IV. MATERIALS AND METHODS

The study area i.e, Pachmarhi Biosphere Reserve (PBR) was repeatedly visited during the study period (2010-2011) as described in the chapter III. The bryophytes were collected from an altitude ranging from 400 to 1350 m (a.s.l). Bryophytes were collected from the diverse habitats selected at altitudinal gradient i.e., Lower (below 800 m); Middle (800 m to 1000 m) and Higher (above 1000 m).

4.1. COLLECTION AND PRESERVATION OF PLANT MATERIAL

The major localities (sites) undertaken for collection were selected across the three altitudinal gradients, viz. Lower altitudinal gradient (400m- 800m), Middle altitudinal gradient (801m-1000m) and Higher altitudinal gradient (1001m-1400m). Attempts were made to collect the plants from different areas of the sites as to cover the overall diversity and distribution of bryophyte flora.

The collection of the plant material was made during the peak period of growth, i.e, the rainy season. However some visits were also made during winter months. The plants were collected along with the substrate in polythene bags, on which the sites, altitudes and habitats were marked. Simultaneously, in the field diary, the specimen no, date of collection, altitude, habitat, locality of collection, associate plants and collector’s name were also noted. The collected plant material was brought to the laboratory and separated species wise and air dried to prepare the herbarium specimens. Specimens were preserved in the standard sized brown herbarium packets with proper accession numbers and field data. The specimens were deposited in the bryophyte herbarium at CSIR- National Botanical Research Institute, Lucknow (LWG).

4.2. IDENTIFICATION OF PLANTS

The identification of liverworts and mosses was carried out at the Bryology Laboratory at National Botanical Research Institute, Lucknow. The plants were identified by using the respective flora of Kashyap (1929, 1932), Gangulee (1969-1980), Chopra (1975) and Bapna and Kachroo (2000). Besides, several research papers and monographs published time to time by bryologists were also consulted (Srivastava and Udar 1976, Srivastava and Srivastava 2002 and Singh and Nath 2007). The specimens of Japanese Exsiccatae and other specimens deposited in the bryophyte
herbarium of NBRI (LWG) belonging to other regions of India like western Himalaya, South India, eastern Himalaya and central India have been critically examined and compared during the course of identification to confirm the status of taxa described in the present work.

Mature as well as young plants were investigated carefully. For identification, the soil adhered to the plant samples were removed with a fine soft colour brush. Plants were soaked in water in petriplates for 2-6 hours, depending upon whether the specimens were fresh (maximum 2 hours in this case) or the air dried ones (maximum 8 hours). In case of mosses and leafy liverworts, medium sized leaves were removed one by one from the stems carefully, so that the tissue of the basal part would not damaged. For thalloid forms, the details of thallus morphology and anatomy were thoroughly studied in detail for proper identification.

The plant morphology was studied by a Stereoscopic Binocular Microscope (Model M6C -10, LOMO, United States and Leica MZ 12, Germany), while the anatomical studies were done by compound Light Microscope (Olympus, Japan and Leica ATC 2000, Germany). Intracellular studies and cellular photography was done by Nikon Advanced Trinocular Research Microscope (Model Eclipse 80i, Japan).

4.3. ANATOMICAL STUDIES

Anatomical details of the liverworts and mosses were done by preparing the temporary slides of various parts of the plants. Morphological studies of structures like like thallus, rhizoids, scales in thalloid forms; leaves, underleaves, lobules for leafy liverworts and for mosses, perichaetial leaves, leaves, capsule, spore, peristome were done. Also, the slides of transverse sections of stem, leaves, thallus, and capsule were prepared. The spores were removed with a fine spatula and cleaned with water to remove debris. The round capsule in mosses was cut off to flatten in order to observe the details of the peristome from both the dorsal and ventral sides.

