MYXOMYCETES OF SHIVAPURI AND GODAWARI (KATHMANDU VALLEY), CENTRAL NEPAL

A Dissertation Submitted for the Partial Fulfillment of Masters of Science in Botany, Institute of Science and Technology, Tribhuvan University, Kathmandu, Nepal

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CERTIFICATE

This is to certify that the dissertation work entitled **"Myxomycetes of Shivapuri and Godawari (Kathmandu Valley), Central Nepal"** submitted by Ananta Bahadur Chuhan has been carried out under my supervision. The entire work was based on the results of his primary fieldwork and has not been submitted for any other academic degrees. I, therefore, recommend this dissertation to be accepted for the partial fulfillment of Masters of Science in Botany from Tribhuvan University, Kathmandu, Nepal.

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LETTER OF APPROVAL

This dissertation paper entitled **"Myxomycetes of Shivapuri and Godawari (Kathmandu Valley), Central Nepal"** submitted at the Central Department of Botany, Tribhuvan University by Ananta Bahadur Chuhan has been accepted for the partial fulfillment of requirements for Masters of Science in Botany.

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ABSTRACT

Myxomycetes are the group of fungi, which are distinct from the rest fungi because of their plasmodial characteristics. Plasmodium is a vegetative phase of Myxomycetes, which consist of many nuclei. Most of the slime moulds are saprophyte growing in damp, shady places, in wood on rotten logs, stumps or in dung in pasture. The study is focused on the exploration of Myxomycetes from the Shivapuri and Godawari hills, the protected forests of Central Nepal. Though Ceratiomyxale has been excluded from the Myxomycetes, this has been included in the thesis at present.

The collection of slime molds was done from first week of September to last week of October in 2007 and 2008 just after the seven days of heavy rainfall which is suitable time for the formation of fruiting bodies of many species. The collected specimens were placed in a small box by a drop of white glue to protect fragile specimens. The specimens were examined carefully by naked eyes for observing the details of morphological characters. The microscopic characteristics of fungi were studied under stereo-microscope. Ornamentation of capillitium and spores were observed through microscope. Though, more than 35 specimens were collected in 2007 and 2008 only 12 species of 9 different genera and 5 orders were identified heaving call. no.1,4,5,7,10,11,15,16,20,27,32,35. Identified species were *Arcyria pomiformis, Arcyria incarnata, Arcyria cineria, Stemoitis* sp., *Didymium flexuosum, Physarum viride*., *Hemitrichia serpula*., *Tubifera microsperma, Ceratiomyxa fruticulosa*., *Fuligo cinerea*., *Mucilago crustacea* and *Didymium iridis.*

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LIST OF ABBREVIATION AND ACRONYMS

°C	degree celcius
µm	micrometer
CDB	Central Department of Botany
cm	Centimeter
DPR	Department of Plant Resources
E	East
Gov.	Government
ICIMOD	International Center for Integrated Mountain Development
Max.	Maximum
Min.	Minimum
mm	millimeter
msl	above mean sea level.
N	North
NARC	National Agriculture Research Center
No.	Number
sp.	species
Sq. Km	Squarekilometer
Temp.	Temperature
TU	Tribhuvan University

CHAPTER ONE

INTRODUCTION

1.1 Background

Slime moulds or slime fungi are a unique group of heterotrophic, eukaryotic organisms, possessing both plant and animal like characteristics. During the early nineteenth century, the slime moulds became universally recognized as a group distinct from the rest of the fungi because of their Plasmodium rather than mycelial assimilative stage, so they are called Myxomycetes (slime fungi). Plasmodium is a vegetative phase of Myxomycetes, which consists of a slimy, naked mass of protoplasm with many nuclei. They are mostly saprophytic and live on the surface of the decaying plants and non-living organic matter. Some species are parasitic on algae, true fungi and higher plants.

1.2 History

De Bary (1884), was first to concentrate on the study of this group. He carried out intensive investigations of slime moulds that led his concept to be the animals like, and named them Mycetozoa (Fungus animals). Later Bessey (1950) and others placed the slim moulds in the Phylum Protozoa of animal kingdom. According to Carlos *et al.* (1998) the American mycologist, Macbride (1899) was the first, who used the term Myxomycetes (Gr. *myxa-slimet, myketes* = mushroom, fungi).

1.3 Occurrence

They are widely distributed throughout the world. Most of the slime moulds are saprophytes growing in damp, shady places, in wood on rotten logs, stumps or leaves and other plant debris, bark of trees, in piles of straw and on dung in pastures. Some species are parasitic on algae, true fungi and higher plants. In mountainous areas where snow packs remain into spring, a number of slime moulds may fruit in great proliferation along the edge of the melting snow banks.

The distribution patterns of many slime molds are diffuse and unpredictable. In this respect these organisms differ from many others that display definite geographic ranges or are bound to specific hosts or habitats. Relatively some slime molds appear limited to sub-tropical and

tropical regions, and another group of species is apparently restricted to monotone habitats and melting snow banks. Outside of these ecological niches, however, most myxomycetes manifest a widely scattered not following any set geographic or climatic patterns. They are frequently found in rainy season.

1.4 Vegetative structure

The vegetative body of slime mould consists of a thin slimy mass of naked (without cell walls) called plasmodium, which creeps about in amoeboid motion over the surface of the substratum. The plasmodium has no definite form and is constantly changing shape. Its size ranges in diameter from a few millimeters to several centimeters. Plasmodia are usually colorless, but in some species plasmodium may be yellow, orange, red, brown or violet. Color is affected by pH, temperature or ingested materials.

Protoplasm of plasmodium is highly granular and is differentiated into two portions of different viscosity. The outer portion is devoid of granules called hyaloplasm. The inner portion is granular and contains numerous nuclei, vacuoles, contractile vacuoles.

According to Alexopoulos (1962 and 1993), there are three types of plasmodia: Protoplasmodium, Aphanoplasmodium and Phaneroplasmodium.

i. Protoplasmodium (Greek protos = first):- It is the most primitive type, microscopic, lack of veins, exhibits slow irregular protoplasmic streaming and produces a single, very small fruiting body. Protoplasmodia are found in the Echinosteliales and Liceales.
ii. Aphanoplasmodium (Greek, aphanes = invisible is rarely observed in nature):- Veins are very thin and a slime sheath is lacking. Aphanoplasmodia are characteristic of Stemonitales.
iii. Phaneroplasmodium (Greek Phaneros = visible):- This type appears as a fan-shaped network of veins containing streaming protoplasm. This type is common in the order physarales.

But in order Trichiales, the plasmodium exhibits characteristics of both Phaneroplasmodia and Aphanoplasmodia.

1.5 Reproductive structure (fruiting body)

1.5.1 Types of fruiting bodies

There are four types of fruiting bodies in Myxomycete. They are sporangia, aethalia, pseudoaethalia and plasmodiocarps. Some fruiting bodies are intermediate between two types and often two or more types may occur within one and the same colony.

Sporangium (in plural sporangia):- The most common type is the sporangium. The sporangium is actually a small spore container which may be sessile or stalked, with wide variations in color and shape. Sporangia usually occur in groups, since they form from separate portions of the same plasmodium. Depending upon the species, a sporangium may be globose (spherical), lenticular, ovoid, cylindrical. Variously lobed, compressed or contorted, or pulvinate; it may be sessile or stalked and colors vary from blackish to brightly pigmented or white, from dull to glossy or brilliantly iridescent.

a. Aethalium (in plural aethalia):- In some species the plasmodium contracts into a more or less mound-shaped (pulvinate) mass of strands, is called aethalium. In aethalium the inner strands become converted into the fertile sporulating region, the protoplasm of outer dies. The outer strands collapse and form a sterile layer called cortex where contains spores. The cortex may be membranous and delicate tough, crusty or spongy. They are a cushion-shaped sessile structure and are presumed to be masses of completely fused sporangia which are relatively large, sometimes exceeding several centimeters in size. This type of fruiting body is produced by *Enteridium* and *Lycogala* in the *Liceales, Fuligo* and *Mucilogo* in the physarales, and *Amaurochaete* and *Brefeldia* in the Stemonitales.

b. Pseudoaethalium:- A dense aggregation of sporangia into a body that superficially resembles an aethalium called pseudoaethalium. This fruiting body is composed of sporangia closely crowded together. They are usually sessile, although a few may be stalked.

c. Plasmodiocarp:- The protoplasm of plasmodium gets concentrated around some of the veins and then secretes a membrane forming fructification, called plasmodiocarp. It is sessile, branched and asymmetrical plasmodium like fructification e.g. *Hemitrichia.*

1.5.2 Parts of fructification

The structural components of a fruiting body include the following:-

(a) Peridium:-

This is the wall enclosing the spore mass and associated structures. It is a delicate non-cellular layer often calcified. It may or may not be evident in a mature fruiting body. In same species the peridium persists as a calyculus, a cup-like structure holding the bottom of the spores mass. In an aethalium, the relatively thick covering over the spore mass is referred to as a cortex rather than a peridium.

(b) Capillitium and Pseudocapillitium

Capillitium consists of thread like elements within the spore mass of a fruiting body. Many species of myxomycetes have a capillitium, either as a single connected network or as many free elements called elaters. Capillitial elements may be smooth, sculptured or spiny or they may appear to consist of several interwoven strands. The capillitia elements are separate from the spores within the spore mass and are not connected to them. Some elements may be elastic allowing for expansion when peridium opens, while other types are hygroscopic and capable of dispersing spores by twisting motion. Some members of physarales have limy capillitium and others have limeless tubules connecting to lime nodes.

Pseudocapillitium originates from collapsed strands of plasmodium or from parts of peridium which resembles capillitium (as in *Dictydiaethalium*). It is associated with aethalia and sometimes pseudoaethalia pseudocapillitial elements are highly variable in size and shape and may appear as bristles, threads or perforated plates.

(c) Spores

They range in size from almost 5 to 15 micrometers. Nearly all of them appear to be round and most are ornamented to some degree. In fact, entirely smooth spores may not exist. Spores ornamentation can be reticulate (covered by a network of ridges), echinate (spiny) verrucose (warted) or asperulate (finly warted). Spore shape and size are very important in identification. Spore color can be classified as either dark (found in the Stemonitales and Physarales) or light to brightly colored (all of others orders). Dark spores include the colors

black, violet, brown and purplish brown. Brightly colored spores may be red, yellow, orange, white, pale gray, pink light or rusty brown. In some species of *Badhamia, Dianema Corticatum,* the spores appear clustured into "spore balls". They are very resistant to unfavorable conditions, particularly to prolonged period of desiccation. Spores are usually held together by a network of tubular threads called capillitium.

(d) Columella and pseudocolumella

The columella appears as an extension of the stalk into the spore mass, although it may not resole the stalk. In a sessile fruiting body, the columella may be an area inside the peridium where it contacts the substrate or appears as a dome-shaped structure A pseudocolumella (pseudo-false) is a columella that does not attach to the stalk. The pseudocolumella is found only in the order Physarales, existing as a lime mass within the spore mass. Capillitial elements may be attached to the columella or pseudocolumella.

(e) Stipe (Stalk)

The stalk or stipe is an important identification characteristic. The stalk may vary in length and color. In some species the stalk is opaque, while in others it is translucent. They may be slender and hair like, hollow or filled with large round cells, lime or debris, granular or spore like structures.

(f) Hypothallus

The hypothallus is a plasmodial remnant forming the base for one or more fruiting bodies. The hypothallus connects the stalk or stipe to the substrate. It may be dull or brightly colored, thin, delicate to tough or coarse membranes and spongy or limy. It is not always in evidence. In some instances, the hypothallus may be composed of calcium carbonate. In the case of the transparent type, the hypothallus may be proteinaceous in composition.

1.6 Reproduction

It takes place by following three methods:- (i) Vegetative reproduction (ii) Asexual reproduction (iii) Sexual reproduction

(i) Vegetative reproduction:- Under unfavorable conditions, the plasmodium is converted into an irregular hard mass, the sclerotium. When conditions are favorable, it germinates again to reform a plasmodium.

(ii) Asexual reproduction:- Under certain conditions for example, a reduced food supply, the plasmodium migrates to relatively dry place where it becomes heaped up and produces one or more reproductive structures or fructifications such as Sporangium, aethalium and plasmodiocarp. When fructifications mature, it will break and spores are scattered. Under favorable conditions spores germinate and form a plasmodium.

(iii) Sexual reproduction:- It takes place by the fusion of gametes. The haploid myxamoebae or swarm cells act as gametes. The fusion occurs in between two myxamobae or two swarm cells or myxamoeba and swarm cell which forms zygote. The deploid nuclei divide repeatedly and the mass of multinucleate protoplasm grows directly into the diploid plasmodium. Sometimes, a number of zygotes fuse to form a new plasmodium.

1.7 Life cycle

The vegetative body of the slime mould is a plasmodium, an amoeboid mass of protoplasm which has many nuclei and no definite cell wall. Under certain conditions (dry), the creeping phase, the common bark-inhabiting slime molds dries into hardened structures producing dark masses of spore-like bodies and clouds of dust like particles when body breaks apart. The spores, capable of surviving unfavorable weather, are spread by wind, water, or other equipment. Under cool, humid conditions, the spores absorb water, crack open and release a single motile or amoeboid cells. Two myxamoebae or swarm cells or myxamoeba and swarm cells fuse and form diploid zygote. Under unfavorable conditions, the swarm cells or myxamoebae may temporarily change into inactive round cysts.

The zygote feeds and grows, its nuclei divide, until a relatively large, soft, multinucleate, creeping mass of protoplasm, the plasmodium is formed.

1.8 Classification of Myxomycetes

Martin (1961) divided the class myxomycetes into two sub-classes as follows:-

Sub-class 1. Ceratiomyxomycetidae

- Spores born externally on individual stalk

Order - Ceratiomyxales

- fruiting bodies in the form of erect or branched, usually white
- fruiting bodies are covered with spores
- peridium absent

Sub-class 2. Myxogastromycetidae

- Spore born internally in fructification

Order – Liceales

- capillitium and columella absent
- psuedocapillitium present or absent

Order – Echinosteliales

- fruiting bodies delicate, minute, white to light brown
- capillitium or columella or both present

Order – Triehiales

- trum capillitium present (rarely extremely reduced)

Order – Stemonitales

- lime absent

Order – Physariales

- lime present in one or more part of fruiting bodies

Alexopolous (1962) classified Myxomycetes in Myxomycotina flowing Martin(1961) system which included 6 orders

Class-Myxomycotina

Order-Ceratiomyxales

Order-Echinosteliales

Order-Liceales

Order-Physarales

Order-Stemoniales

Order-Trichiales

Farr (1981) in "*How to know the true slime moulds*"propoposed three sub-classes:

Sub-class-Cratiomyxomycetidae

Order-Ceratiomyxales

Sub-class-Myxogastromycetidae

Order-Liceales

Order-Echinosteliales

Order-Trichiales

Order-Physarales

Sub-class-Stemonitomycetidae

Order-Stemonitales

In recent system Myxomycetes includes 5 orders

Order-Echinosteliales

Family-Clastodermataceae

Family-Echinosteliaceae

Order-Liceales

Family-Cribariaceae

Family-Liceaceae

Family-Lycogalaceae

Family-Reticularaceae

Order-Physarales

Family-Didymiaceae

Family-Physaraceae

Order-Stemonitales

Family-Stemonitidaceae

Order-Trichales

Family-Arcyriaceae

Family-Dianemataceae

Family-Trichiaceae

The order Ceratiomyxale (ceratiomyxaceae) has been excluded from the Myxomycetes and placed under Protosteliales in Protosteliomycetes.

