MATERIAL AND METHODS

As many as 504 collections of Gasteromycetes were made by the author from the various localities of the Eastern Himalayas and adjoining hills during the last five years* (1977-81). Several fungal explorations were undertaken during the monsoon seasons (June to October) when the rainfall is maximum and the conditions are most favourable for the growth of fungi. The first foray was undertaken in the month of October 1977 to Darjeeling hills (West Bengal); the second from June to September 1978 to Khasi and Jaintia hills (Meghalaya), Haflong, Silchar and Garampani (Assam), Imphal and Ukhrul (Manipur) and Agartala and Ambassa (Tripura); the third from August to October 1979 to Darjeeling hills, Siliguri (West Bengal) and Khasi, Jaintia and Garo hills (Meghalaya); the fourth again to Darjeeling hills and Siliguri (West Bengal), and many localities around Thimphu in Bhutan. The fifth and final foray was undertaken again to Bhutan and to West Kameng District (Arunachal Pradesh). It may be mentioned here that localities from where collections yield was rich were visited more than once in order to have best possible representation of these fungi from study area.

The map covering Eastern Himalayas and adjoining hills

*This work is part of the five years research project to study the specified fungal flora of Eastern Himalayas. This project was financed by the Department of Science and Technology, Government of India.
used for illustrating the distribution of species of various genera has been redrawn from the map printed by "Printing Group of Survey of India". The map shows only the places which have been actually visited or otherwise important geographically. The map depicted here is not strictly authentic from the point of view of boundaries and accurate distances.

Collecting gasteromycetous fungi:

Majority of these fungi are terrestrial in their habitat and are not difficult to collect in the field. The peculiar shape, large size and colour of most of the species of Gasteromycetes at once attract the eye of the collector. The terrestrial species can easily be taken out from the soil with the help of sharp knife, dagger etc. However, lignicolous members can be removed with the help of a sharp chisel and small hammer, which are important tools of their collection in the field. The collector of the hypogaeous members of this group of fungi, has to put sufficient efforts as these are mostly found up to one foot deep in the soil, below the surface. To locate these fungi several suitable spots were dug out in the forests with the help of a dagger and the success was all by chance.

The preliminary examination of small specimens in the field with the help of a hand lens gave very valuable information regarding many features. The fresh and fleshy specimens were put in hard cardboard boxes while the dry and hard were
put in thick brown paper envelopes especially made for collection purposes. The data pertaining to their habit, habitat, colour*, any exudate, locality, date of collection, type of forest was noted down at the spot in a field note-book especially kept for this purpose. The collections brought from the field were immediately and carefully screened to remove extraneous matter and then the preliminary study of fresh specimens was carried out. Morphological features were noted with the help of a stereoscopic binocular microscope at a temporary laboratory set up at the camp station. The bigger and hypogaeous specimens collected from the field were cut into samples of small size and slices.

**Drying and preservation of the specimens:**

After completing the preliminary studies, most of the specimens were dried, but several specimens especially the members of Phallales and hypogaeous genera were preserved in alcohol-formalin solution prepared as follows:

- Formaldehyde solution: 5 ml
- Alcohol: 25 ml
- Distilled water: 70 ml

On bright sunny day, all the specimens were initially dried by spreading over a dry blotting sheet. After that, the specimens were dried by placing them in a hot-air drier which is

* Methuen's Handbook of colours by A.Kornerup & J.H.Wanscher, 2nd ed. was largely consulted to note the accurate shades of various colours.
an improvised, foldable and portable wooden structure measuring 102 x 48 x 48 cm when fully assembled. It has three wire-gauze partitions, dividing it into two chambers and a small outlet at the top. A kerosene oil wick-type stove is placed at the bottom of the drier and the specimens contained in thick brown paper were placed for drying on the wire-gauze trays. The hot air current produced by the burning of the stove dries the specimens lying above on the wire-gauze trays, and escapes through the outlet at the top. This apparatus works very well in humid conditions and during rainy weather. The temperature within the drier remains approximately at 60°C. The dried specimens were properly put in small transparent envelopes of cellophane paper which were then clipped. The cellophane envelopes containing dried specimens were then, in turn, wrapped in 21 lbs bond paper packets measuring 15 x 12 cm. However, bigger dried specimens were first wrapped in kraft paper and were then placed in cardboard boxes. A herbarium label was pasted on each packet or box bearing the necessary collection data. The envelope-type packets were then arranged in bigger (39.5 x 17 x 13 cm) cardboard boxes in a vertical fashion-like index cards, and a few naphthalene balls were put in each box in order to save the material from the attack of worms, insects etc. These bigger cardboard boxes were, in turn, placed permanently in steel cupboards.

The collections included in the present work, unless otherwise indicated, have been exclusively made by the author.
The abbreviations of the herbaria used in this work are according to Lanjouw and Stafleu (1964). All the collections have been deposited in the herbarium of Botany Department, Panjab University, Chandigarh, India (PAN)*. A sizeable part of some of the collections have also been deposited at the herbarium, Department of Botany, University of Liege, Sart-Tilman, Liege, Belgium (LG); Herbarium, Department of Botany, Escuela Nacional de Ciencias Biologicas, Instituto Politecnico Nacional, Mexico, D.F. (ENCB); herbarium of the Oregon State University, Oregon, U.S.A. (OSC); and herbarium of Professor H.J. Brodie, Dept. of Biology, University of Victoria, P.O. Box 1700, Victoria, British Columbia, Canada (DBUV).

