CHAPTER 3

MATERIALS AND METHODS

3.1. STUDY AREA (Plate. 1)

For the taxonomic studies of Eurytomidae, the specimens were collected from all the fourteen districts of Kerala. The methods used for collection may vary from case to case (which were explained in detail in the next session of the same chapter). Kerala is the south west State of India and occupy a unique position in the world map with a wide costal area. It shares its boundary with Tamil Nadu in eastern and southern sides and with Karnataka in Northern and Northeastern sides.

Kerala have an overall land area of 38864 square Kilometer, which forms 1.18% of the total land area of India. The most peculiar feature of Kerala is the presence of Western Ghats and coastal areas and Arabian Sea in entire length and blessed with high faunal diversity in terrestrial and aquatic habitats. The state lies between the north latitude 8.32187 and 12.75490 and east longitude 74.89400 and 77.15012.

Geography

Considering the geographical features of Kerala, the state can be divided in to three regions viz. high lands, mid lands and low lands. The high lands also called eastern high lands having an elevation above 76 meter which is mainly part of slopes of Western Ghats and generally having an average slope of 900 meter and with a number of peaks having height over 1800 meters. The highest peak is ‘Anamudi’ with 2695 meter height and located in Idukki district. The high lands mainly include forest areas and plantations, which cover an area of 18653.5 square Kilometer, which is 48% of the land area of the state. The fourty four rivers originate from this area and among
them 41 are flowing towards west and three to east. The high lands spread in almost all districts of Kerala except Alappuzha with major cultivated crops tea, coffee, rubber and cardamom.

Mid lands are areas present between the elevation ranges of 76 and 7.6 meter. Mid lands lie between high lands and low lands or between slopes of Western Ghats and coast. This land is highly suitable for cultivations of Areca nut, banana, cashew, coconut, ginger, pepper, rice, tapioca, vegetables etc. due to it its soil texture. The mid land covers an area of 16231.2 square kilometer and constitute 41.76% of the land area of the state.

Low land comes below the elevation of 7.6 meter. This part mainly includes the coastal areas formed mainly by deposition of sediments brought down by rivers which originates from high lands and also by the sand deposited due to sea waves. It covers an area of 3979.3 square kilometer and about 10.24% of the total land area of Kerala state. The district Alappuzha lies exclusively in low land and the main crops in these areas are Coconut and Paddy.

**Field Surveys conducted/Participated**

During the study period, field surveys to all fourteen districts of Kerala State were conducted. Collections were also made by being a part of the faunistic surveys conducted by Zoological Survey of India, Western Ghat Regional Centre, Kozhikode, to different types of ecosystems like deciduous forests (Plate. 3. Fig. 1&2), grasslands (Plate. 3. Fig. 3&4), Marshy wetlands (Plate. 3. Fig. 5) etc. As a part of AICOPTAX Project in which I am working as Senior Research Fellow, faunistic surveys to different States of India were also conducted and participated.
3.2. METHODS OF COLLECTION

a. Net Sweeping (Plate. 2. Fig. 2)

Net sweeping was found to be one of the best methods for collecting eurytomids as like that of many parasitic Hymenoptera. The main advantage of net sweeping is that we get plenty of specimens with high diversity in a short period of time while comparing with other techniques for collection. One of the main disadvantages of net sweeping is that we didn’t get any data regarding the host, and sometimes the new or interesting species will be represented by single specimen or very low numbers and the chance for getting the same species in the same locality also will be less. Even though these disadvantages are there, net sweeping is considered as the best method for collecting diverse specimens of Eurytomidae.

The insect net used for collection is a modified type designed by NOYES, 1982. The modifications are done for increasing the number of specimens collected in a set of sweeping. The sides of net frame were made by aluminum measures 48cm x 46cm x 48cm. The triangular shape of the frame allows a larger area of vegetation to be covered while sweeping. The handle measures about 106-122cms. The long handle allows sweeping underneath and overhanging bushes easier and extends the area covered in each sweep. 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 with 80cm length, which have fine meshes that permit easy passage of air but at the same time prevent escape of smaller insects of less than 1mm size. The open end of the bag is fixed in the triangle shaped end of the frame by sewing with canvas. The results obtained by using this type of net shows that the insects collected are roughly 10 times more than the insects obtained by the conventional type of round nets.
For getting better results the area which is selected for sweeping is also very important. Normally insect activity will be high in areas with highly diverse vegetation. Grass lands with good collection of flowering plants surrounded by bushes and trees normally forms a good site for collection of Eurytomids. The flowering or seeding stages of vegetable fields and other agricultural fields also show high results. The sweeping was done as described by NOYES 1982. The specimens from the net can be sucked up by using an aspirator (Plate. 2. Fig. 4).

