



CHAPTER III

MATERIALS AND METHODS

Materials for taxonomic study

1. A plant press, size 30 x 46 cm.
2. The sheets of newspaper and corrugated cardboard ventilators.
3. A pair of hand pruners and plastic bags.
4. Preserving agents and insecticides.
Formula : Mercuric chloride 75 gm
 95 % Ethyl alcohol 5 l.
5. Collector's number cards
6. Herbarium sheets, 27 x 43 cm.
7. White paper covers, 27.5 x 43 cm.
8. Brown paper covers, 30 x 44.5 cm.
9. Latex mixed with synthetic glue in ratio 1:1
10. Needle and thread
11. Labels, 10.5 x 13.5 cm.
12. Hand-lens and dissecting microscope with camera lucida
13. Camera and films

Materials for palynological study

1. Paper envelopes
2. Sieving crucible
3. Pyrex beaker, 50 ml.
4. Hot plate
5. Warm plate
6. Slides and cover glasses
7. Centrifuge and centrifuge tubes

8. Vials
9. Label stickers
10. 10 % Potassium hydroxide
11. Distilled water
12. Glacial acetic acid
13. Acetic anhydride
14. Concentrated sulphuric acid
15. 70, 95, 100 % Ethyl alcohol
16. Benzene
17. Silicone oil, viscosity 2000 cts.
18. Paraffin
19. Light microscope and micrometer
20. Scanning electron microscope (model JEOL JSM-35 CF)

Methods for taxonomic study

1. Reviewed literatures dealing with Convolvulaceae emphasized on ten genera; Aniseia, Bonamia, Cuscuta, Evolvulus, Hewittia, Merremia, Jacquemontia, Neuropeltis, Operculina, Xenostegia 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.
2. The herbarium specimens of those ten genera available in the Forest Herbarium, Royal Forest Department (DKF); The Herbarium of Department of Agriculture (BK) and The Kasin Suvatabhandhu Herbarium, Department of Botany, Chulalongkorn University (CU) have been thoroughly studied.
3. Field collections and flowering period observations of those ten genera were made in many provinces of Thailand as many as possible. Five duplicated of plants specimens with flowers or fruits were collected. The field notes on morphological characters of each specimens such as color, form and size of flowers; kind of fruit and ecological

information etc. were recorded. The transparency slides were also taken.

4. The details of morphological characters of each specimen were observed in the laboratory. The full descriptions and line drawings were made. The herbarium specimens were prepared and have been kept in the Kasin Suvatabhandhu Herbarium, Department of Botany, Chulalongkorn University.

5. The keys to genera and to species based on their significant characters were made.

Methods for palynological study

1. Boiled sample in 10 % potassium hydroxide.
2. Washed with distilled water.
3. Removed water by mean of glacial acetic acid.
4. Removed of most organic matters by acetolysis mixture.
5. Washed with glacial acetic acid and distilled water respectively.
6. Dehydrated by 70, 95 and 100 % ethyl alcohol respectively.
7. Mounted the acetolysed pollen grains in silicone oil, viscosity 2000 cts.
8. Examined and studied the sample slides by the light microscope and took the photographs. Recorded the distinguished character of pollen grains on wall, apertures, the polar axis, equatorial axis, polar field index, shape and size.
9. The dry-non acetolysed pollen grains were mounted on stubs then coated with gold before observation through JEOL JSM-35 CF scanning electron microscope. Photomicrographs were taken.