synthetic glue in ratio 1 : 1
- label pad, about 10.5 x 13.5 cm
- needle and thread
- sand bags

1.3 Identification equipments

- dissecting microscope
- dissecting needles
- razor blades
- petri dishes
- Related taxonomic literatures

2. Method

2.1 Literatures review

- Reviewed literatures dealing with *Argyreia* emphasized and related genera which distributed in Thailand and neighbouring countries such as Malaysia, Indonesia, India, Burma and Indo-China in order to use as a guidance for further study and collection.

- The related literatures were searched from the libraries at the Professor Kasin Suvathabhandhu Herbarium, Department of Botany, Chulalongkorn University (BCU) and The Forest Herbarium, Royal Forest Department (BKF).

2.2 Exploration and collection

- Field collections and flowering period observations of *Argyreia* was made in many provinces of Thailand as many as possible.

- The herbarium specimens of *Argyreia* available in Professor Kasin Suvatabhandhu Herbarium (BCU), Bangkok Herbarium (BK), The Forest Herbarium (BKF), The Herbarium, Department of Biology Faculty of Science Chiang Mai University (CMU), The Herbarium, Department of Biology Faculty of Science Khon Kaen University (KKU), The Herbarium, Department of Biology Faculty of Science Prince of Songkhla University (PSU), and Queen Sirikit Botanic Garden Herbarium (QSBG) have been throughly studied.

- Three duplicated of plants specimens with flowers or fruits were collected. The filed notes on morphological characters of each specimens such as color, form and size of flowers; kind of fruit, ecological information, habit and some diagnostic characters of each species were noted.

3. Laboratory study

- The details of external and internal morphological characters of each specimens were studied in the laboratory. Plant specimens were identified using both keys and descriptions from taxonomic literatures. The full descriptions of each species and line drawings were made.

- The herbarium specimens were prepared and have been kept in the Kasin Suvatabhandhu Herbarium, Department of Botany, Chulalongkorn University (BCU) and The Forest Herbarium, Royal Forest Department (BKF)

- Dichotomous keys to species based on their significant characters were made.

- In addition, other information, including ecological data, distribution, vernacular name for each species, were prepared.

Materials for palynological study

1. **Materials for field work and sample collection**
   - 1.1 Paper envelops
   - 1.2 A plant press, size 30X46 cm
   - 1.3 A pair of hand pruners
   - 1.4 Plastic bags
   - 1.5 Hand-lens
   - 1.6 Camera
   - 1.7 Collector's number card
   - 1.8 Color slides film (Kodak 100)
   - 1.9 Color printed film (Kodak 100)
2. **Materials for pollen slide preparation and pollen morphological study**
   - 2.1 10 % Potassium hydroxide
   - 2.2 Glacial acetic acid
   - 2.3 Acetic acid anhydride
   - 2.4 Concentrated sulphuric acid
   - 2.5 70 %, 95 %, absolute ethyl alcohol
   - 2.6 Distilled water
   - 2.7 Benzene
   - 2.8 Acetone
   - 2.9 Silicone oil AK 2,000
   - 2.10 Paraffin
   - 2.11 Immersion oil
   - 2.12 Paper envelops
   - 2.13 Label stickers
   - 2.14 Sieving crucible
   - 2.15 Pyrex beaker 50 ml., 100 ml.
   - 2.16 Vials
   - 2.17 Hot plate
   - 2.18 Warm plate

2.19 Centrifuge and centrifuge tubes

2.20 Microscopic slides and cover glasses

2.21 Micrometer, scale 1:100 micron

Light Microscope (model Nikon AFX 35) at the Prefessor Kasin Suvatabhandhu Herbarium, Department of Botany, Faculty of Science, Chulalongkorn University

2.22 Scanning electron microscope (model JEOL:JSM-5410 LV) at Technological Research Equipment Center, Chulalongkorn University.

2.23 Black – white negative films (Kodak TMAX 100 for LM and Kodak VP 120 for SEM)

2.24 Pollen materials: fresh materials, herbarium specimens

Methods

1. Field work and sample collection

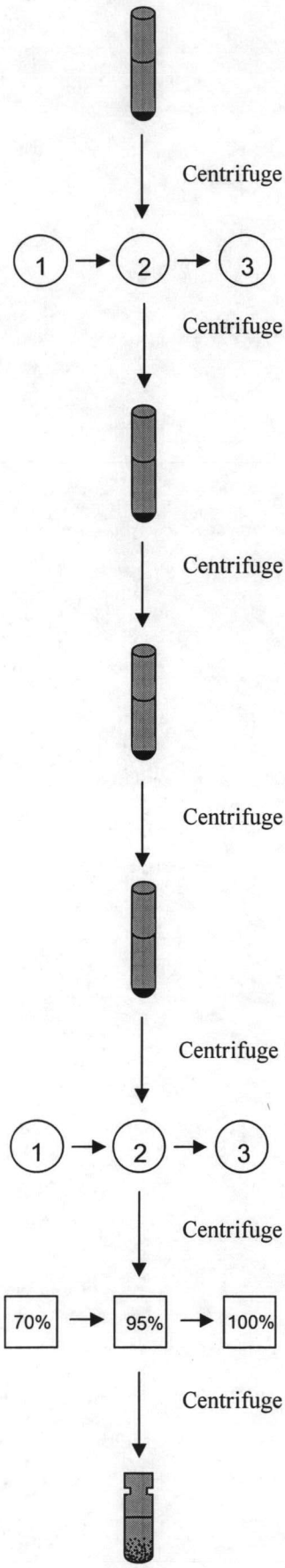
Field collection for herbarium specimens and pollen materials were made through many provinces in Thailand during May 2001 to January 2003. One to ten mature flowers of each species were kept and dried in paper envelopes. In addition, three duplications of plant specimens were collected.

2. Pollen slide preparation and pollen morphological study

Most of pollen samples were taken from fresh specimens were collected in the field. Some of them were obtained from the specimens deposited in BCU, BKF and CMU.

For LM, the pollen samples were treated by the acetolysis method (Fig.1) and preserved in silicone oil AK 2,000 (Andersen, 1960). The acetolysed pollen grains were mounted on microscopic slides and sealed with paraffin. Pollen morphological observations and photographs were made under a Nikon AFX 35. Permanent type slides of all samples are deposited at BCU.

For SEM, acetolysed pollen grains were dried and directly mounted on stubs with double-sides adhesive tape. The pollen grains were, then, coated with gold by using a Bazer-sputter coater for 5 minutes, and observed by SEM model JEOL JSM-5410 LV. SEM micrographs were taken with 1,000 to 15,000 magnification at kV.



1. Boiled pollen samples in 10% (KOH). (1 min.)
2. Washed with distilled water and centrifuge 1 min. (3 times).
3. Removed water by means of glacial acetic acid.
4. Removed of most organic matters with acetolysis mixture (conc. Sulphuric acid: Acetic anhydride; 1:9) and boiled 1 min. at 60-80 °C
5. Washed with glacial acetic acid
6. Washing with distilled water and centrifuge 1 min. (3 times).
7. Dehydrated by 70%, 95%, absolute ethyl alcohol.
8. Transferred acetolysed pollen grains from centrifuge tube to vial by means of benzene before preserved in silicone oil.

Figure 1. Acetolysis Method (Erdtman, 1960)