The specimens collected by the aspirator, were killed by placing small cotton soaked with four or five drops of Ethyl acetate through the entry tube of the aspirator and immediately closing the tube. The aspirator was kept for 20-30 minutes to ensure that all the specimens in the aspirator were killed. The dead insects were transferred to the vials having 70% alcohol. A temporary label with locality, date of collection and name of collector was placed in the vial.

b. **Malaise trap** (Plate. 2. Fig. 1)

The suitable design of Malaise trap has been well defined by TOWNES in 1972. It is a tent like device made by terelene cloth with fine mesh, which works on the basis of the positive photo tactic and negatively geotropic behavior of insects. The malaise trap is one of the best methods to collect insects by passive method. The major parts of malaise trap are the tent like device with alcohol holding bottle, ropes and steel rods for fixing the trap in correct position. The flow of wind and the presence of sun light has significant role in determining the efficiency of the collection. Due to this reason the malaise trap should be fixed in an area with good sunlight especially on the area where alcohol carrying bottle is present. The passage of a slow wind through the open end of the tent is also good for getting smaller insects.
The insects which stuck accidentally and small insects which fly with the direction of wind are stuck up with the separator present in the tent and crawl up through the cloth or fly up due to photo tactic behavior and finally enter in to the bottle with 70% alcohol. The transferring of collections was made every week by draining the alcohol container and refilled it with fresh 70% alcohol. There are several modifications of the original malaise trap. For the collection the malaise trap made by Rescholar Equipments, India was used. The main advantage of this trap is that, it only needs to remove the collections once in a week and the collections can also be done by a non entomologist.

c. **Yellow pan trap** (Plate. 2. Fig. 3)

This trap is also called by the name Moericke trap, which works on the principle that insects are attracted to the yellow color. The pan used measures 6 - 7 cm deep and 30cm circle with bright yellow color. The tray was filled with water and a few drops of detergent solution are added to reduce the surface tension of water. The trap was placed in suitable habitat, with preference to shadow areas. Normally the tray was set in the morning and the collections were taken out in the evening. A small hand net was used to empty the tray and before transferring the specimens to 70% alcohol, the specimens were washed with fresh water to avoid deposition of detergent on the specimens which lead to damage or reduced visibility of the body parts. The washed specimens were transferred to the vials containing 70% alcohol. The yellow pan may not give good result if it is kept in a habitat with high number of yellow flowers (Narendran, 2001).

d. **Rearing**

The suspected hosts collected from the field were placed in suitable containers with cotton cloth lid till the emergence of the parasitoids. The host
materials include eggs, larva, pupa, galls, seeds, etc. It is a rewarding method for the collection of insect parasitoids. In some cases specialized emergence cages are required for larger plant parts. The emerged parasitoids were collected by using aspirator (Plate. 2. Fig. 4) or light trap method depending up on the nature of the parasitoid. The time of emergence of parasitoids may vary from host to host and it also depends up on the nature of host. The advantage of the rearing method is that we will get the informations about the host and its associates, its biological information, etc. In this method, there is also a high possibility of getting both sexes, and in the case of Eurytomidae or even in Chalcidoidea there are no distinguishable characters or key for the correct identification of males up to species level due to the high similarity at species level.

