CHAPTER 2
MATERIALS AND METHODS

The materials for present study are collected through extensive field trips in different parts of India during the period 2008–2013. About 200 days are spent in the field with 52 field trips. 25 species of *Murdannia* are collected. Specimens collected, except North East endemics are maintained in the Botanical Garden of University of Calicut, Thenjhipalam for future references. Besides these personal collection by the author, materials deposited at various herbaria, ASSAM, BLAT, BSD, BSI, BSJO, CAL, CALI, DD, DEV, JCB, MH, PBL, RRLB, RHT, TBGT, MEL and herbarium of North Bengal University, West Bengal are also examined to study the variation, ecology and distribution of species (list of herbaria consulted with their details are provided at the end as Appendix II). Acronyms of herbaria are in accordance to the Index Herbariorum (Thiers, 2011). Four hundred and fifty five populations are observed in the field.

Specimens are critically studied both in the field and in the laboratory. Floral characters are recorded in the field itself owing to their deliquescent nature. Data sheets are prepared for each population studied. The data sheet covers information on habitat, habit, nature of root system, type of pubescence, nature of inflorescence, colour of petals, structure, position and orientation of stamens and staminodes and other features that are not faithfully represented in herbarium specimens. A model of data sheet used is provided at the end as Appendix III. Ecological notes are made for every population studied.

Identification of each species is done in consultation with types and protologues. In the case of non availability of type specimens, cibachromes,
microfisches and images obtained from K, US, BM, BR, C, G, L, and P are consulted. Detailed morphological studies are made using all the available materials and variations are recorded. Descriptions of each taxon are prepared after proper determination and examination of a wide range of specimens. Observations are done under Leica MZ 75 Stereo Microscope and illustrations are made. The most typical form is illustrated in all cases. Dried specimens soaked in water for 1–2 hours and boiled in water prior to microscopic observations. Photographs are taken using Leica EZ 4HD Stereo Microscope.

Literature and relevant information related to the taxa under investigation are collected from various sources such as institutional libraries and online information retrieval systems. The literature retrieval systems of BHL of New York Botanical garden (http://www.biodiversityheritagelibrary.org), botanical literature from Missouri Botanical garden library (http://botanicus.org) are also utilized. The citations of taxa follow IPNI. The database of the International Plant Name Index (http://www.ipni.org) and world checklist of Selected Plant Families, a database of Royal botanic Garden Kew (http://apps.kew.org) are also utilized. The nomenclature of each species is updated as per the Melbourne Code (McNeill et al., 2011). Author citations follow Brummitt and Powell (1992). For description of taxa, the terminology follows Lawrence (1951) and Radford (1968). The terminology for testa ornamentation, inflorescence and floral morphology follows Faden (1991).

The distribution maps are prepared based on specimens examined during the present investigation. Metric system has been followed in all kinds of measurements. Data on phenology is mostly from field studies and rarely from the literature and herbarium sheets.
The foliar anatomy and their taxonomic utility in the systematics of the genus are also attempted in the present study. This study is conducted on 24 species. All the samples are procured afresh from cultivated materials at botanical garden. The detailed methodology used in anatomical studies is discussed in the chapter 5.

Scanning Electron Micrographs are taken to study the surface ornamentation of seeds. Dried seeds are directly fixed to aluminium stubs, layered with 10-15 nm of gold by sputtering Blazers and samples are examined using Hitachi Su 600 and digital electro photo micrographs obtained. SEM of seeds is taken at NIIT, Kozhikode.

Morphometric analysis of 26 Indian species is performed with NTSYS software. A total of 61 characters are selected for the cluster analysis from each species. All data inferred during the current study either from the field or herbaria as well as the experimental garden, are used for the present analysis.