(Source: Krik, Cannon, David & Stalpers, 2001. Dictionary of Fungi)

1.9 Problem statement

Slime moulds or slime fungi are a unique group of heterotrophic, eukaryotic organisms, possessing both plant and animal like characteristics. During the early nineteenth century, the slime moulds became universally recognized as a group distinct from the rest of the fungi because of their plasmodial rather than mycelial assimilative stage, so they are called Myxomycetes (slime fungi) plasmodium is a vegetative phase of Myxomycetes which consists of a slimy, naked mass of protoplasm with many nuclei, is animal like. They are

mostly saprophytic and live on the surface of the decaying plants and on non-living organic matter in damp earth. Some species are parasitic on algae, true fungi and higher plants.

Because of the diverse land structure Nepal is rich in ecosystem, genetic and species diversity. As it is good decomposer of the ecosystem, there is possibility of diversity of lower fungi (Myxomycetes). Nepal has diversity and endemism in higher fungi; it might be there in Myxomycites too.

Floral exploration of Nepal along with the higher fungi have been conducted by the different researchers but very few in the lower fungi (Myxomycetes). Though, there is total of 34 genera and 146 species have been reported by the researcher (Adhikari & Manandhar 1996) the exploration is not sufficient and need to explore the different areas in different seasons to find out the new species of Myxomycetes.

1.10 Objectives

The study is mainly focused on the mycofloral exploration in the hills around the Kathmandu valley. The specific objectives are as follows.

- Collection and study of Myxomycetes from different places of Kathmandu valley.
- Search for some interesting, new or hitherto unrecorded species around Kathmandu valley.
- Study of internal structure of Myxomycetes and identification.
- Study of habitats and physical appearance of Myxomycetes.
- There are damp places and many decayed logs, wood, leaves in Shivapuri, which is suitable for the growth and development of Myxomycetes.

1.11 Justification of the study

The research work had been done in many Mycoflora and others higher plants but the work done regarding Myxomycetes is less in Nepal. Because of the diverse land structure and climate Nepal is rich in diversity. There is possibility of diversity of slime moulds (Myxomycetes) too. It also plays the important role in the ecosystem as like others mycoflora. It is universally recognized as a group distinct from the rest of fungi because of

their plasmodial rather than mycelial assimilative stage. By the exploration of new species of slime molds the different ecosystem structure and function could be determined. There is endemism in higher fungi, so, it might be there in Myxomycetes too.

The saprophytic slime moulds live on the surface of decaying plants and on non living organic matter in damp earth. Godavari and Sivapuri are the protected areas where are many more decayed logs, leaves, humus and damp places that is suitable for the slime moulds. There is variation in land structure in altitude; therefore there might be diversity in slime moulds.

1.12 Limitation of the study

- There is difficult to preserve collected specimens due to delicate body structure for a long time to study.
- There was lack of electronic microscope for the detail study of internal structures.
- There was insufficient literature.
- It was difficult to prepare permanent slide of internal structures
- Godawari and Sivpuri were suitable place for the study due to aboundent numbers of logs, decayed, leaves, humid and damp soil etc.

CHAPTER TWO

STUDY AREA

2. Kathmandu Valley

Kathmandu valley lies in the central region of our country between the Mahabharat and Himalays. It is a saucer shaped valley which ranges from 1336m to above from sea level. It includes several green mountains around it, like Phulchoki, Shivapuri, Godawari, Dakshinkali and Nagarjun etc. But study area has been selected to Godawari, and Shivapuri.

2.1 Godawari

2.1.1 Physiography and Climate

It is situated in the south-east corner of Kathmandu valley and 10km far from the town. It lies at $27^0$33' to $27^0$86' N latitudes and $85^0$22' to $85^0$26' E longitudes. Altitudinal variation of the hill ranges from 1515m to 2831m, where typical monsoon climate with rainy summer and dry winter is found in this region. The average monthly temperature varies from 16.1^0C in winter to 27.1^0C in summer and average monthly minimum temperature varies from 2.8^0C to 18.9^0C in winter and summer respectively. Over 52.14% of the total rainfall is encountered during monsoon June to September. Out of 1933.4 mm annual rainfall, there is 1497.5 mm rainfall during monsoon.

The vegetation of Godawari comprises of upper cool subtropical and lower warm temperate zone. *Schima-Castanopsis* (*Schima wallichii, Castanopsis indica*) dominate the lower periphery of the hill. *Quercus-Laurel* is in the middle elevation of the hill and *Quercus-Rhododendron* (*Quercus* and *Rhododendron* sp) type is on the upper limit of the hill (Phulchoki). Some major dominant species are *Quercus* sp. *Cinnamonum* spp, *Lithocarpus spicata* and *Mahonia nepaulensis* are also dominant.

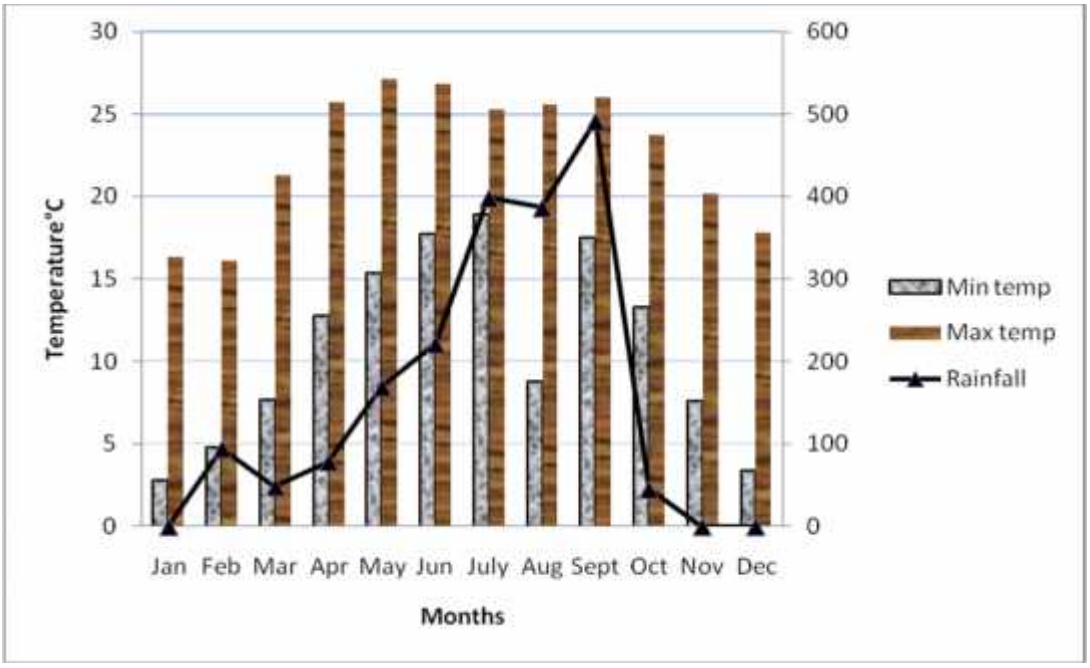

Figure 1. Five years (2003-2008) average minimum and maximum temperature and rainfall of Godawari station. (Source: Department of Hydrology and Meterology Gov/Nepal)

2.2 Shivapuri

2.2.1 Physiography and Climate

Shivapuri watershed and wild life conservation area was established in 1985, situated in the Nothern part of the Kathmandu valley when covers the total area of 14.4 km^2 and lies in between $27^0$46' to $27^0$51' N latitudes and $85^0$15' to $85^0$28' E longitudes Shivapuri has a steep mountains topography. The highest point of Shivapuri summit is at 2732m altitude while its lowest point is at about 1360m. There is sub-tropical to warm temperate climate with dry winter. The average monthly maximum temperature varies from 12.7^0C in winter to 23.5^0C in summer and average monthly minimum temperature varies from 4.3^0C to 16.7^0C in winter and summer respectively. The annual rainfall is 2872.2 mm and 2404.4 mm rainfall is during monsoon.

2.2.2 Vegetation

Vegetation of Shivapuri hill; upper cool subtropical and lower warm temperate zone having three major types of vegetation. *Schima-Castanopsis* (*Schima wallichii, Castasopsis indica*) dominate the lower periphery of the reserve on the Southern aspects. Second major vegetation's *Quercus-Laurel* is in the middle elevation. Third major vegetation *Quercus-Rhododendron* (*Quercus* and *Rhododendron* sp) type is on the upper limit of the hill. Similarly, *Quercus* sp, *Cinnamonum* sp, *Lithocarpus spicata* and *Mahonia nepaulensis* are also dominant.

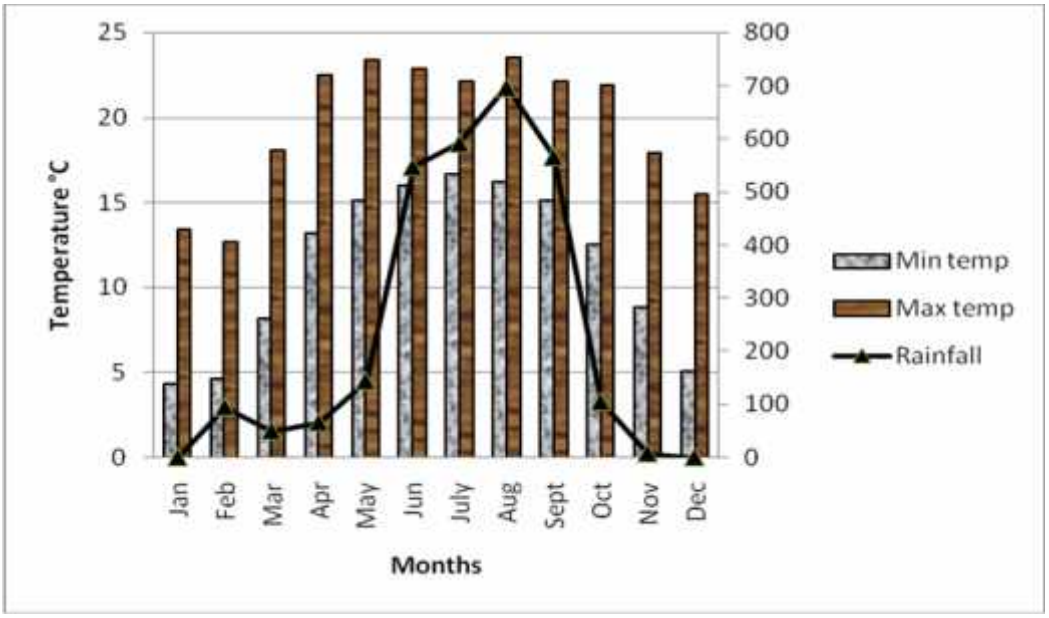

Figure: 2. Five years (2003-2008) average minimum and maximum temperature and rainfall of Kakani station. (Source: Department of Hydrology and Meterology Gov/Nepal)

Both of the study sites were protected areas, so the luxuriant of logs and litters were available. Both of the study sites were similar in slopes and altitude. The humidity and moisture of both study areas were mostly similar.

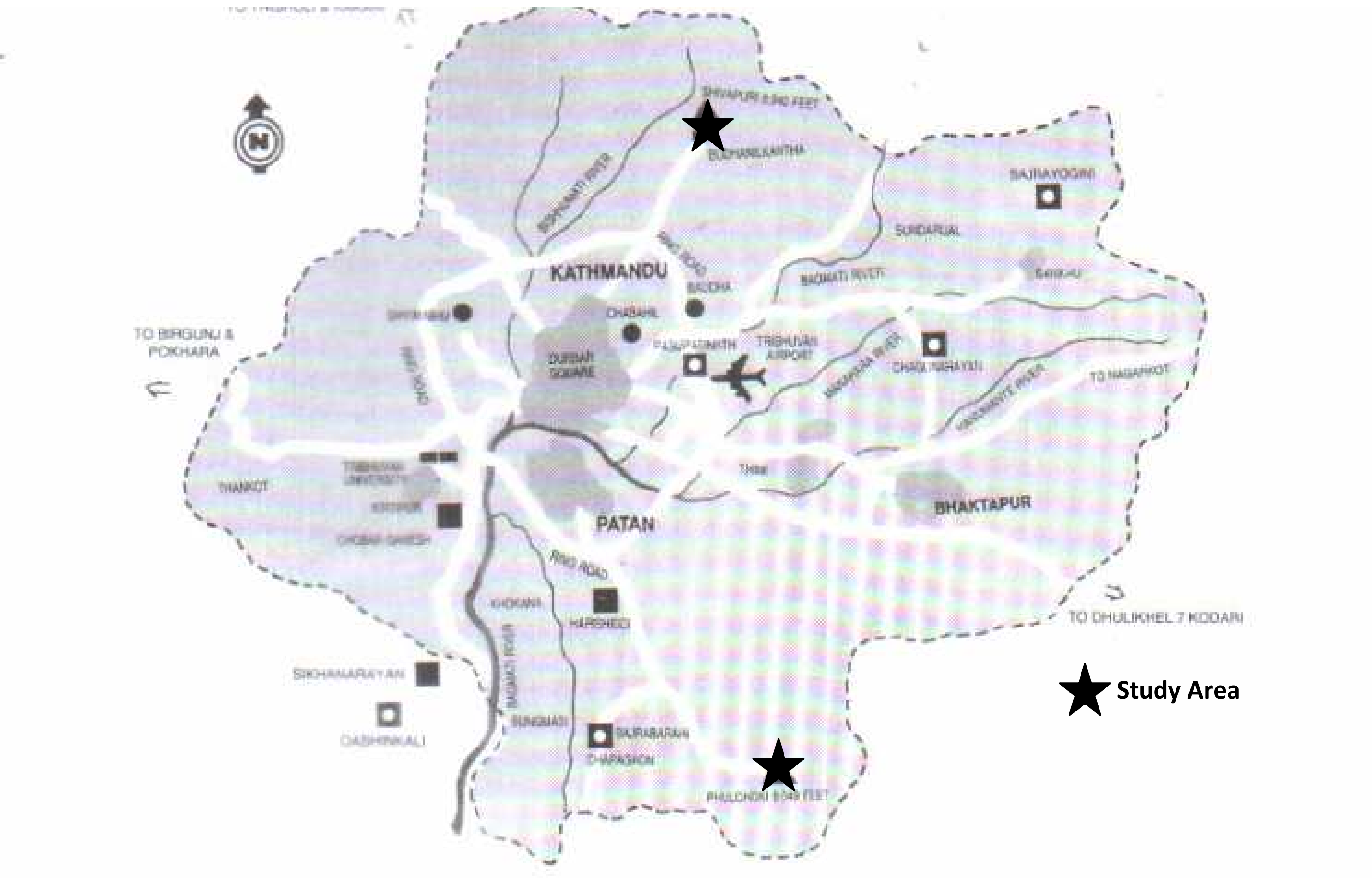

Figure:3 Map of study area

CHAPTER THREE

LITERATURE REVIEW

3.1 Exploration of Myxomycota

Bhatt (1965-1966) enumerated 118 specied of fungi from different parts of Nepal in which he reported one Myxomycota.

Poelt (1965) reported 26 genera and 55 species of Myxomycetes from Khumbu Himal and its adjoining areas including two species new for Nepal.

Singh (1971) reported two species, *Diderma hemisphaericum* on decaying leaves of *Cyanodon* and *Didymium iridis* on decaying twig from Kathmandu Valley.

