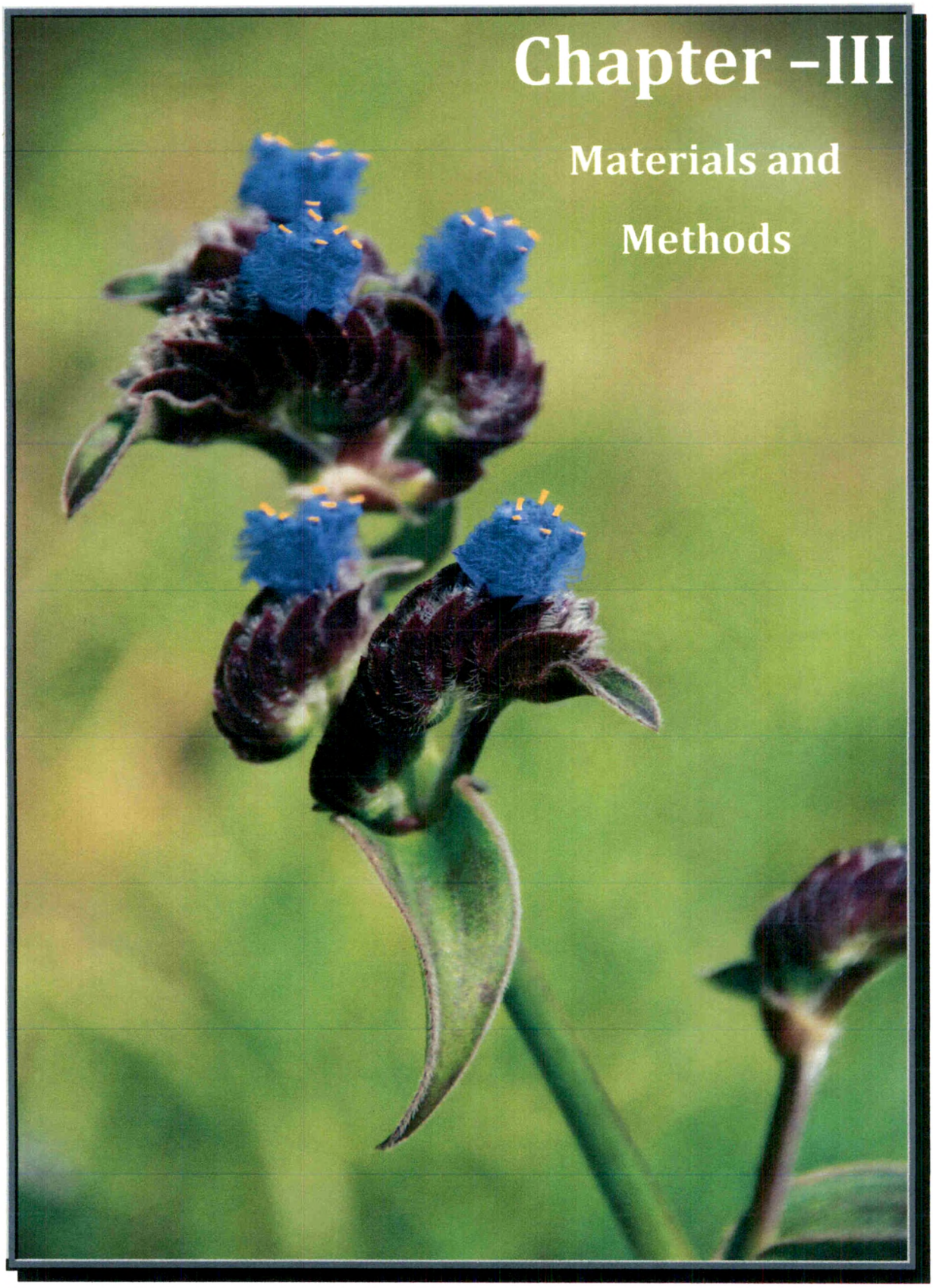
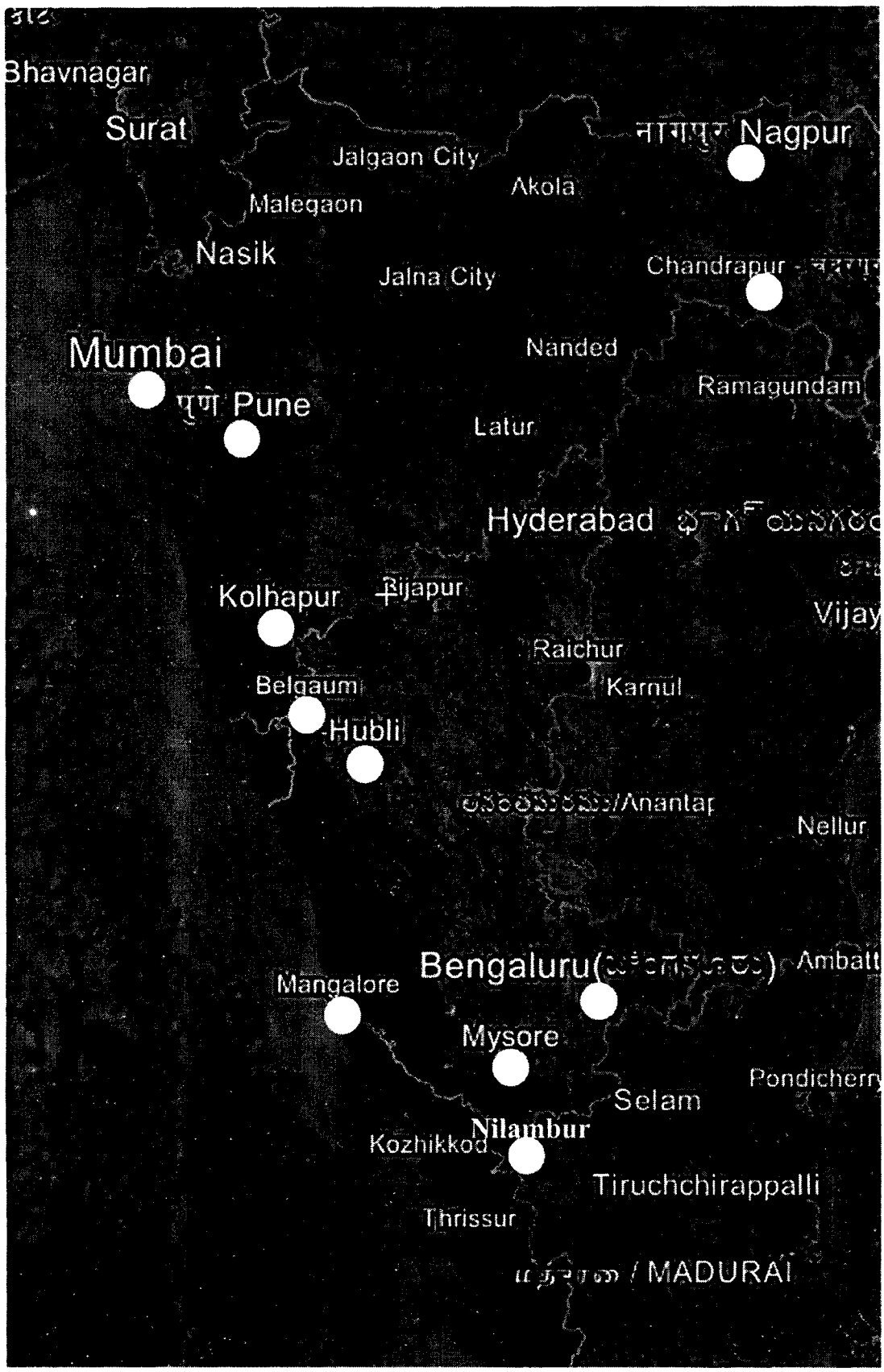