Efforts were also made to procure the specimens of the earlier recorded species in the study area, from the different herbaria. Some specimens from abroad, i.e. Herbarium, Department of Botany, University Liege, Sart-Tilman, Liege, Belgium (LG); herbarium of Royal Botanic Gardens, Kew, University of Michigan (England), were also examined for additional as well as for comparative study.

**Microscopical studies:**

The microscopic studies of spores and capillitium were carried out by taking a little bit of dry or wet material from the fructification and then placing in a drop of 3% KOH on a microslide. It was then gently teased under stereobinocular microscope and spread over the microslide with the help of fine

*The abbreviations indicating different herbaria where part(s) of various collections have been deposited are shown within parentheses just after the collection number in the text.*
needle and forceps. Colour and other features of capillitial threads and spores in KOH were noted. The capillitial threads were also studied to note the septation by putting 2% aqueous phloxine on one side of the coverslip and removing excess of it from other side with the help of a piece of blotting paper. Spores and capillitium were observed by slightly warming them in lactophenol which was prepared after Ainsworth (1971) as follows:

Phenol (pure crystals) 20.0 g
Lactic acid (S.G. 1.21) 20.0 g
Glycerine 40.0 g
Water (distilled) 20.0 g

This reagent (lactophenol) was used to study the colour of capillitial threads, nature of pores on their walls, and ornamentation and colour of spores etc. Lactophenol can also be used as a stain by adding little dye such as cotton blue in which the ornamentation of the spores becomes distinctly visible.

Melzer's reagent was used to note the amylloid or dextrinoid character of spores which was prepared after Singer (1962) as follows:

Iodine 0.5 g
Potassium iodide 1.5 g
Chloral hydrate 22.0 g
Water 20.0 ml

Minute ornamentation on the spore wall was best observed with the help of aqueous cresyl blue.
To note the guttulate nature of the spores, Sudan III, which was prepared by dissolving small quantity of the dye in 100 ml of 95% alcohol, and then strongly stirring the solution and filtering it after 24 hours, was used.

Ammoniated congo-red (prepared by dissolving a little dye in 100 ml of 1% ammonium hydroxide) was used to study the nature of the pores and other structures on the walls of capillitial threads.

Cotton blue in lactic acid was used to study the 'cyanophilous' or 'acyanophilous' nature of the spores or hyphae. This stain was prepared after Le Gal (1947), as follows:

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Lactic acid 50% 30.0 g
Cotton blue 0.05 g
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The terms 'cyanophily' and 'acyanophily' were first used by Kotlaba and Pouzer (1964), whereas the stain (cotton blue in lactic acid) was chiefly used by Le Gal (1947) in the taxonomy of the Discomycetes. This stain was subsequently used by the workers such as Nannfeldt and Eriksson (1953), Eriksson (1954) and Kotlaba and Pouzer (1963) in the taxonomy of the Hymenomycetes. However, in this work I have applied this stain and terms (i.e. acyanophily or cyanophily) in the global structures (spores, paracapillitium and global membranes) of genera Vascularium F. Smarda, Morganella Zeller, and Lycoperdopsis P. Henn. (Lycoperdales).

To study the anatomical details of peridium and peridiole, thin, free hand, transverse sections were cut from dry as well as preserved material. For this purpose, the dry material was
revived by soaking first in water and then placing it in 3% KOH for 24 hours or longer, if required. Sections were then cut with the help of a fine blade or razor. Good and thin sections were selected under stereobinocular microscope. These were washed in water to remove excess of KOH and then were transferred to lactophenol or 50% lactic acid, and were then warmed, for 5-10 seconds over a spirit lamp flame. The sections were covered by a coverslip and were then observed so as to note the colour and nature of various structures, such as hyphae, basidia, cystidia, setae and basidiospores etc. It may be mentioned here that sections were best studied by boiling them in 50% pure chloral hydrate dissolved in half of its weight in water (suggested by Dr. Demoulin, private communication). The different structures present in sections were very clearly visible after this treatment. Sections were stained in cotton blue (in lactophenol) and were mounted in lactophenol.

Permanent mounts of the sections and spores and capillitium were prepared by transferring them to a slightly acidic 50% glycerine or lactophenol and then allowing these mounts to dry as such for 2-3 days and then adding more 50% glycerine or lactophenol, if required, finally ringing these with Canada balsam or nail-polish.

The measurements of spores and capillitium were usually taken in 3% KOH, sometimes in lactophenol, with or without staining. The spores were usually measured including spines,
but in case of strongly echinulated or warted spores, measurements with or without spines or warts were taken. The anatomical structures were measured in cotton blue or in lactophenol.

The descriptions of the species included in this work are based on my own collections made from the Eastern Himalayas and adjoining hills.

All the diagrams are original and are based on a representative collection of a species. The collection numbers have been mentioned from which a particular diagram or structure (spores, capillitium, setae, etc.) of a species has been drawn. The diagrams were prepared with the help of a "Erma" make camera-lucida. However, it may be mentioned here that in case of anatomical drawings, it was not found possible to draw them from a single section, because all parts in same section were sometimes not fully clear, thus the sketch was drawn with the help of a good representative section, but the details of the various structures such as hyphae, cells, cystidia, basidia, etc. were drawn with the help of camera lucida from various sections and the diagrams were then constructed accordingly. In this way the figures are slightly diagramatic, but are correct to the scale. Much care has been taken to make them as accurate as possible.

The spores and capillitium were drawn at x2000 magnification. The anatomical sections of the anatomy of peridium and peridiol were drawn at magnification x 800. A scale is inserted with each figure to give information of its measurements.