Onsberg (1973) studied Myxomycetes in Nepal and recorded two species of Myxomycetes in which *Lycogala fuscavioceum* was recorded as new for science.

Nisha *et al.* (1977), Reported 5 species of Myxomycetes from Kathmandu Valley.

Hagiwara & Bhandari (1982) listed 17 genera and 35 species of Myxomycotina collected from central region (Langtang and Kathmandu valley)

Hagiwara (1982) enumerated 12 species of Dictyostelid cellular slime molds from Gosainkunda area with reference to their altitudinal distribution influenced by various phytogeographical factors.

Hagiwara (1983) described 4 new species of these slime molds (*Dictyestelium exiguum*), *D. gracile, D. longosparum* and *D. magnum*) collected from Langtang valley.

Hagiwara (1990) studied altitudinal distribution pattern of Dictyosterid cellular slime moulds of Langtang valley.

Nannenga - Bremekam and Yamamota (1988) described a new species (*Stemonitis laxifilia*) while studying specimens collected from Kathmandu valley and adjoining area.

Hagiwara (1988) enumerated 18 species of Dictyostelid cellular slime moulds from different forest soil samples.

Yamamota & Hagiwara (1980- 1988) jointly recorded 14 genera and 50 species of Myxomyletes

Nisha *et al.* (1977) studied Myxomycetes in Kathmandu valley and recorded 5 species, *Physarum nutans, Physarella oblonga, Stemonitis confluens, Arcyria incarnata, Stemonitis fusca*.

Singh & Nisha (1976) reported 14 species of Myxomycetes (II) *Arcyria - 5 species*, *Ceratimyxa* - 1 species, *Comatrichia species*, *Hemitrichia* - 1 species, *Physarella* - 2 species collected from Phulchowki, Gadawary, Tokhav Nagarjoon, Thankot & other places of Kathmandu valley.

Adhikari (2009) published *"Researches on Nepalese Mycoflora"* which embraces historical review of Myxumycotina (134 genera, 146 species and 11 new species).

3.2 Checklist to Myxomycetes of Kathmandu valley

This list of Myxomycetes recorded from Kathmandu valley, described by earlier workers is as follows.

Acytostelium sp. from soil, Kathmandu Valley (Hagiwara, 1988).

Cribraria cancellata (Batsch) Nann.- Brem. [= *Dictydium cancllatum* (Batsch) Macbride] on decaying wood, Kathmandu valley (Singh, 1971); on *Pinus roxurghii* (= *P. longifolia*) log, Dschiddara (Poelt, 1965); on wood Ulleri - Ghorepani (Yamamoto & Hagiwara, 1990); in *Abies - Rhododendron* forest, Thodung and Lamjura - Junbesi (Poelt, 1965).

C. microcarpa (Schrad.) Pers, on wood, Phulchoki (Yamamoto & Hagiwara, 1990).

Badhamia affinis Rostafinski, Kathmandu (Poelt, 1965); on bark, Lamahotel (Yamamoto & Hagiwara, 1990).

Diderma hemisphaericum (Bull.) Hornem, on decaying leaves of *Cynodon dactylon* and decaying branches of *Chaemecyparis lawsoniana*, Kathmandu valley (Singh, 1971).

Didymium iridis (Ditmar) Fr. [= *D. nigripes* Fries *var. xanthopus* (Lister) Lage,] east Nepal (Poelt, 1965) and on dead twigs, Kathmandu valley (Singh, 1971)

Physarella oblonga (Berk et Curt.) Morgan, Jiri (Poelt, 1965), on decaying and dead wood, Thankot and Hatiban (Singh & Nisha, 1976), Kamalapokhari (Nisha *et al.* 1977; Birethanti - Tikhedhunga (Hagiwara & Bhandary, 1982).

Physarum nutans Pers., on dead wood, Baneswar, Kathmandu (Nish, Singh & Joshi 1997); Chitre- Banthanti (Hagiwara & Bhandary, 1982).

P. ovisporum G. Lister, on living leaves of *Cucurbita maxima*, Thankot (Singh & Nisha, 1976).

P. wingatense Macbride, on decaying leaves of *Chamecyparis lawsoniana*, Putali sadak, and on leaves of *Jasminum* sp. and *Cynodon dactylon*, Godavary (Singh & Nish, 1976).

Clastoderma pulchella (Babington et Berk.) Rost., in *Rhododendron* forest belt, Jun besi (Poelt, 1965); on decaying wood, Godavary (Singh & Nisha, 1976); on mosses and dead wood (Hagiwara & Bhandary, 1982).

Clastoderma pulchella (Babington et Berk.) Rost., in *Rohododendron* forest belt, Junebesi (Poelt, 1965); on decaying wood, Godavary (Singh & Nisha, 1976); on mosses and dead wood (Hagiwara & Bhandary, 1982).

Stemonitis flavogenita Jahn, on deaed wood, Kathmandu valley (Singh, 1971) and on wood Dhuche- Lamahotel (Yamamoto & Hagiwara, 1990).

S. fusca Roth, on dead wood, Balkumari, Kathmandu (Nisha *et al.* 1977); on bark and wood, Gokarna, Chitra and Lamahotel (Yamamoto & Hagiwara, 1990).

S. herbatica Peck., on leaves and dead wood, Nagarjoon (Singh & Nisha, 1976); Gokarna and Terai (Yamamoto & Hagiwara, 1990).

S. laxifila Nann. - Brem et Yamamamoto, on mossy bark, Swayambhu, Kathmandu (Nannenga Bremekamp & Yamamoto, 1988; Yamamoto & Hagiwara, 1990).

S. splendens Rost., in *Abies - Rhododendron* forest, Thodung and Jiri (Poelt, 1965); on dead wood, Kathmandu valley (Singh, 1971) and Singompa (Hagiwara & Bhandary, 1982); place not mentioned (Yamamoto & Hagiwara, 1990.

Stemonitis sp. Kathmandu (Bhatt, 1966) and on dead wood, Putali sadak, Kathmandu (Singh & Nisha, 19769).

Arcyria cinerea (Bull.) Pers., on dead wood, Phulchowki (Singh & Nisha, 1976), Ghorepani-Chitre (Hagiwara & Bhandary, (1982), Singompa (Yamamoto & Hagiwara, 1990). In *Abies-Rhododendron* forest, Thodung (Poelt, 1965).

A denudate (L.) Wettst., on dead wood, Kathmandu valley (Singh, 1971); Banthati, South of Annapurna (Hagiwara & Bhandary, 1982). Sybru-Lama hotel and Gokrna (Yamamoto & Hagiwara, 1990).

A. ferruginea Sauter, in *Abies- Rhododendron* forest, Thodung (Poelt, 1965; on dead wood, Thankot (Singh & Nisha, 1976) and on wood, Singompa (Yamamoto & Hagiwara, 1990).

A. incaranta Pers, on dead wood, Bal Kumari (Kathmandu Valley) (Nisha *et al.* 1977).

A. insignis Kalch et Cooke, on dead branch of *Lantana camara,* Godavary (Singh & Nisha, 1976).

A. nutans (Bull.) Grev., on dead wood of Lantang camara, Putalisadak, Kathmandu valley (Singh & Nisha, 1976).

A oerstedtii Rost., on dead wood, Kathmandu valley (Singh &Nisha, 1976) and wooden roof of a hut, Singompa and Gosainkunda (Hagiwara & Bhandary, 1982)

Hemitrichia serpula (Scopoli) Rost., on dead wood, Kathmandu valleu (Singh & Nisha, 1976) and Singompa (Hagiwara & Bhandary, 1982; on twig and wood, Gokarna and Lamahotel (Yamamoto & Hagiwara, 1990) and on moss, Syabru (Yamamoto & Hagiwara, 1990).

CHAPTER FOUR

MATERIALS AND METHODS

4.1 Materials

Altimeter, Basket, Small paper box cotton, Polythene, Envelop, Microscope, Stage and ocular micrometer, Slides and slide cover, Glue, Knife and Marker.

4.2 Methods

The following methods were performed during the study of Myxomycetes.

4.2.1 Collection of specimens

Godawari and Shivapuri were selected for general survey of slime molds. The collection of slime molds was done in between September and October after the seven days of heavy or steady rainfall. This allows time for the formation of ripe fruiting bodies of many species. Plasmodia may be in evidence after a shorter interval. These places were visited many times during the collection of specimens.

Each collected specimen is immediately placed in a small box by a drop of white glue to protect fragile specimens. Then boxes were carried in basket with handle. Some of the larger or less fragile slime moulds (*Stemonitis*, *Arcyria* etc.) were placed inside envelop and laied on top of the basket specimens to avoid crushing. Some specimens like *Hemitrichia* were directly collected in a polythene bag. At the time of collection color of fruiting bodies, type or identity of substrate and ecological conditions (kind of habitat) height were noted and photographs were also taken in natural habitat.

4.2.2 Preservation of collected specimens

After returning to home or in laboratory, boxes were opened to air dry the substrates. But some specimens have substrates of high moisture that wet to envelop, were changed for the preservation of materials it avoided from heat. They were placed in dark and aerated place and fumigated with insecticide, such as naphthalene or paradichlorobenzene periodically so insect will not damage or destroy the specimens during storage case. Specimens were deposited in the mycology laboratory of CDB, TU.

4.2.3 Lab work

The specimens were examined carefully by naked eye for observing the details of morphological characters of fungal colony. The detailed characteristic features of fungus associated with each specimen were studied under compound and stereo microscope. Measurement of sporangiosphore, plasmodium was done.

Fruiting body (sporangiosphore) was covered by slide cover. The preparation was tapped vigorously to break the same so that the contents of the fruiting body could be released and observed. After this squash preparation, diameter of spores and capillitium was measured with the help of the micrometer. Measurement was done by using following formula.

One division of stage micrometer = 10 µm or 0.01 mm.

Factor of calibration for ocular division is calculated for a particular combination of objective and ocular lens by

$$\text{One ocular division } (\mu m) = \frac{No.of\ division\ in\ stage \mid 0.01}{No.of\ division\ in\ ocular} \mid 1000$$

After calibrating ocular micrometer, it was used to determine the size of micro organism by the following formula.

$$\text{Size (in } \mu m) = \text{No. of ocular division} \times \text{calibration factor}$$

CHAPTER FIVE

ENUMERATION OF SPECIES

5.1 Key to the identification

Key to the identification of fungal species has been described below:-

5.1.1 Key to the orders

1(a) Fruiting bodies; erect, simple or branched, usully white pillars or sometimes convoluted or crust like, covered with spores that are attached individually by minute filaments; peridium absent …….…….Ceratiomyxales.

1(b) Fruiting bodies not as in 1a; spore mass enclosed inside fruiting bodies by a wall (peridium) ……....2

2(a) True capillitium and columella absent; pseudo capillitium present or absent when present, then consisting of pale nearly smooth to sculptured tubules or perforated membranes, sometimes fraying in to filament……..Liceales.

2(b) True capillitium present; pseudo capillitium absent (except in *Fuligo* and *Mucilago),* columella absent…….3

3(a) Spores mass more or less brightly colored, rarely brown, columella absent; capillitium light colored to light brown or dark red, usually faintly to prominently sculptured, rarely smooth ……..Trichials.

3(b) Spores mass predominantly purplish brown to black, occasionally pale violet or red brown, columella present or absent; capillitium not sculptured, predominantly dark, if pale, then usual containing lime……..4

4(a) Lime present …….....Physarales.

4(b) Lime absent ……....Stemonitales.

5.1.2 Keys to families and genera

1(a) Fruiting bodies sporangiate and very small (except in *Licea variabilits),* rarely exceeding 1mm in dram; pseudo capillitium absent …….Liceaceae.

1(b) Fruiting bodies mostly aethaliate; pseudoaethaliate; pseudocapillitium present (except tubifera) ……....Reticulariaceae.

2(a) Fruiting bodies pseudoathaliate; sporangial components evanescent except for a apicalcaps which form a tessellate layer, ……*Dictydiathalium.*

2(b) Fruiting bodies pseudoaethaliate on a thick hypothallus; consiting of clustured sporangia; peridian persistent or evanescent only in the upper part…….....*Tubifera.*

Order- Trichiales

1(a) Fruiting consisting of small, sessile, heaped sporangia, dull brownish red or yellowish brown; peridium in large part persistent, not sharply differentiated in to basal cup (calyculus) and evanescent upper portion; capillitium worted or spiny…….*Aryodes.*

1(b) Sporangia gregarious to crowded but not heaped, mostly stalked; peridium usually more or less sharply divided into basal cup (calyculus) and fugacious upper part; capillitium marked with works, spins, cogs, reticulation, or rarely, spirals…….*Arcyria.*

2(a) Capillitium reticulated, hence with few if any free ends…….*Tlemitrichia.*

2(b) Capillitium consisting of simple or branched elaters, not united in a net, hence with many free ends ……...*Trichia*.

Order- Physarales

1(a) Lime present in the capillitium……Physaaceae ……..…2

1(b) Lime absent capillitium …….Didymiaceae

2(a) Fruiting bodies aethaliate…….……*Fuligo*

2(b) Fruiting bodies sporangiat, plasmodiocarpous, or pseudoaethaliate……3

3(a) Capillitium a mixture of two distinct systems …….4

3(b) Capillitium uniform……..…6

6(a) Peridium differentsated into persistent calyculus and a more fragite, dehiscent upper portion, sometimes with definite lid, dehiscence circuscissite or irregular; sprongia more or less globlet shaped, avoid, or cylindric…….*Crtaterium*

6(b) Peridium not differentiated as in 6a and then with uniform membranous peridium……..…7

7(a) Capillitium (with few exception) a net work of more or less uniform, limy tubules; spores often united in balts…….*Bodhamra*.

7(b) Capillitium consisting of variously shaped lime nodes and limeless, thin connecting threads; spores free…….*Physarum*.

8(a) Peridium limeless, usually iridescent……*Diachea*.

8(b) Peridium limy……...9.

9(a) Fruiting bodies aethaliate, covered by a spongy or flasky, white to creamy certex seated on a well- developed hyporthallus lime crystalline……..*Mucilago*

9(b) Fruiting bodies not aethaliate……...10.

10(a) Lime crystals forming a loose powder or a crust of compacted individual crystals not united into scales.....*Didymium.*

Order- Stemonitales

1(a) Peridium presistant, more or less lustrous to brilliant metallic-iridescent ….……..*Lamphroderma*.

1(b) Peridium evanescent cat least in the upper portion or, if persistent, then sporangia cylindric…….……..2.

2(a) Capillitium dense, ending in a well defined surface net at the periphery; sprongia slender more or less cylindric, stalk and usually tuft or densely crowed……...*Stemonitis.*

Order-Ceratiomyxales

Fruiting bodies showing spores, tip of one branch showing spores attached by filaments……..*Ceratiomixa*

5.1.3 Keys to species

Tubifera

1a. Many sporangia containing a columella like central strand or spine; often with lateral branches extending to peridium…….……*T. casparyi.*

1b. Columella-like structures absent……2.

2a. Sporangia or pseudoathalia borne on a thick, raised to stalk-like hypothallus spores 5-6 mm in diam……..*T. microsperma.*

Arcyria

1(a) Sporangia usually bright red or rose, sometimes turning brown with age; capillitium moderately expended and remaining more or less erect at matuarity……..*A. incarnata*

1(b) Sporangia yellow rarely greenish or brownish, globose to avoid or short cylindric and them constricted in the centre, calyculus shallow……..*A. pomifomis*.