Chapter –III

Materials and Methods



Map I.
Satellite image showing various collection localities in the present study as white circles

A. COLLECTION, IDENTIFICATION, DISTRIBUTION AND PRESENT STATUS OF FAMILY COMMELINACEAE FROM THE STUDY AREA:

During the study period (2008 to 2010), about 40 exploration tours of 1 - 4 days duration were conducted in different seasons to collect the species of family Commelinaceae from various localities in the study area (**Map I**). In total 37 species including one new species were collected from the study area of which about 20 species are maintained in the Botanic Garden of Department of Botany, Shivaji University, Kolhapur as germplasm and used for further studies. The species collected are reported in the descriptions of the species in the Chapter - IV 'Results and Discussions' along with their localities.

All the species under study were screened for their range of morphological variation. To make comparative account of morphological characters and to study range of variation, at least 10 individuals of each species from each locality were examined. The studies were performed with actual field observations. While collecting the specimens, the data such as habit, habitat, frequency distribution, uses, and peculiar characters were recorded in the field book. The photographs of most of the species were taken in the field and/ or after cultivation in the Botanic Garden by using the camera OLYMPUS SP 550 uz. The illustrations of 12 species were made with proper scale. Some of the specimens were processed for drying using regular drying method with blotting or newspapers (Santapau, 1955 and Jain and Rao, 1977). After proper processing the specimens were mounted on herbarium sheets and deposited in Herbarium of Botany Department, Shivaji University, Kolhapur (SUK).

