CHAPTER III
MATERIALS AND METHODS
(Plate 1)

III.1 AREAS OF STUDY

Study area includes various parts of Kerala and some adjacent areas falling in Karnataka and Tamil Nadu. Kerala, located on the Malabar Coast, shares its borders with Karnataka to the north and Tamil Nadu to the northeast. Kerala is geographically located in the southern peninsular part of India and stretches for an area of 38,863 Sq.Kms. Kerala lies between North latitudes $8^017'$ N and $12^047'$ N and East longitudes $74^027'$ and $77^037'$ E. In between high Western Ghats on the East and the Arabian Sea on the West, the width of state varies from 35 to 120 Km. Kerala can be divided into three regions: 1) High lands 2) Mid lands and 3) Low lands, according to their geographical features.

The High lands slops down from the Western Ghats which rises on an average height of 1500 meter elevation above sea level with a number of peaks well over 2500 meter in height. This is the area of forests and of major plantations like Tea, Coffee, Rubber, Cardamom etc. This is a hot spot area as far as the biodiversity of the fauna and flora concerned.

The Mid Lands lying between the Mountains and Low Lands is made up of undulating hills and valleys. This is an area of intensive cultivation. Cashew, Coconut, Areca nut, Tapioca, Banana, Paddy, Ginger, Pepper, Sugarcane and Vegetables of different varieties are grown in this area.

The Low Lands or Coastal area which is made up of river deltas and back waters. The shore of the Arabian Sea is essentially a land of Coconut and Paddy. Kerala with its rich and diverse flora provides an excellent habitat for insect fauna throughout the year. The Torymid fauna is found to be distributed throughout Kerala.
Specimens for the study are collected from various habitats like Grass lands, bushes, forests, Paddy fields, Plantations, Riverine areas etc.

### III.2 CLIMATE OF KERALA

Kerala’s climate is heavily influenced by the seasonal heavy rains brought by the monsoon. The climate in Kerala can be divided into three periods, viz., summer, monsoon and winter. The summer begins approximately in the second half of February and lasts till the end of May. The monsoon period starts in June and ends in September - October. This is followed by the winter which lasts till the beginning of February. Kerala’s average maximum daily temperature is around 36.7° C and the minimum is 19.8° C. Kerala’s average humidity is 81% and Kerala receives an average rain fall of 3107 mm.

### III.3 PERIOD OF COLLECTION

Collections were made during 2006-2011. Specimens, galls and ootheca were collected throughout the year, mainly collected after the rainy season from September to May. Collections were made at different times of the day. Collections were best between 8 am to 11 am.

### III.4 METHOD OF COLLECTION OF TORYMIDAE

**A) SWEEPING WITH SWEEP NET (Plate:1:A)**

This is the best method for collecting Torymidae as well as several other groups of insects. The sweeping was done using a triangular type of sweep net instead of conventional round type. The sides of the frame measures 48, 46 and 48 cms. The handle measures about 106-122 cms. The frame can be fitted to one end of the handle and can be easily separated when not in use. The net bag is made up of durable white cotton cloth or terelene cloth which have fine mesh that will permit easy passage of air but at the same time prevent escape of smaller insects of less than 1 mm in size. Since the mouth of the frame is wide enough, the collector can put his hand and head into the bag and remove the insect with the help of an aspirator (Plate:1:D). The specimens from the net can be sucked up by using the
aspirator. Once the specimens are collected in the aspirator, they are killed by placing a small wad of cotton wool soaked in ethyl acetate. The conventional use of killing bottle using potassium cyanide, chloroform etc is abandoned these days by entomologists and instead bottles with ethyl acetate are used for killing insects. This is because ethyl acetate gives a better relaxing effect than the chloroform or cyanide and chances of poisoning by breakage of killing bottles which contain potassium cyanide is thus avoided also. Major part of my collection work was done with the help of sweep net. The net was made from an industry in Calicut.

B. MALAISE TRAP (Plate: 1: B)

Another method of insect collection is by using Malaise Trap. This trap which makes use of the negatively geotactic and positively phototactic behaviour of insects is originally invented by Malaise. Later several modifications of the original malaise trap are now in use. Basically the trap resembles a tent made of fine-mesh terelene gauze with a specially adapted collecting bottle at the top. This bottle has a capacity to hold 550 ml. The trap can be kept on grasslands, near paddy fields, inside forests etc. This trap requires no power for operation and on a sunny day will collect well over a thousand insects without attention. Most of the insects collected by this trap will Hymenoptera- Parasitica and Diptera with a smattering of Homoptera, Thysanoptera and small numbers of some other insect orders. The complete trap is about 183 cms wide, 106.7 cms high at one end and 198 cms high at the other end. The insects when fly into the sides of the trap by chance crawl upwards to the roof (negatively geotactic behaviour) where they enter a collecting bottle (situated in a direction of sunlight usually) containing 70-90% alcohol. The trap was imported from Czech Republic.