3.3. STORING AND PRESERVATION

a. Unmounted Material

The unmounted specimens were stored in air tight vials containing 70% alcohol and kept in refrigerator for better result. The preservatives were periodically changed and replaced with fresh alcohol.

b. Relaxing Material

Relaxing is required for the specimens which are killed by using Ethyl acetate and these specimens becomes more harder due to delay in transferring to alcohol. This technique is also used for remounting of specimens which are already mounted. For relaxing, the specimens were kept in relaxing chamber with glacial acetic acid. Relaxing chamber is nothing but an air tight rectangle box with a cotton bed. After 6-8 hours, due to the activity of glacial acetic acid, the specimens become soft and the mounting or spreading of legs and wings become easy without breakage.
3.4. MOUNTING

a. Card mounting

The methodology followed for mounting the eurytomid specimens in the present work was following NOYES, 1982 and NARENDRAN, 2001. The specimens were mounted on triangle card having length 14mm and a maximum width of 5mm. The materials used for card mounting are as follows:

1. Absolute alcohol
2. Cavity block
3. Blotting paper
4. Fine zero point brush
5. Table lamp with 60W bulb
6. Mounting cards (Ivory paper)
7. Entomological pins (No. 4)
8. Water soluble gum
9. Stereo zoom microscope

For best results the specimens should be mounted immediately after killing. But in continuous field trips this may not be possible hence the specimens are kept in 70% alcohol. While mounting, the specimens should be dried first by placing it in absolute alcohol for 10 minutes. In the case of freshly killed specimens, they are first transferred to 70% alcohol for 10-20 minutes and then transferred to absolute alcohol to avoid the collapsing of gaster in the case of soft bodied specimens. Generally the eurytomids have
hard body so if directly transferred to absolute alcohol, normally collapsing
does not occur except in some species of Tetramesa and Sycophila.

The dried specimens were then transferred to blotting paper which was
placed under table lamp with 60W bulb for one minute. The softly dried
specimens were then placed under the stereo zoom microscope along with
blotting paper and spread the legs, wings and antenna in correct position to
make all body parts visible. By using an entomological pin a small drop of
water soluble glue was placed on the tip of the triangle card with the help of a
needle under microscope. The glue must be very less and adequate for
holding the specimens, normally the amount of glue used is 2/3 volume of the
mesosoma of the mounted specimen. A zero point brush was moistened with
a drop of alcohol and specimen was picked with the brush on its left
mesopleuron and placed it on the glue, making sure that the right
mesopleuron was getting fixed with glue with a good visibility of the dorsal
side of the mesosoma and gaster. The specimen was tilted so that it lied on its
sides at about 45° to the card. Make sure that all the parts of the specimens
were touched or supported by the card, if not such parts may be broken later.
The specimen was gently pressed by using the brush to make it tightly
attached with glue. Then the card was pinned using an entomological pin
(Bohemia Insect pins No. 4: 38x0.55mm) using a pinning block.

The first label having data on the locality, date of collection and name
of collector was taken from the temporary label which was made in field. The
first label should be in the format, first the country name in capital letters
followed by the name of the State both should be in the same line. The next
line include name of the district of the collection locality and the exact
collection locality. The exact date of collection was indicated in the next line
and followed by the name of collector. If any host data is available then it was
added as second label. The specimen mounted on card was then placed under
table lamp with 60W bulb for 5-6 hours to make it completely dry. The registering of the specimen was done after identification up to species level, with details of serial number, register number, scientific name, name of the person who identified the species, name of the locality, date of collection, name of collector and remarks. There was another label with scientific name and the name of the person who identified the specimen, which was added to each mounted specimens. Labels were printed in Microsoft word with Times New Roman as font and with a font size 5.

The sorting and mounting of specimens were done by using Labomed CZM6 microscope with an additional 2x objective lens. The card mounted specimens were observed under Leica M 60 and Leica S8 APO with LED illumination (Germany). The properly mounted and labeled specimens were stored in insect boxes and kept inside insect cabinets. Naphthalene balls and 1, 4- Dichlorobenzene were used as insecticide and fungicide to avoid damage of specimens caused by other small insects and fungi.

3.5. PHOTOGRAPHS AND MEASUREMENTS

Photos of the different parts of the specimens were taken by using DFC 450 camera attached with Leica S8 APO microscope (Plate. 2. Fig. 5). The editing of the images in a permissive level was done in Adobe Photoshop CS 5. The measurements were taken from the photographs taken by DFC 450, by using the specialized software Leica LAS V 4.0.