All the specimens and field identifications were confirmed satisfactorily with the help of regional and national floras, available literature, herbaria of various institutes and discussions with the experts. The herbaria viz. Herbarium of Shivaji

University, Kolhapur; Herbarium of BSI, Pune; Herbarium of NBRI, Lucknow and Blatter Herbarium, Mumbai were visited for confirmation of the identifications.

The keys were prepared for easy identification of tribes, genera as well as species under genera studied. The nomenclature adopted was based on the latest taxonomic literature and is in accordance with the recommendations made by ICBN. The correct name is followed by citation of important pertinent literature. This is followed by basionyms and synonyms used in common earlier works. The nomenclature has been followed by short diagnostic description. Flowering and fruiting months are cited for each species. A short ecological note on phenology, distribution, a critical note on identity and variation is given at the end.

The references quoted in the prologue of the species are cited in the bibliography in order to avoid repetitions. *Exsiccata* is provided wherever the herbarium sheets are prepared or the departmental herbarium is consulted. The species grown in Botanic garden are multiplying continuously and hence not given numbers to them.

B. ANATOMICAL STUDIES:

For the anatomical studies the material was selected from the plants grown in Botanical Garden. The studies were performed for various parts (root, stem and leaf) of plants under study by taking hand cut sections and/ or sections taken with the help of microtome. For the cuticular studies the peels of fresh as well as preserved leaves were taken. The sections were stained with aqueous saffranin and light green stains to visualize the important tissues. The sections and the peels were observed under light microscope and the photographs were taken by using ZEISS AXIOSKOP 40 Fluorescence microscope.

C. PALYNOLOGICAL STUDIES:

The palynological studies were performed for 8 species of Commelinaceae. The pollen grains were collected from the living specimens grown in Botanical Garden. The collected pollen grains were acetolyzed for 48 hours using standard acetolysis technique (Erdtman, 1969). All the samples were observed under light microscope and photographed by Scanning Electron Microscope (SEM).

D. CYTOLOGICAL STUDIES:

For the meiotic studies the material (young inflorescences) was collected in different seasons according to the flowering period of the respective species. The scales were removed carefully and young inflorescences were fixed in Cornoy's fluid (45 ml absolute ethyl alcohol + 15 ml glacial acetic acid). Inflorescences were washed well in water, treated in 1N HCl and smeared in 2% propionic orcein. The appropriate plates were photographed using LEICA DM 2000 Fluorescence microscope along with camera.

The somatic chromosome number of 3 species was determined from growing root tips. The roots of one inch in length, were taken out and washed thoroughly before taking them for pretreatment. To obtain proper separation of metaphase chromosomes, aqueous saturated solution of 1, 4- (para) Dichlorobenzene (pDB) was employed. The root tips were pretreated with PDB for 4 – 8 hours at 8 – 10^0C temperature in refrigerator. These root tips were taken out from the refrigerator and kept at room temperature to monitor proper separation of chromosomes. The tissue was treated in 1N HCl and stained with 2 % aceto- or propionic orcein. The root tips were squashed under the cover glass in 45 % acetic acid. The squashes were observed under light microscope and desirable slides showing well separation of chromosomes were used for photography. The photographs were taken using LEICA DM 2000 Fluorescence microscope along with camera.

For karyotypic analysis the method of Levan *et al* (1964) was adopted. The symmetry of karyotype was analyzed by using Stebbin's (1961) system of classification. The values of F and TF were calculated by using formula given by Huziwara (1962).

E. SEED MORPHOLOGY:

The seed surface morphology of 22 species of Commelinaceae was carried out by using micro photographic technique and Scanning Electron Microscope (SEM). The seeds were collected from various localities and shade dried and used for the study purpose. The seed surface characters are discussed using available literature.

F. CLADISTIC ANALYSIS OF GENUS MURDANNIA:

For the cladistic analysis of the species of genus *Murdannia* under study, 24 characters were selected. The procedures and assumptions were according to the morphological data collected for those 24 characters. The data were collected from the field observations of the specimens as well herbarium specimens. For the present study the steps were followed which are typical for the analysis of morphological data (Stuessy, 1990 and Wiley *et al.*, 1991). Cladogram was prepared by using 'PAST' software. Genus *Gibbasis* was taken as an out group (Evans *et al.* (2000) for comparison purpose.