2(a) Capillitium mostly spinulose, lacking spirals; sporangia scattered to closely gregarious or fascicled……...*A. cinerea.*

Hemitrichia

1(a) Spores coarsely reticulate; fruiting bodies consisting of bright yellow or yellow brown; reticulate plasmodiocarps……...…*H. serpula.*

Didymium

Plasmodium absent, sessile, anastomosing or ring shaped plasmodiocarp spores-warted ……..... *D. flexuosom*

Columella white, stalk usually yellow or yellowish to bright reddish brown peridium pale ……...…….*D.* iridis

Fuligo

Aethalia white, stender, often thin and resembling plasmodiocarps, sometime arranged in extensive network, many spores ellipsoid, 14 - 16 x 10 -12|⇑m, some globse and mostly 13-14 in diameter often occuring on litter piles .....*F. cinerea.*

Mucilago

Aethalium composed of long and branched, brittle cartex-like covering of lime crystals, 2-6cm long 1-6cm broad, 1cm thick, capilitium purple brown or colorless stout thrends, spores dark violet, strongy spinulose, 10-13 ⇑m. in diamter. .....*M. fruiticulosa*

Physarum

Sporangia and capillitial lime nodes yellow to deep orange or greenish ..... *P. viridi*

Ceratiomyxa

Sporophores while, fasciculated branchlets about 1 mm in height, spores colorless, ellipsoid, smooth surface, 10-13× 6-7 ⇑m .....……..*C. fruiticulosa*

5.2 Description

1. *Arcyria pomiformis* (Wiggers) [=*A. pomiformis* (Leers) Rostof.] in Ferr, 1962 Mycologic 54: 516 – 530. in Emoto. *The Myxomyceter of Japan* 64: 1977 pl. 32,

Shivapuri (2388-2384 m), on rotten wood or on rotten bark place of tree, 2006/8/3, 01, Ananta Chauhan ,

Plasmodium white, sporangium scattered, stalked, subglobose or ovoid dimater 0.3- 0.6mm dull yellow. Calyculos furrowed below remainder nearly smooth with faint reticulation and popilose. Stalk slender, yellow, brown 0.2-0.5 mm long capillitum enclosing spores like cells within a loose network of dull yellow threads 3-5⇑ dimater, marked with spines transverse bands. Spores nearly colourless, few scattered warts, 7-8⇑m diameter.

Distribution: Comopolitan

2. *Arcyria incarnata* (Rostaf) 1875, *Pamietu Towartz Nauk Sci Paryzu* 6 (1): 278, Martin & Alexopoulus (1969), in Emoto. *The Myxomycetes of Japan* 64: 1977 pl. 32.

Sivapuri (2369m - 2375m), on rotten wood or falling twigs, 2006/8/3, 16, Ananta Chauhan.

Sporocarpe coorded, Stipulate, sporahgia is 1-2 mm high bright red or rose, gnegarious, cylindrical stalk weak, 0.1-0.3mm long. Capillitium branched elastic expands long, 3-5⇑m diameter, pale red or reddish brown irregularly branched, broad perforated spirally arranged spinules, sometimes minutely scattered spinules. Spores pale pink, a few scattered spinules. Spores pale pink, a few scattered warts, 6-8 ⇑m diameter.

3. *Arcyria cinerea* (Bull) Pers. 1801. in Emoto. *The Myxomycetes of Japan.* 62: 1977. Pl. 31.

Shivapuri (3100-3514m), 2006/8/3, 05, Ananta Chauhan.

Plasmodium greyish sporocarps distorded or in small groups. Sporangia stalked 1-4 mm, gregarious, scattered, single or united in clusters of 2-6, cylindrical rarely glubose, 0.5-0.8mm

diameter. Stalk cylindrical 0.2-2mm, calyculus smooth, spinulose innerside. Capillitium a compact network of gray threads 2-4⇑ diameter, marked with spirally arranged spinules or transverse bands. Spores colorless, scattered warts 6-8⇑ diameter.

Distribution: Formosa, Korea, Cosmopolitan

4. *Stemonitis cinerea* (Bull.) J.F. Grnel 1782. in Emoto. *The Myxomycetes of Japan.* 62: 1977. Pl 31.

Godwari (2368-2385m), on rotten wood, 2006/8/10, 07, Ananta Chauhan.

Plasmodium white. Sporangia small loosely clustered shortly stalked, cylindrical brown. Height 2-4mm, stalk 0.1-0.5 mm slender above forming columella. Capillitum a network of anastomosing brown threads springing from the columella. spores small, reticulation connected by faint lines or warts 5-4 ⇑ dimaters

Distribution: Europe, North America

5. *Physarum viride* (Bull) Pers. in Emoto. *The Myxomycetes of Japan* 170; 1977. Pl. 85.

Shivapuri (2460-2510m), on rotten wood, 2007/9/1, 10, Ananta Chauhan.

Plasmodium pale grayish white total height 2 mm. sporangia yellow stalked, usually scattered, globose 0.3 - 0.8 m diameter. Sporangium wall membraneous, dehicing in small pieces stalk subulate, longitudinally furrowed grayish while or yellow, rarely charged with lime Columella none. Capillitium a coarse network of fine, branched hyaline threads connecting fusiform or orange lime knots. Spores brown purple nearly smooth 7-10⇑ diameter.

Distribution: Europe, Africa, Australia, south America, North America

6. *Hemitrichia- serpula* (Scap.) Rost. in Emoto. *The myxomucetes of Japan.* 94; 1977 pl- 47.

Shivapuri (2250-2446m), on rotten wood, 2007/9/1, 11, Ananta Chauhan.

Plasmodium yellow. sporangia elongated or curved and branched plasmodiocarps 0.3-0.7mm wide, usually net like bright yellow, often seated on reddish brown hypathallus sporangium wall of two layers, outer membranous or cartilaginous and inner with fainth reticulation. Capillitium elastic network of branching and anastomosing yellow or orange threads 5-6⇑ diameter, marked with 4, rearely 5-6 regular spiral bands, spinulose, rarely smooth spores yellow, reticulated with narrow ridges spherical, 10-12 ⇑ diameter.

Distribution - Europe, Africa, Australia, New Zealand, North America, (cosmopolitan)

7. *Ceratiomyxa fruticulose* (Muller) Macbride. in Emoto. *The Myxomycetes of Japan* 4: 1977 pl 2.

Shivapuri (2627-2687m), on rotten wood. 2008/7/31, 20, Ananta Chauhan.

Plasmodium white, sometimes pale yellow translucent, sporphores white, simple fasciculated branchlets 1-1.5mm in height diameter 0.8-1.5 mm, sometimes anastmosing in band and forming irregular lobes. Spores colorless, oval, smooth on surface and size 10-12⇑ x 6-7⇑

Distribution: Korea, China, Malay Peninsula, Indonesia, Europe, North America, South America, Australia.

8. *Fuligo Cinerea* (Schw. Morgan. in Emoto. *The Myxomycetes of Japan.* 138: 1977. Pl. 69.

Shivapuri (2627-2687 m), on fallen leaves or twings, 2008/7/ 4, 27, Ananta Chauhan.

Plasmodium white. Aethalium pulvinate clustered, cushion like (20-26mm) sporangia densely confluent, covered with cortex usually calcareous white smooth. Sporargium wall of acthallitium is membraneous. Capillitium hyaline branched or simple threads and large white lime knots. Spores purple brown, spinulose (12-15⇑m x 8-10 ⇑m)

Distribution: Formosa, Korea, Europe, India, Australia, North America.

9. *Mucilage crustacean* Wiggers. in Emoto. *The myxomycetes of Japan* 230: 1977: pl 115.

Shivapuri (2627-2687m), on fallen leaves, 2007/8/4, 32, Ananta Chauhan.

Plasmodium white Aethalium long and branched compressed sporangia in loose or compact cluster from branching strands of membraneous hypothallus enclosed with thick brittle cortex- like covering of lime crystal, gray white color, 2-6cm long, 1-6 cm broad about 1cm thick sporangium wall membraneous colorless or purplish. Columella membraneous, hollow. Comparessed capillitum purple brown or colourless stout threads, branching and nastomosing spores dark violet, strongly spinulose 10-13⇑m diameter

Distribution: Europe, Australia, North America, South America

10. *Tubifera ferruginosa* (batsch) Gmelin 1947: *Taxonomic notes on Myxomycetes.* 11. *Mycologia,* 39: 453-462. (1947), in Emoto. *The Myxomycetes of Japan* 16: 1977 pt 8.

Shivapuri, Godawari (1820-1947 m), on rotten wood, 2007/9/2, 15, Ananta Chauhan.

Plasmodium white rosy to brownish red sporangia cylindrical, closely clustered, polygonal due to mutual pressure, convex or canonical above, about 4 mm long and 0.3 mm diameter, reddish brown or deep yellow brown occasionally iridescent, seated on broad common spongy hypothallus, forming reddish brown cushion. Sporangium stalked wall membraneous minutely warted or papilose on inner side often with pouch like protuberance. Spores reddish brown, minutely reticulated, rarely smooth or broken ridges 4-6⇑m diameter.

Distribution - Formosa, Korea, India, North America, Abundant in topics.

11. *Didymium iridis* (Ditmar). Fries Wang *et al. Biol Bull. Nat* (Taiman. Normal Umi 16: 8 (1-12) Aldric, H.C. 1971. Synaptonomal Complexes and Meosis in *Didymium iridis. Mycologia*. 63:308-16. in Emoto. *The Myxomycetes of Japan* 240, 1977 pl. 120.

Sivapuri (2812-3210m), on fallen leaves, 2006/8/3, 35, Ananta Chauhan.

Plasmodium yellow or brown. Sporangia gregarious, globose or somennat depressed, 1-3 mm, stalked, sporangium wall membranceou more or less curved with white crystals. Columella turbinate or globose white, capllitium slender colorless or pale yellowish brown, branching threads. Spores mass brown, faintly warted or smooth, 7-9⇑m diameter.

Distribution - Formosa, Korea, China, Europe, North America, South America, Africa

12. *Didyamium flexuosum* Yamoshiro Lin, Citt. And Y . E. Chen. *Taiwania.* 43 (178): 177-184 1998. in Emoto. *The Myxomycetes of Japan* 236: 1977 pl 118.

Godawari, Sivapuri (2900-3200m), on fallen leaves 2006/8/20, 07 Ananta Chauhan.

Plasmodium not observed. Sporangia sessile, subglobose, pulvinate elongate branching anastomosing or ring-shaped. Plasmodiocarp 0.2-0.5mm broad, sporangium wall membraneous white grey lime crystal columella membraneous composed of based protuberance of the sporangium. Capillitum consisting of a coarse network of pale brown branched threads. Spores black, warted, incomplete broken reticulations 8-10⇑m diameter.

Distribution: Japan

5.2.1 Illustration

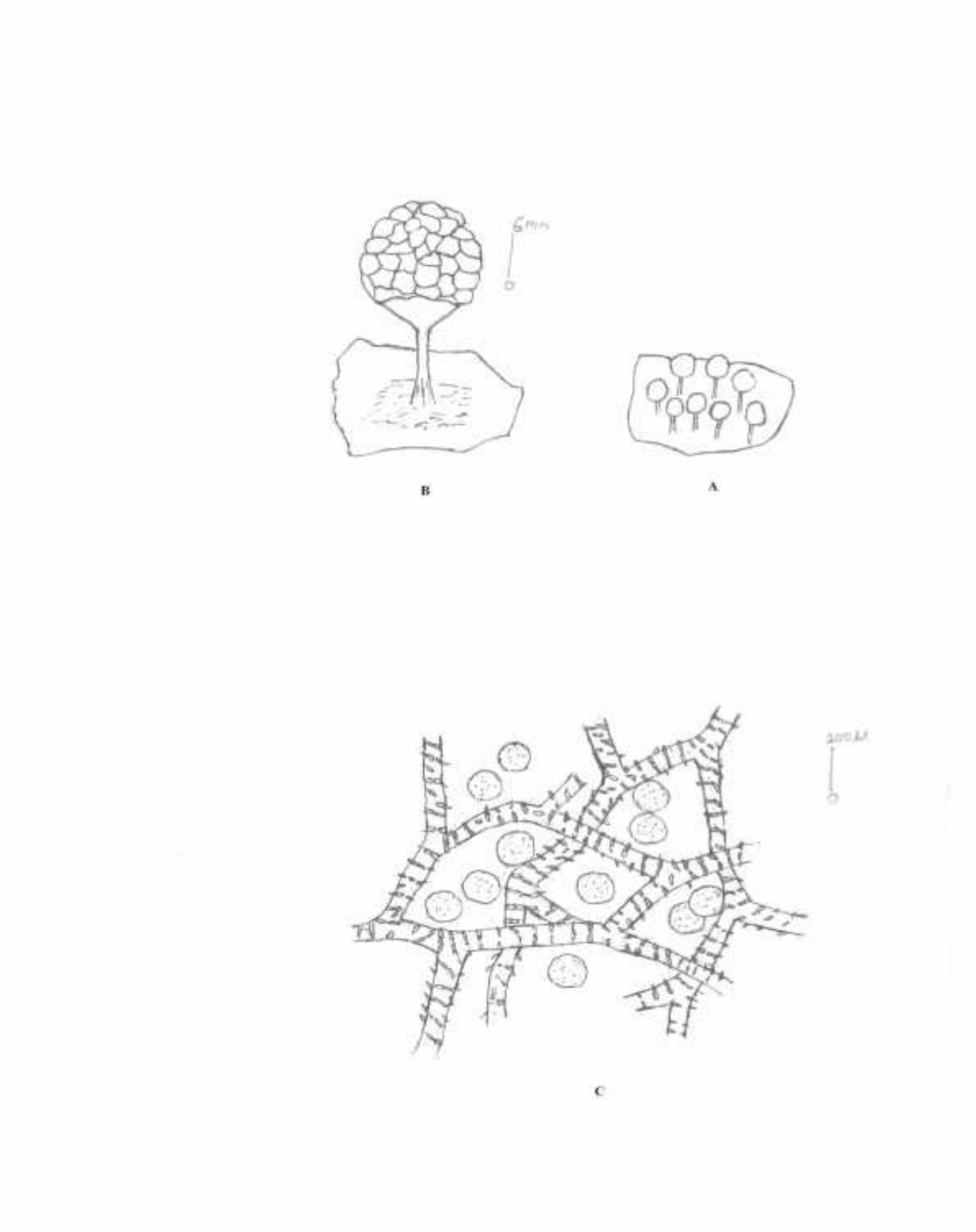

Figure: 4. *Arcyria pomiformis* A) In natural habitat. B) Single fruiting body. C) Arrangement of capillitium of with spores