C. YELLOW PAN TRAP (MOERICKE TRAP) (Plate: 1: C)

This trap is based on the principle that many insects are attracted to yellow colours. This is a simple tray measuring 60-70 mm deep and about 30 cms square. It is painted bright yellow on inner side and black on outer side. The tray is placed on the ground in a suitable habitat such as grass land, paddy fields, gardens etc. The tray is filled with water to which a few drops of detergent is added to break the surface
tension. The yellow pan is emptied once in a day using fine mesh net in order to filter the specimens. The yellow pan will not be of much use if kept in a place where lots of trees or plants with yellow flowers are present. We can use yellow plastic dishes also as yellow pans traps.

**D REARING**

This is the most useful method of collecting Torymidae since this method gives the following useful pieces of information:

a) The host data, host association and other biological information etc about the parasitoids and their hosts.

b) Rearing parasites from hosts will enable one to positively associate males with their females which otherwise is often difficult because males of many species of a group resemble extremely closely (sibling).

Rearing may be easy or difficult depending on the type of host. It is relatively easier to rear parasitoides from eggs, scales, pupae, seeds, fruits, galls, mines etc. In the case of host larvae, one has to feed them long enough for the parasite to develop. Simple emergence cages or any glass vial or containers can be used for rearing quiescent hosts. During my work I could collect ootheca of different species of Mantids as it is the host for many species of torymidae. Plant galls were also collected to rear torymids.

The best method for collecting large number of diversified specimens is by sweep net followed by malaise trap and yellow pan trap. However for knowing host associations the best method is rearing the parasites from their hosts.

**III. 5 MOUNTING**

After drying, the collected specimens are mounted in two different ways. They are:

**A. Rectangular Card Mounting**

Materials used for card mounting are:
a) Fine zero point brush  
b) A pair of fine needles or pins  
c) Water soluble glue  
d) A pair of fine forceps  
e) Mounting cards

In this method the specimen is mounted on a rectangular card in such a way that the specimen is made to lie on one side on the card with a drop of glue sticking it to the card. The specimen will lie 45° to the plane of card. This enables one to see the features from front (face), dorsal side, and from lateral aspect. This method gives better protection of the specimen than all other methods mentioned earlier above. The white background of the card enables one to see the various parts of the specimen more clearly (however in some cases the reflection of white card hinders clear vision). Water soluble glue is used for mounting specimens on cards.

B. Card-point mounting

In this method a specimen is glued to a triangular card point on lateral or ventral part of the specimen. Though this may facilitate examination easier, the small area of attachment provides little protection for the specimen and it may drop off while handling.

III. 6 LABELLING AND REGISTERING (Plate: 1: F)

Temporary labels are written in the field at the time of collecting the specimens. After mounting the specimens, permanent small rectangular labels were given which contain the information such as name of the country (in capital letters), name of the state, name of the locality from which the specimens are collected, name of the person who collected the specimen, date of collection, name of hosts, collection number etc. The smaller the labels the better it will look. The labels can be written by using a Rotring (0.1 or 0.2) microtip pen or by computer printing. If it is a new species it should be marked as holotype and paratype with red and yellow coloured paper respectively. All specimens collected may be registered in register book with the following details:
III.7 PRESERVING AND STORING (Plate: 1: E & G)

The unmounted materials are stored in 70% alcohol in small bottles (Plate: 1: E), which are kept in a refrigerator or in air-conditioned room. The, bottles are labeled. The preservative was periodically changed and replenished to prevent deterioration. The specimens are dry-mounted or slide-mounted before completing five years in alcohol. The card mounted materials are preserved in insect boxes specially made for the purpose (Plate:1: G). Naphthalene balls are placed on the inside corners of the boxes in order to protect the specimens from other injurious insects. Thymol crystals are used as fungicide. Besides, the collection boxes are periodically subjected to warming by using table lamps to avoid fungal growth.

III.8 RELAXING OF SPECIMENS

In order to prevent breakage of highly rigid and brittle specimens, they are subjected to relaxing chamber containing a few drops of glacial acetic acid in a layer of cotton wool which is again covered over by a second layer of cotton wool without glacial acetic acid. Specimens to be relaxed are usually kept on a piece of tissue paper in a glass dish and kept over the cotton wool. Then the relaxing chamber (any suitable small glass or plastic box) is tightly closed and kept for 5 to 10 hours.

III.9 IDENTIFICATION, DRAWING & PHOTOGRAPHY (Plate: 1: H&E)

The labeled specimens are studied with the help of microscopes. Observations and drawings of the specimens were done with the help of leica MZ 6 stereozoom
microscope with camera lucida (Plate:1:H). Photographs of the specimens were taken with the help of leica EZ 4D stereozoom microscope with inbuilt camera (Plate:1: I), which is attached to a computer. Identification of the specimens was done with the help of taxonomic keys and literature.

III.10 STUDY OF HOLOTYPES FROM DEPOSITORIES

Some types (holotypes and paratypes) of known species were studied from depositories like DZCU & ZSIK. Photographs of the available holotypes were taken from ZSIK.
A. Sweep net  
B. Malaise trap  
C. Yellow pan trap  
D. Aspirator  
E. Preserving vials  
F. Labelled specimens  
G. Insect boxes  
H. Leica MZ 6 Stereozoom microscope with camera lucida  
I. Leica EZ 4D Stereozoom microscope with inbuilt camera