3.6. GPS

GPS by GARMIN etrex®H was used to gather information on latitude and longitude of collection localities of this study. The distribution maps of genera and species were generated using DIVA- GIS 7.5. Geographical coordinates of the collection localities has been provided in appendix.
3.7. IDENTIFICATION

Most of the literatures are available in Universal Chalcidoidea Database, Natural History Museum, London. The remaining literatures were obtained from the personal collections of Dr. T. C. Narendran and Dr. P. M. Sureshan.

The generic level identification was made by running the keys of Boucek, 1988 and Narendran, 1994. The species level identification was made by the keys of Narendran, 1994. Since several species in the monograph of Narendran, 1994 underwent generic transfer, the exact identity of species was made by comparing with original description and also by examining the type specimens. For the new and poorly described species under the genera *Neobephrata, Philolema, Prodecatoma, Sycophila, Systole* and *Tetramesa* complete descriptions were provided and for the already described species with good description, a short diagnosis with major characters were provided.

3.8. TERMINOLOGY (Plate. 4 & 5)

**HEAD**

**Antenna:** The paired sensory appendages present between compound eyes

**Clypeus:** The medial sclerite of the head just above the labrum.

**Scrope:** The groove on head to accommodate the scape.

**Sculpture:** Specialized marking or pattern of impressions on the surface of body.

**Scape:** First segment of antenna after the base radicula.

**Pedicel:** Second segment of Antenna.
**Ring segment 1 & 2:** The third and fourth segment of antenna and these are the smallest segments.

**F1 to F6:** The funicular segments one to six. Ring segments are followed by funicular segments.

**Clava:** The last three segments of antennae.

**Frons:** The area of head between toruli and front ocellus.

**Face:** The area of head between toruli and clypeus.

**Gena:** The lateral part of head after compound eye up to post genal carina, and normally called the cheek.

**Malar space:** The shortest lateral distance between compound eye and base of mandible.

**Malar sulcus:** The vertical groove present in malar space.

**Mandible:** Paired highly sclerotized chewing lateral appendage of mouth parts, with teeth.

**Toruli:** The paired socket in front of head which accommodated the radicula of antenna.

**Carina:** A ridge or raised area.

**Preorbital carina:** The carina present between compound eye and scrobe.

**Post orbital carina:** The carina present between compound eye and post genal carina.

**Post genal carina:** The carina present between gena and occiput. Normally it is considered as the ventral edge of gena.
Ocelli: It is the simple eyes present on dorsal part of head, with a triangle shaped arrangement.

POL: The distance between posterior ocelli.

OOL: The shortest distance between one posterior ocelli and compound eye.

AOL: The shortest distance between posterior ocelli and anterior ocellus.

Vertex: It is the roof of head, the area between anterior ocellus to occiput.

MESOSOMA

Prepectus: The triangle shaped structure or sclerite present between lateral sides of pronotum and mesepisternum.

Mesopleuron: Lateral part of mesothorax.

Tegula: Small, almost rounded sclerite which cover the base of fore wing.

Scutellum: The middle region between mesoscutum and propodeum.

Pronotum: The first segment of mesosoma dorsally.

Mesoscutum: Pronotum followed by mesoscutum, it is the second segment of mesosoma.

Propodeum: It is the first segment of gaster which is immovably fused with mesosoma.

Petiole: The stalk like structure to connect the propodeum and gaster tergites.

Natauli: the longitudinal groove on mesoscutum, and usually it makes a median lobe on mesoscutum.
FOREWING

SMV: Sub marginal vein.

MV: Marginal vein.

PMV: Post marginal vein.

STV: Stigmal vein.

CC: Costal cell.

METASOMA

T1 to T7: Gaster tergites one to seven.

Tergites: The dorsal segments on gaster.

Sternites: The ventral segments of gaster.

Ovipositor Sheath: The bi layered protective sheath of ovipositor.

3.9. ABBREVIATIONS

QMB : Queensland Museum, Brisbane, Australia

BMNH : The Natural History Museum, London SW7 5BD, England

ZSIK : Zoological Survey of India, Western Ghat Regional Centre, Kozhikode.

ZSIC : National Zoological Collections, Zoological Survey of India, Kolkata.

PCAB : Priyadarsanan Collection, Ashoka Trust for Research in Ecology and the Environment (ATREE), Hebbal, Bangalore 560 024, India

CNC : Canadian National Collections of Insects and Arachnids, Ottawa, Canada

Col : Collector