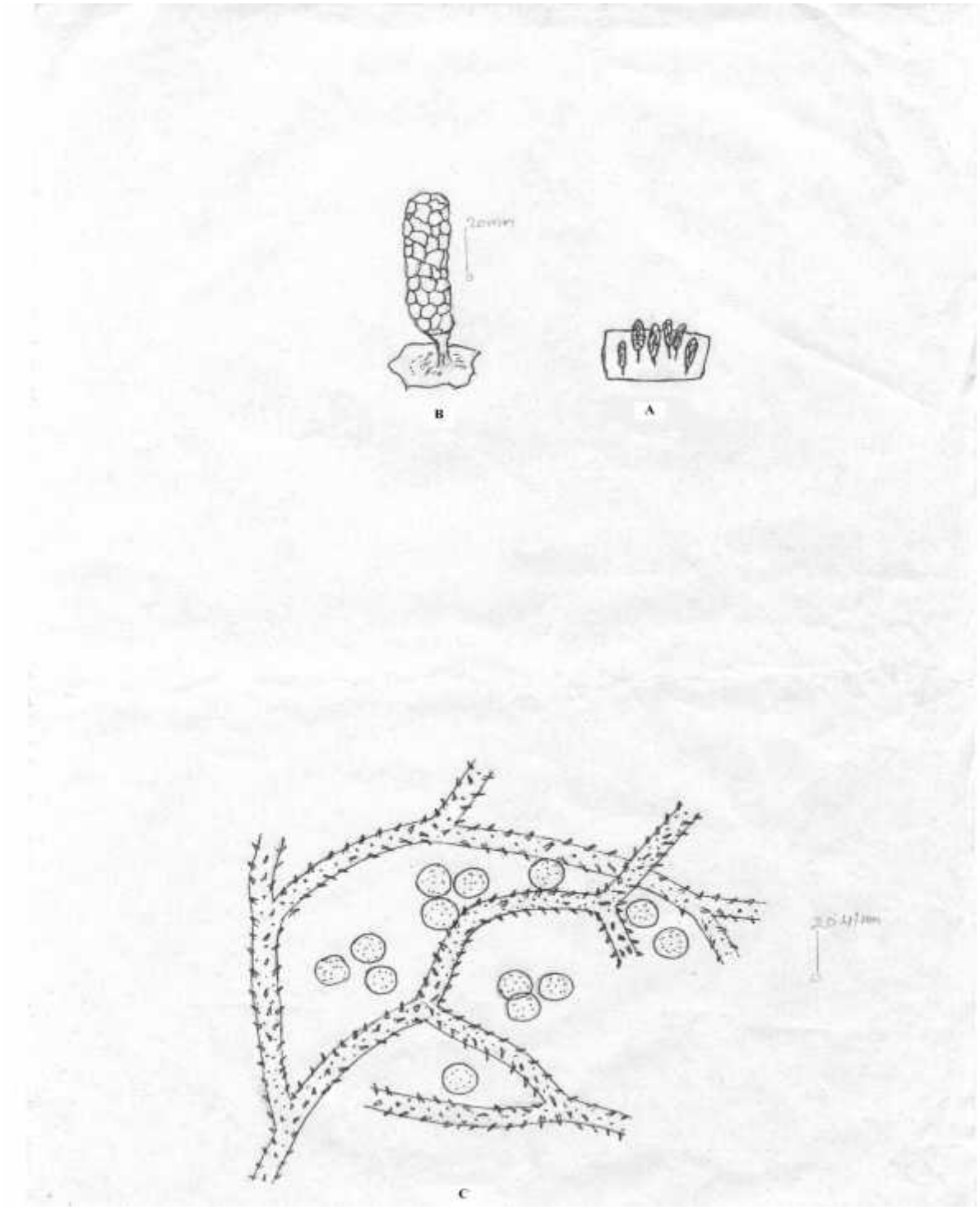

Figure: 5. *Arcyria incarnata* A) In natural habitat. B) Single fruiting body. C) Arrangement of capillitium with spores

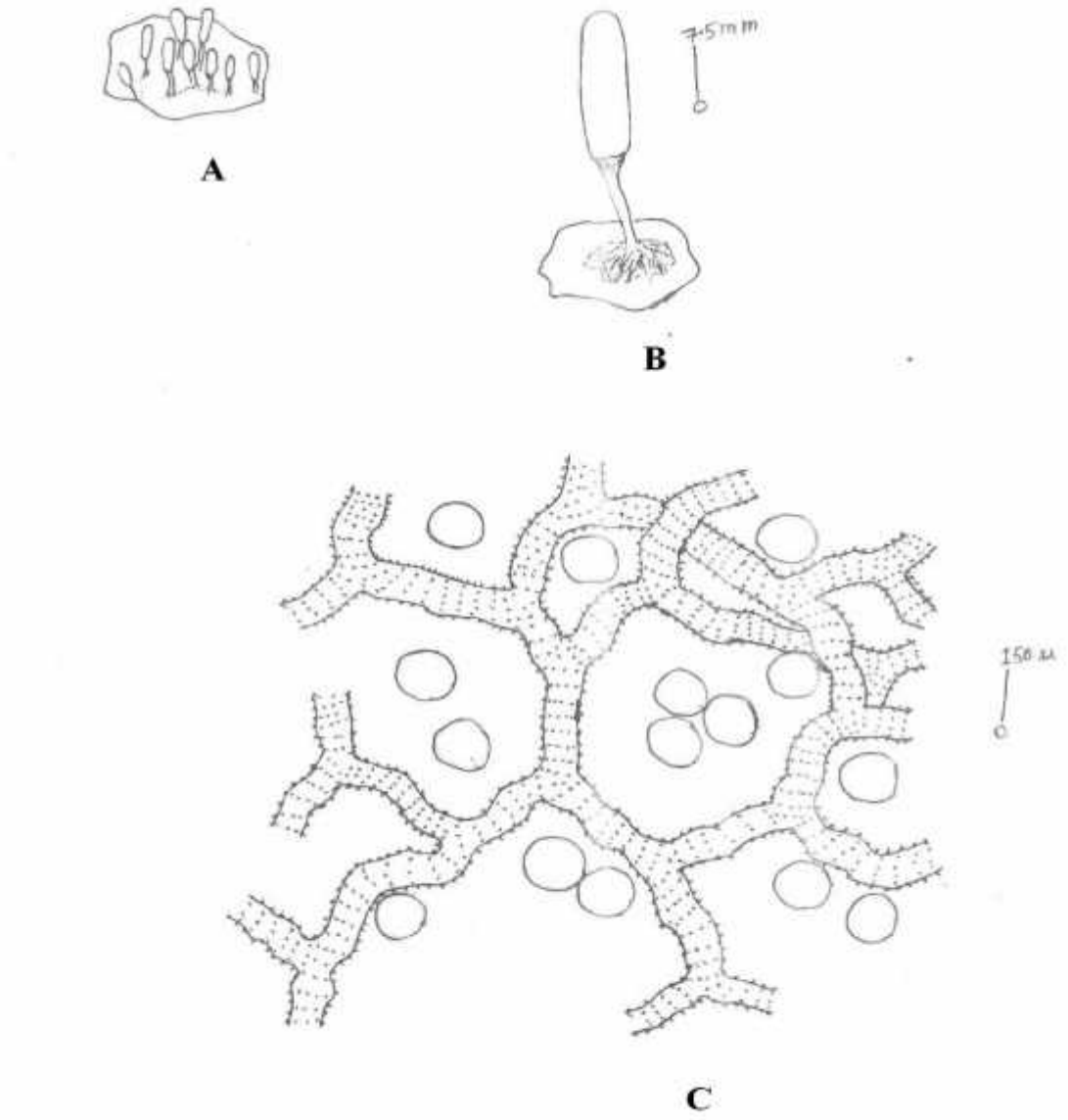

Figure: 6. *Arcyria cinerea* A) In natural habitat. B) Single fruiting body. C) Arrangement of capillitium with spores

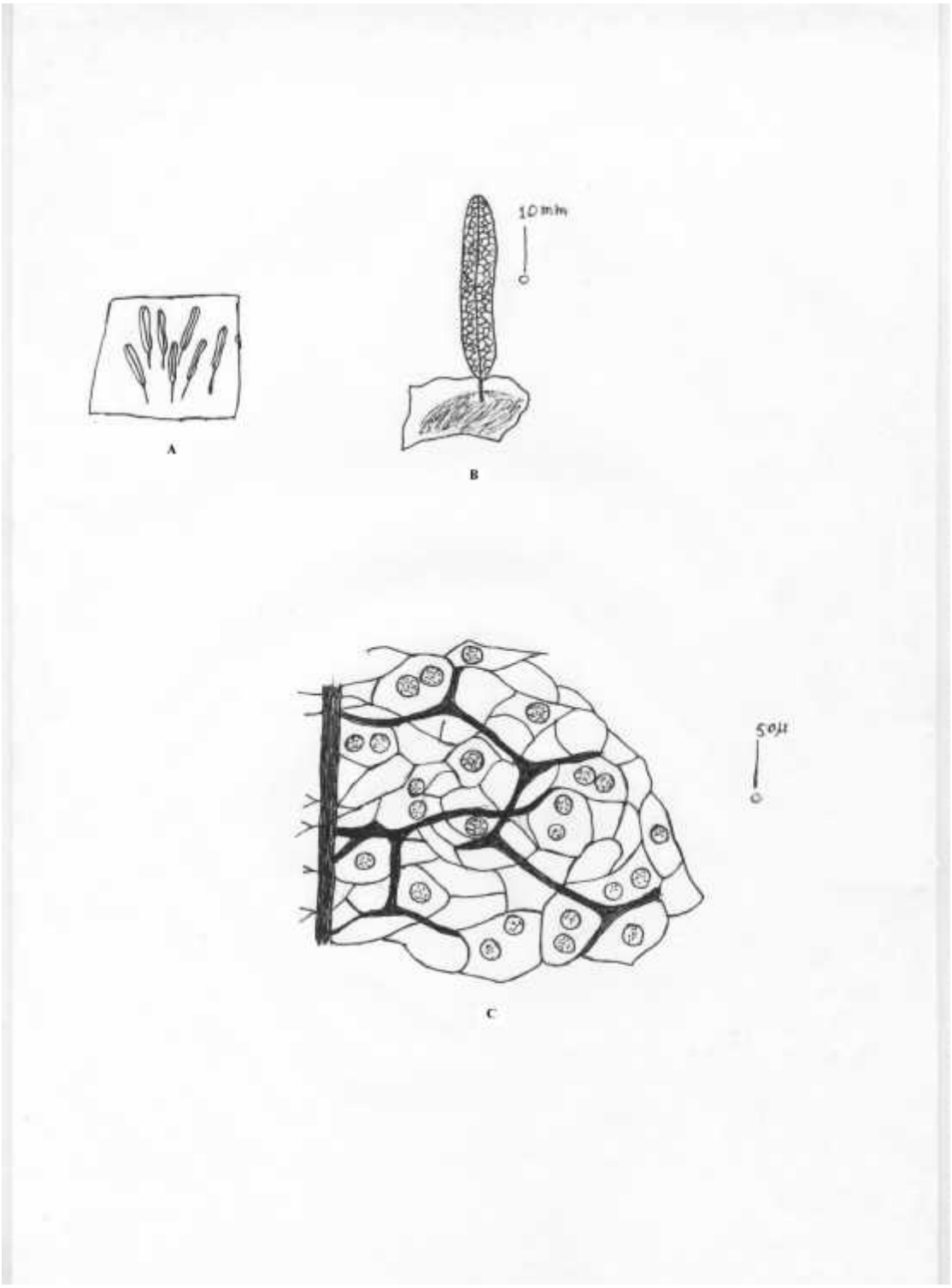

Figure: 7. *Stemonitis cinerea* A) In natural habitat. B) Single fruiting body. C) Arrangement of capillitium with spores.

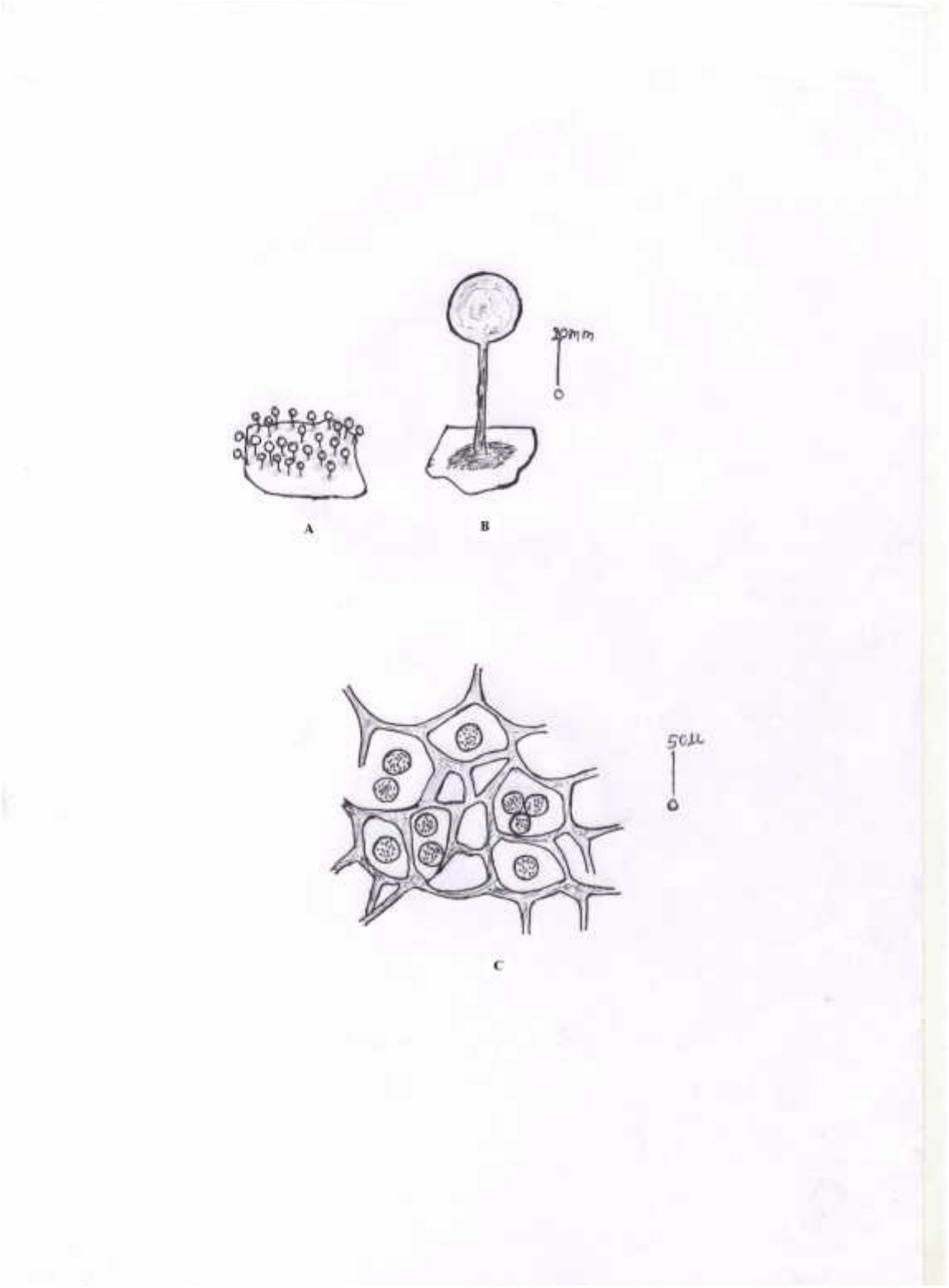

Figure: 8. *Physarum viride* A) In natural habitat. B) Single fruiting body. C) Arrangement of capillitium with spores