The measurements of the plants, leaves, cells, capsule, spores etc. were taken by an ocular micrometer duly calibrated using a stage micrometer. At least 5-6 measurements of each character per sample were taken. Quantitative characters were observed as minimum and maximum.
4.4 SEM STUDIES

To study the sporoderm pattern and peristome structure of some of the bryophytes Scanning Electron Microscopy was carried out (Phillips XL 20; Birbal Sahni Institute of Paleobotany, Lucknow). Well cleaned spores and washed peristome sections were dehydrated in glacial acetic acid, acetic anhydride and an acetic acid solution (acetic anhydride + HN03 in 1:3 ratio) by rotations (2000-3000 rpm) in the centrifuge (Acetolysis method, Erdtman 1960). In separate glass stub, the spores of each of the different species of mosses, liverworts and hornworts were poured and separately affixed on the aluminium stubs through double sided adhesive tape and glow discharged. A thin layer of gold-palladium was coated in PS-2 coating unit for 15-20 minutes. The mounted samples were then stereo-scanned under SEM (Model Phillips XL 20) at under suitable magnification and at an accelerating voltage of 10-30 KV. Micrographs were taken at different magnifications. Likewise, stubs of peristome were prepared for different species.

4.5. PREPARATION OF CAMERA LUCIDA DRAWINGS

Line drawings for different moss species were prepared exhibiting its habit, leaves, leaf cells, capsule, peristome, spores and transverse sections of axis. Illustrations of plants were made by using Camera Lucida. As the line drawing illustrations of the thalloid liverworts studies were already done in our laboratory and described elsewhere in detail, therefore not taken into account for camera lucida drawings.

4.6. PHOTOPLATES

Photographs of some of the plants were also clicked using a stereoscopic Zoom Binocular Microscope (Leica MZ2, Germany), Nikon Advanced Trinocular Research Microscope (Eclipse 80i, Japan) and Nikon Coolpix 4500- Digital Camera.

4.7. MAPS

The maps were created by using Google Maps website by searching the exact localities. Appropriate locations were marked on the Maps provided by EPCO, Madhya Pradesh.
4.8. BRYOPHYTE DIVERSITY ASSESSMENT

Diversity of bryophytes was assessed along an altitudinal gradient ranging from 400 m to 1350 m. The study area was divided into 3 gradients along the altitude i.e., Lower altitude (below 800 m), Middle altitude (between 800 m to 1000 m) and Higher altitude (above 1000 m). In all 30 sites were selected with around 10 sites at each altitudinal gradients. At each site, six habitats viz. soil, wet rocks, dry rocks, soil covered rocks, stony walls (terricolous habitats) and epiphytic habitat was identified.

**Growth and Morphological forms**

The growth forms of bryophytes are influenced by the immediate environmental conditions and habitat characteristics. At each habitat, the growth forms of the bryophytes were also studied following Gimingham and Smith 1971 and Mägdefrau, 1982). Also the morphological forms for the studied taxa were identified.

**β Diversity**

Beta (β) diversity or between habitat diversity is the measure of the change in species diversity (dissimilarity) between habitats or communities (Mac Arthur 1965, Whittaker 1960 and Magurran 1988).

The β diversity was calculated by the formula:

\[ \beta = \frac{\text{Total no. of species observed in all habitats}}{\text{Mean no. of species in all habitats}} \]

**Similarity Index**

The Sorenson’s Similarity Index (SSI) (Sorenson 1948) was used to compare the similarities between the species growing at different habitats sites and at different altitudes.

\[ S = \frac{2C}{A_n + B_n} \times 100 \]

(Where, C= No. of species common to both sites (A and B); A= Total no. of species. at site A; B= Total no. of species at site B.)
The calculations of ‘S’ were performed at 2 levels-

(i) **At the habitat level:** SSI was calculated for the six habitats taken in the study uniformly at all the 30 sites.

(ii) **At the altitudinal level:** Similarity Index was also calculated across the three altitudinal gradients viz. lower altitude, middle altitude and higher altitude.

**pH**

pH of the soil and bark were also measured by preparing a slurry of soil and distilled water in a ratio of 1: 2.5. The solution was shaken well and kept for 2hrs. The solution was filtered by Whatman’s filter paper No.1. Decant was used to measure pH using a pH meter.
Estelar