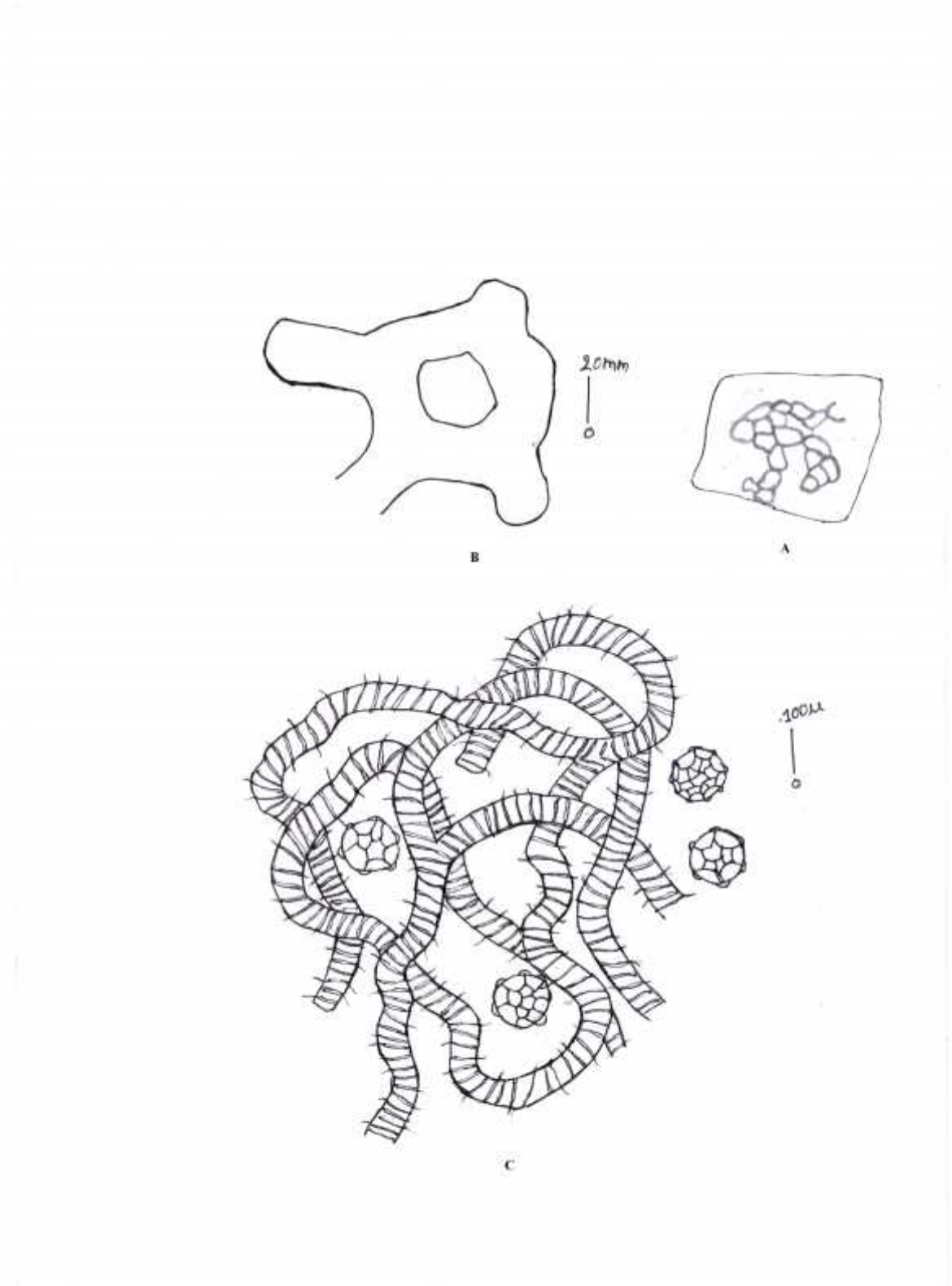

Figure: 9. *Hemitrichia* serpula A) In natural habitat. B) Single fruiting body. C) Arrangement of capillitium with spores

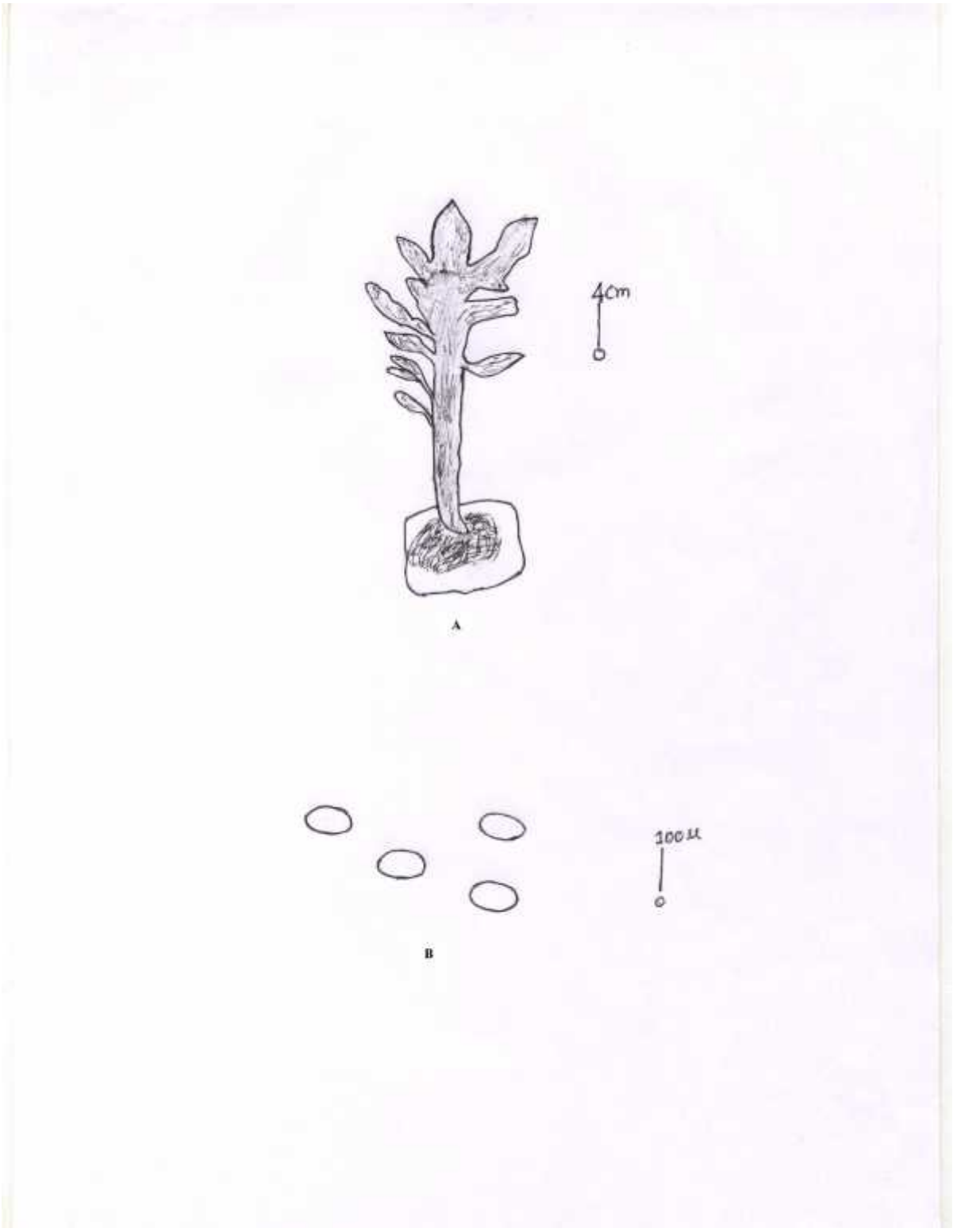

Figure: 10. *Ceratiomyxa fruiticulosa* A) In natural habitat. B) Arrangement of spores

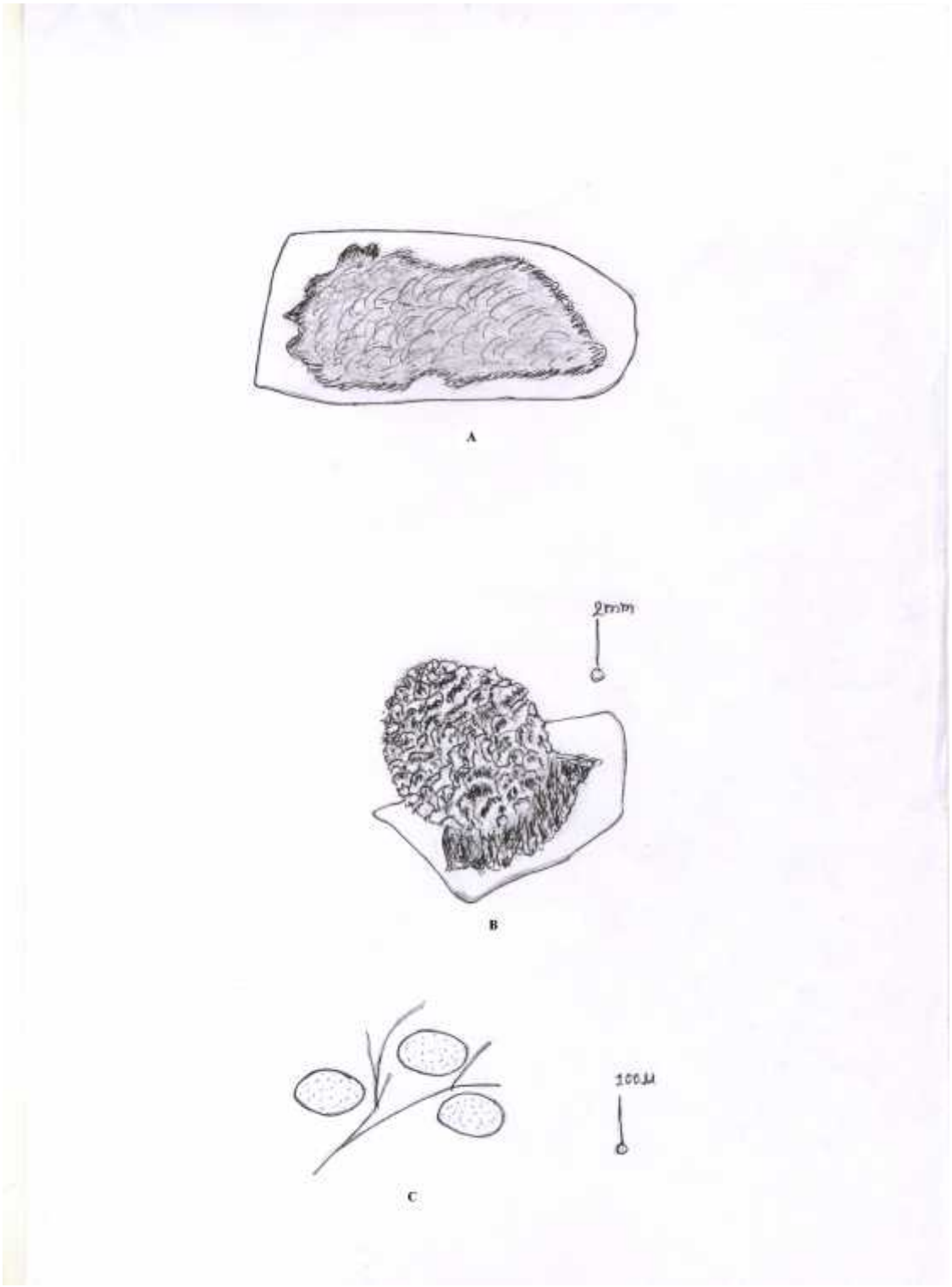

Figure: 11. *Fuligo cinerea* A) In natural habitat. B) Cushion shaped structure in natural habitat. C) Arrangement of capillitium with spores

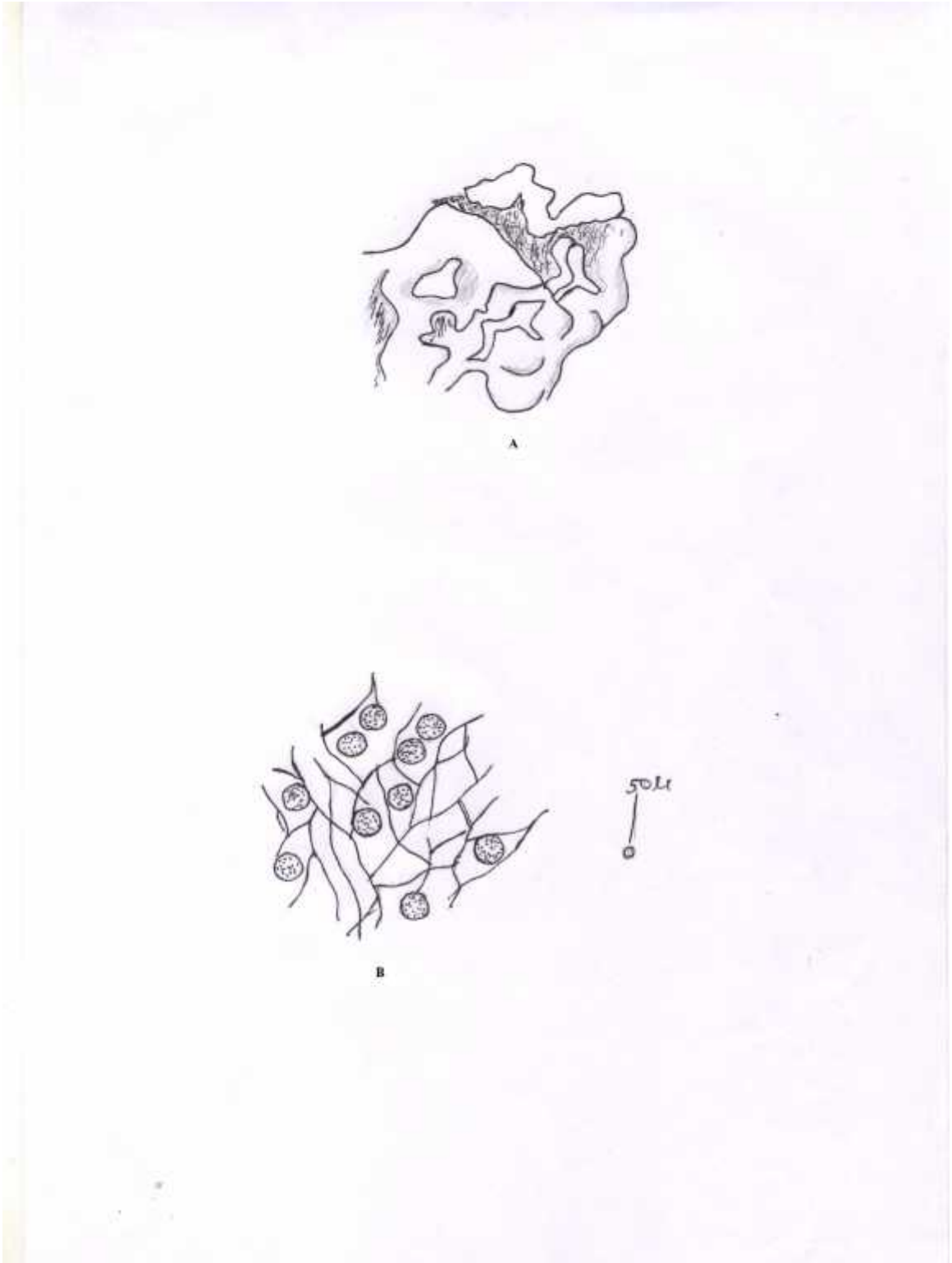

Figure: 12. *Mucilago crustacea* A) In natural habitat. B) Arrangement of capillitium with spores

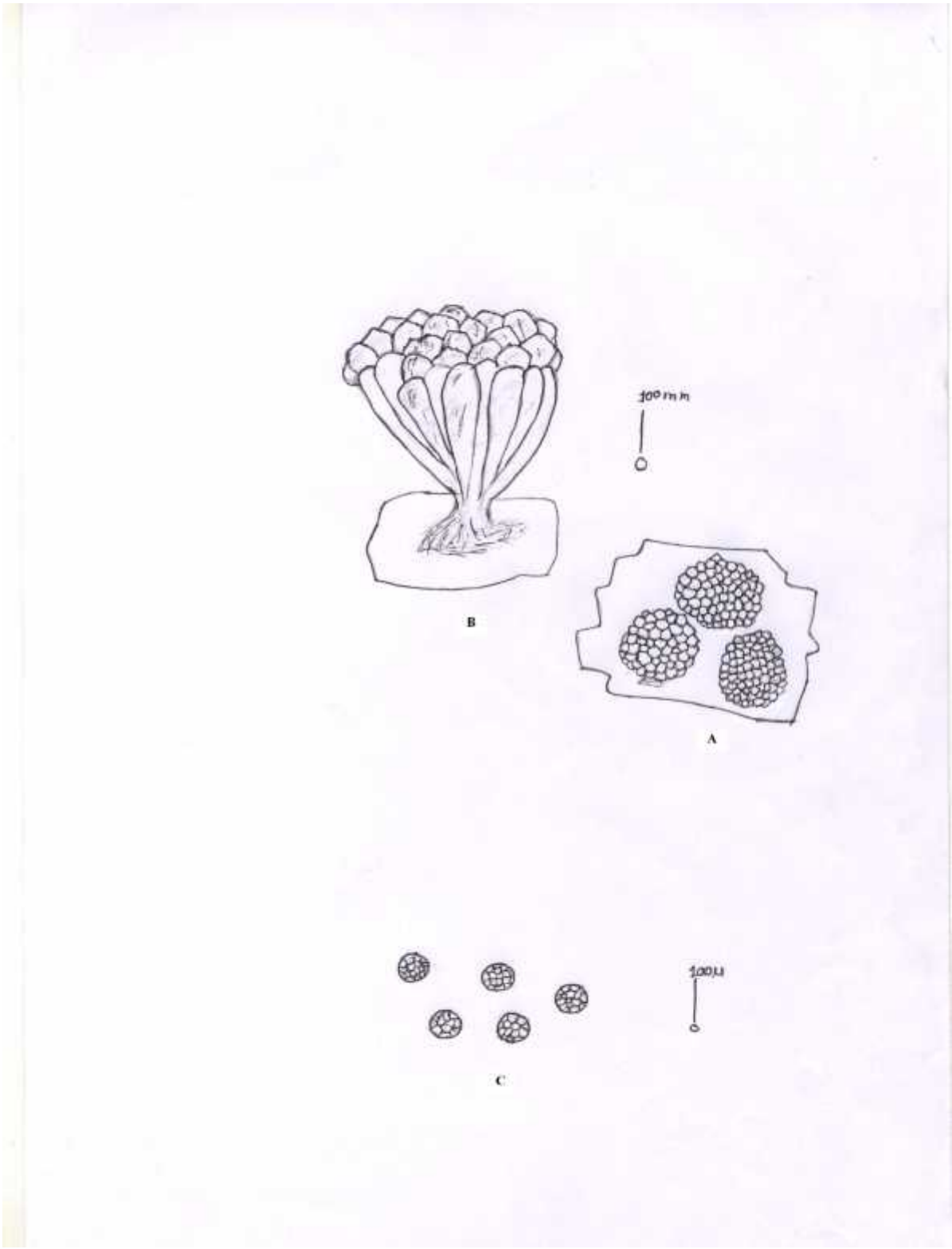

Figure: 13. *Tubifera microsperma* A) In natural habitat. B) Single fruiting body C) Arrangement of capillitium with spores

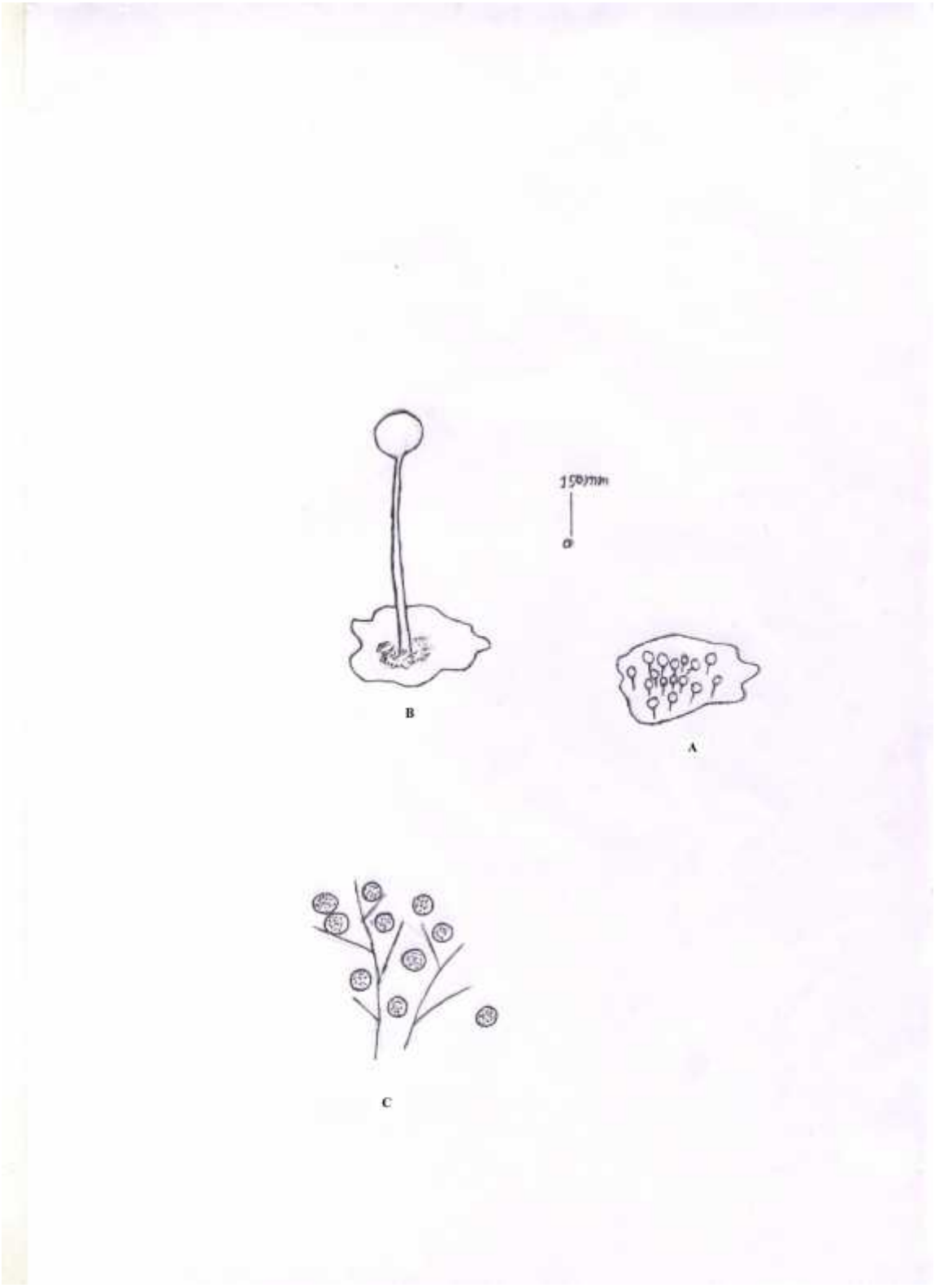

Figure: 14. *Didymium iridis*. A) In natural habitat. B) Single fruiting body . C) Arrangement of capillitium with spores

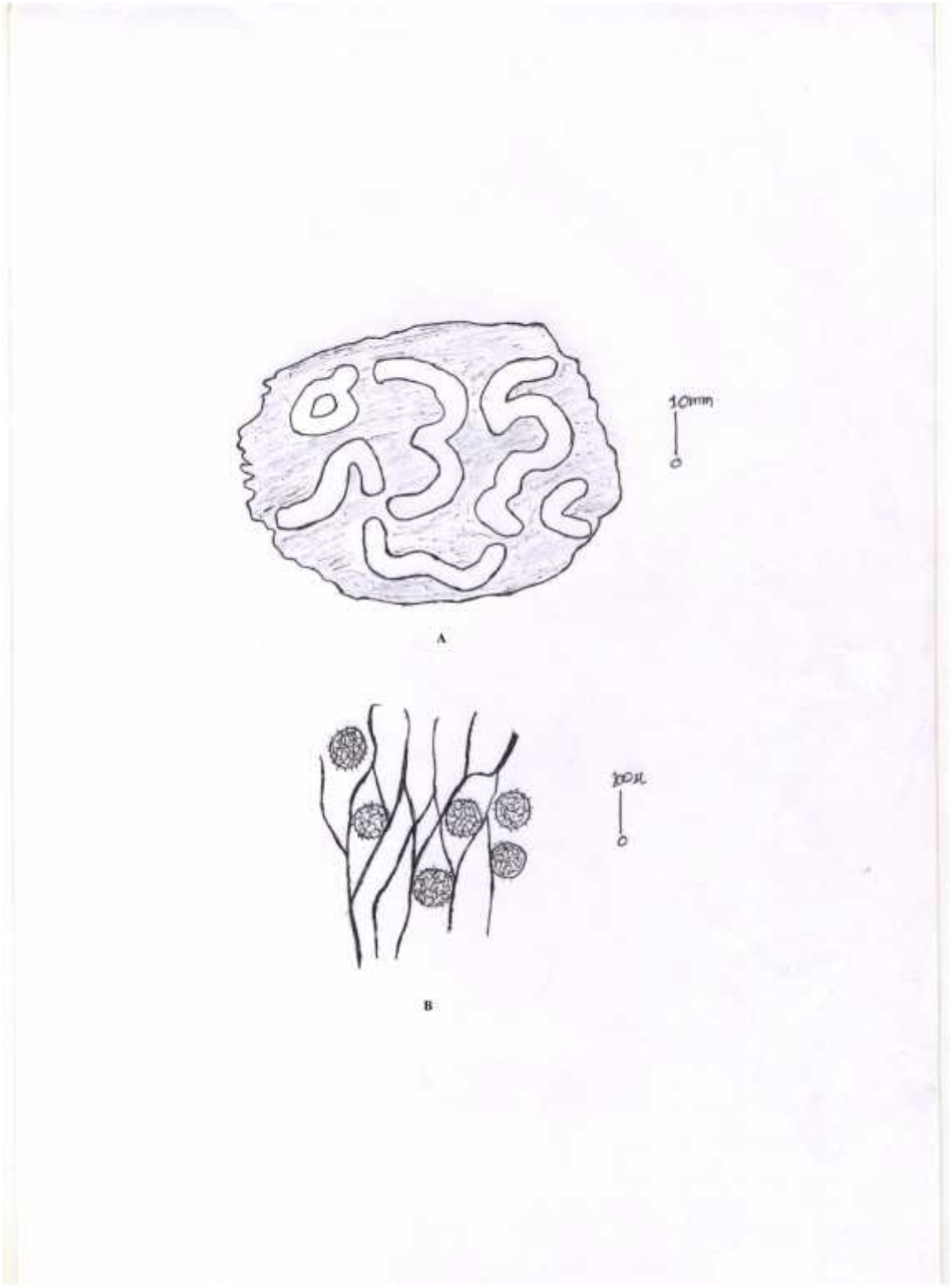

Figure: 15. *Didymium flexuosum* A) In natural habitat. B) Single fruiting body . C) Arrangement of capillitium with spores

CHAPTER SIX

RESULTS AND DISCUSSION

6.1 Results

Several field trips to collect the specimens were made during the work period. The specimens were mostly collected from September to December, the study area being chiefly Godavari and Shivapuri at an altitude ranging from 1300 m to 2732 m. Most of the species were found in moist soil, bark of tree and decayed leaf. They were of different colors but in some of them, released dust when it is touched. Best effort and endeavor has been put to identify the myxomycetes. The identification has been based on "*The Myxomycetes of Japan*" Emoto (1977) and "*How to Know the True Slime Molds"* Farr (1981).

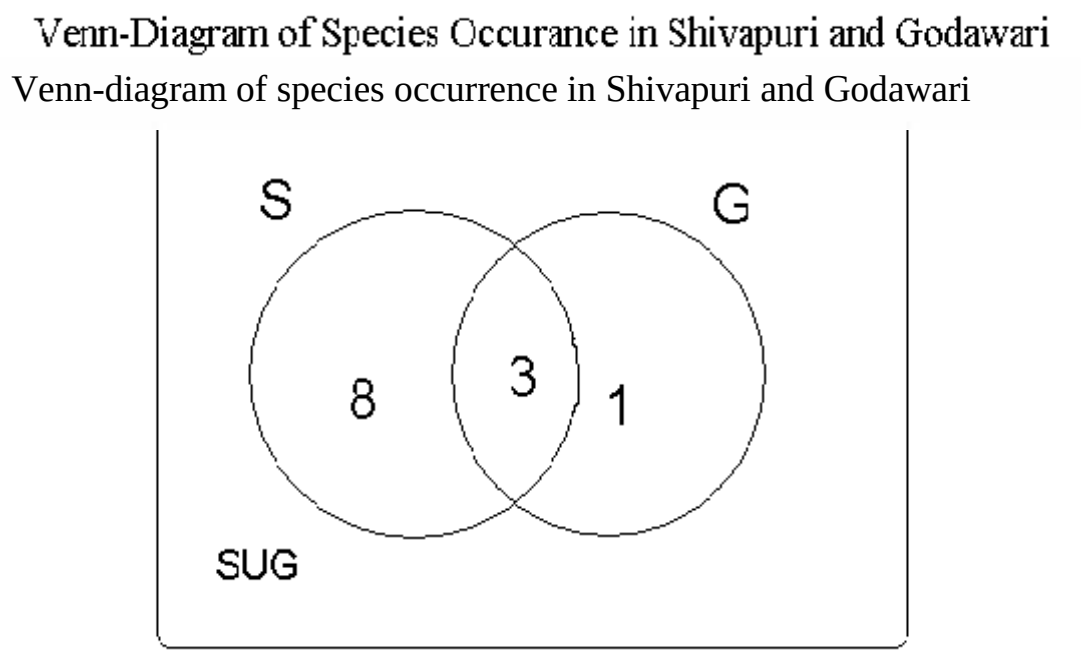

Figure: 16. Venn-diagram of identified species occurrence in Shivapuri and Godawari studied areas.

Table: 1 Summary table for identified species of Myxomycetes.

SN	Call No.	Location	Host	Colour	Fungi
1	01	Sivapuri	rotten wood/on the bark of living tree	dull yellow	*Arcyria pomiformis* (Leers) Rostoginski
2	04	Godawari	rotten bark	black	*Stemonitis cinerea* (Bull.) J.F. Grnel
3	05	Sivapuri	rotten wood, fallen twig	pink or red	*Arcyria incarnata* Persoon
4	07	Godawari, Sivapuri	fallen leaves	white	*Didymium flexunosum* Yamashiro.
5	10	Sivapuri	rotten wood, rotten fruit	yellow	*Physarum viride* (Bull) Pers
6	11	Sivapuri, Godawari	rotten wood	yellow	*Hemitrichia serpula* (Seop) Rost
7	15	Sivapuri, Godawari	rotten wood	purple brown	*Tubifera microsperma* (Berk & M.A. Curtis) G.W. Martin
8	16	Sivapuri	rotten wood/bark	greyish	*Arcyria cineria* (Bulliard) Porsoon
9	20	Sivapuri	soil	white	*Ceratiomyxa fruiticulosa* (Muller) Macbfide
10	27	Sivapuri	fallen leaves	white	*Fuligo cineraea* (Shw.) Morgan
11	32	Sivapuri	fallen leaves	Grayish white	*Mucilago crustacea* Wiggers
12	35	Sivapuri	fallen leaves	white	*Didymium iridis* (Ditmar) Fries

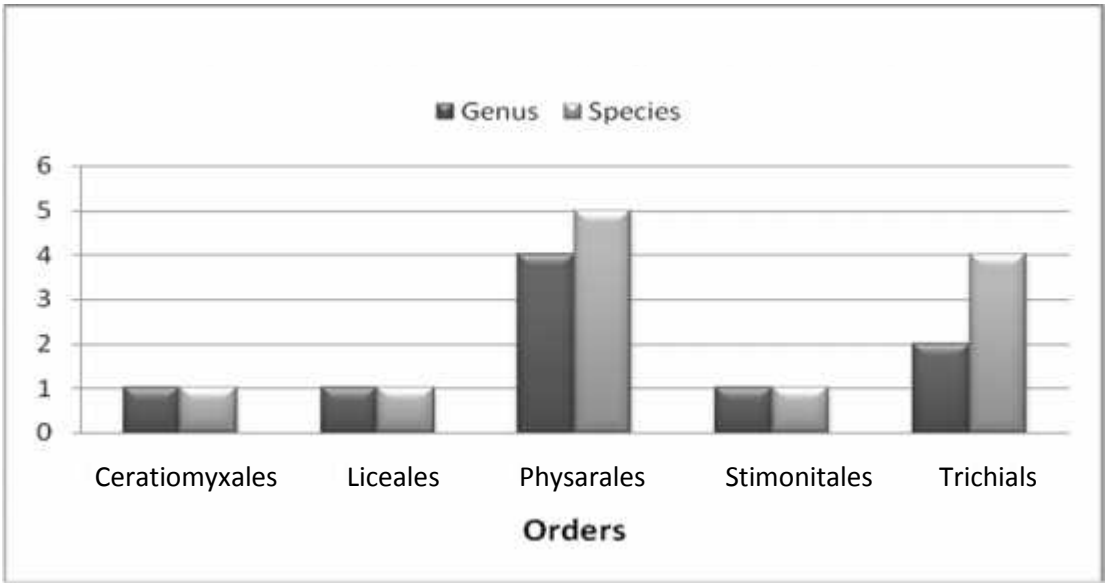

Figure: 17. Number of genus and species occurance in different orders.

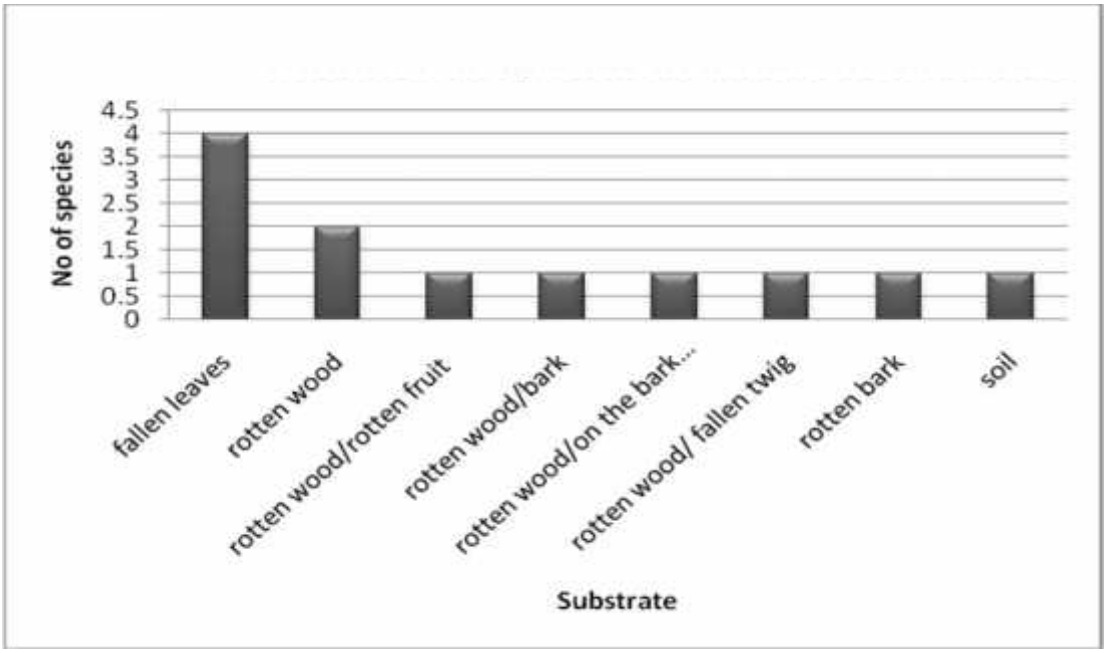

Figure: 18. Number of species occurance on different substrates.

6.2 Discussion

Nepal is a mountainous country with diverse microflora due to variation in climate and topography. The topography is too much rugged in nature due to varying altitude between 56 m (flat land to the south) and 8848 m (Himalayan belt in the north). The climate of study areas warm humid in summer and cool dry in winter. These complex geomorphology, altitude and climate have flourished the country with diverse interesting flora from north to south and east to west. The phytogeographic elements in turn have made the country a treasure house for luxuriant growth of tremendous amount of fungi (Adhikari 1990). The slime molds live in cool, shady, moist places on decaying wood leaves or other organic matter retaining abundant moisture. Bark mulch in a flower garden or shrub bed certainly fits that description. The same type of organism is often seen in the woods on decaying logs. Slime molds feed on decaying organic matter, bacteria protozoa and other minute organism which it engulfs and digests.

Godavari and Shivapuri (Kathmandu valley) were chosen as the collection sites because high humidity and moderate temperature was found to be favorable for the maturity of fungal fruiting body. There was the most dark, moist and humus rich soil which is suitable for the Myxomycetes. The moist and protected forest with luxuriant logs and litters enhanced the diversity of Myxomycetes in both study areas.

The months from September to December was found to be most favorable for development of the fungus along with sexual and asexual stages. The collection of fungi other than the mentioned month is futile because during the rainy season the fruiting bodies get washed away and the extreme cold of the winter development does not take place. The specimens were collected on different months and time because it was observed that various fungi have different maturing time. Therefore collection of fungi in different trips was found to be fruitful. The numerous trips to the field were undertaken in each month during favorable season. The Myxomycetes are very delicate so it was difficult to preserve and collection of Myxomycetes along with fully developed fruiting bodies was not easy job. Thus collection was carried out in two seasons (2 years). Therefore, working period was prolonged from 2007-2008.

The collection of Myxomycetes at Godavari and Shivapuri was done in many trips. During the collection 35 specimens of Myxomycetes were collected and out of them only 12 species

of 9 genera and 5 orders were identified due to lack of literature and identification tools for lower fungi. In particular, the taxonomy of rest of unidentified fungi remained incomplete due to lack of references and keys.

From the total collected specimens, most diverse species out of identified species were *Arcyria*, which were 3 in number. It was followed by *Didymium* which was two in number. Similarly, one-one species of *Ceratiomyxa, Mucilago, Physarum, Hemitrichia, Stemonitis, Tubifera* and *Fuligo* were reported.

Arcyria pomiformis (1), *Archyria incarnata* (5) and *Arcyria cineria* (16) were abundantly found in Shivpuri. *Didymium flexunosum* (7), *Hemitrichia serpula* (11) and *Mucilago furruginosa* (15) were found in Shivapuri and Godawari where as *Didymium flexuosum* (7), *Cerxtiomyxa, Fruiticulosa* (20), *Fuligo cinearea* (27) *Physarium viride* (10) and *Mucilago crustacea* (32) were found only in Shivpuri. *Stemonitis cinearea* (4) only in Godawari. Adhikari and Manandhar (1996) also reported these species in fungi of Nepal.

Where as previous record was found as follows:

Acytostelium sp.

Cribraria cancellata (Batsch) Nann.- Brem.

C *microcarpa* (Schrad.) Pers.

Badhamia affinis Rostafinski

Diderma hemisphaericum (Bull.) Hornem.

Didymium iridis (Ditmar) Fr.

Physarella oblonga (Berk et Curt.) Morgan

Physarum nutans Pers.

P. ovisporum G. Lister

P. wingatense Macbride

Clastoderma pulchella (Babington et Berk.) Rost.

Clasto derma pulchella (Babington et Berk.)

Stemonitis flavogenita Jahn

S. fusca Roth.

S. herbatica Peck

S. laxifila Nann.

S. splendens Rost.

Stemonitis sp.

Arcyria cinerea (Bull.) Pers.

A. denudate (L.) Wettst.

A. ferruginea Sauter.

A. incaranta Pers.

A. insignis Kalch et Cooke

A. nutans (Bull.) Grev.

A. oerstedtii Rost.

Hemitrichia serpula (Scopoli) Rost.

Mucilogo sp.

CHAPTER SEVEN

CONCLUSIONS AND RECOMMENDATION

7.1 Conclusion

From the present research work following conclusions can be drawn.

- Altogether there were 35 specimens collected in two seasons (September-December of 2007 and 2008) in which 12 species of 9 different genera of 5 orders were identified.
- *Mucilago crustacea,* on fallen leaves, which was found at Shivapuri, might be new taxa for Nepal.
- Three species of *Arcyria* were reported only from Shivapuri area.
- Most of the Myxomycetes were found in rotten wood, leaves and bark where as some were in soil as well as in living plants.

7.2 Recommendations

- Although, the basic work for all sorts of investigations in applied mycology is study of taxonomy, but the exploration of Myxomycetes in comparison of other higher fungi was found in less priority. Therefore, priority of exploration of lower fungi should be given.
- The priority on cytological and genetic investigations of mycoflora should be given.

7.3 Further research

- Identification of rest of collected species are still to be done.
- Soil Myxomycetes of Kathmandu valley can be studied in different seasons.
- Exploration of Myxomycetes around Kathmandu valley in contest to seasonal variation could be done.
- *In-vivo* and *in-vitro* study of lifecycle could be done.
- Comparative study of pathogenic and non pathogenic Myxomycetes could be done.

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ANNEX I

Five years (2003-20080) average min-max temperature and rainfall of Kakani station.

	Temperature		
Months	**Mean max**	**Mean min**	**Rainfall**
January	13.4	4.3	0
February	12.7	4.6	94.8
March	18.1	8.2	50.2
April	22.5	13.2	65.8
May	23.4	15.1	144.2
Jun	22.9	16	547.6
July	22.1	16.7	592.3
August	23.5	16.2	697.8
September	22.1	15.1	566.7
October	21.1	12.5	105
November	17.9	8.8	7.8
December	15.5	5.1	0

Average annual rainfall (mm):- 2872.2

Monsoon rainfall (mm):- 2404.4

ANNEX II

Five years (2003-2008) average min-max temperature and rainfall of Godawari station.

Temperature

Months	**Mean max**	**Mean min**	**Rainfall**
January	16.3	2.8	0.0
February	16.1	4.8	94.3
March	21.3	7.7	48.4
April	25.7	12.8	78.7
May	27.1	15.4	169.2
Jun	26.8	17.7	221.3
July	25.3	18.9	398.2
August	25.6	18.8	386.5
September	26.0	17.5	491.5
October	23.7	13.3	45.3
November	20.2	7.6	0.0
December	17.8	3.4	0.0

Average annual rainfall (mm):- 1933.4

Monsoon rainfall (mm):- 1497.5

PHOTO PLATES

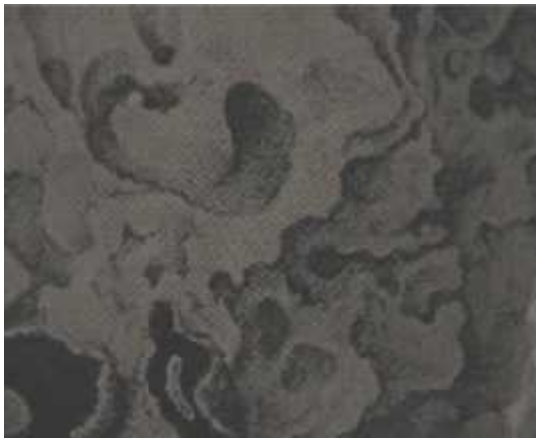

Photo: 1. *Mucilago crustacean*

Photo: 2. Internal structure of *Mucilago crustacea*

Photo: 3. *Fuligo cineraea*

Photo: 4. Internal structure of *Fuligo cineraea*

Photo: 5. *Fuligo cineraea*

Photo: 6. *Fuligo cineraea*

Photo: 7. *Arcyria pomiformi.*

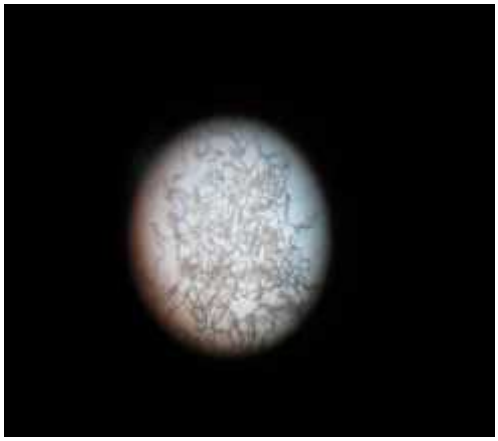

Photo: 8. Internal structure of *Arcyria pomiformis*

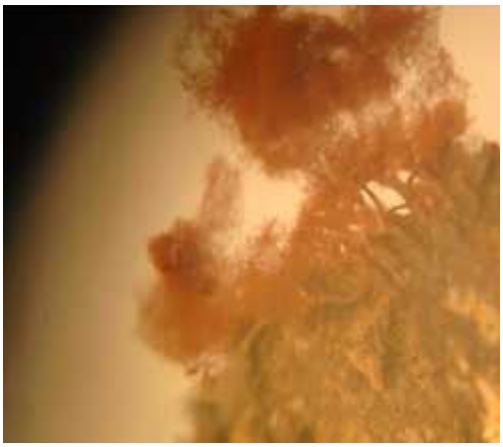

Photo: 9. Arcyria incarnata

Photo: 10. Internal structure of *Arcyria oerstedtii*

Photo: 11. *Arcyria cinerea*

Photo: 12. Internal structure of *Arcyria cinerea*

Photo: 13. *Stemonitis cineraea*

Photo: 14. *Stemonitis cineraea*

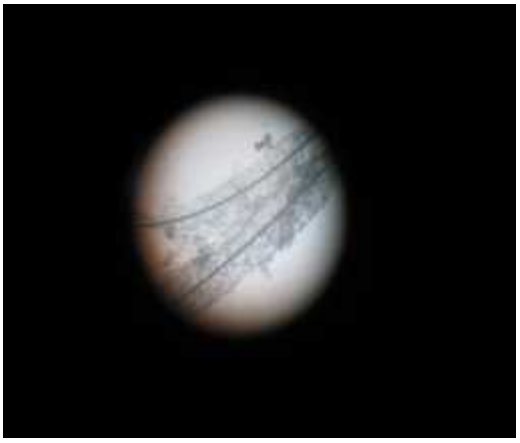

Photo: 15. *Stemonitis cineraea*

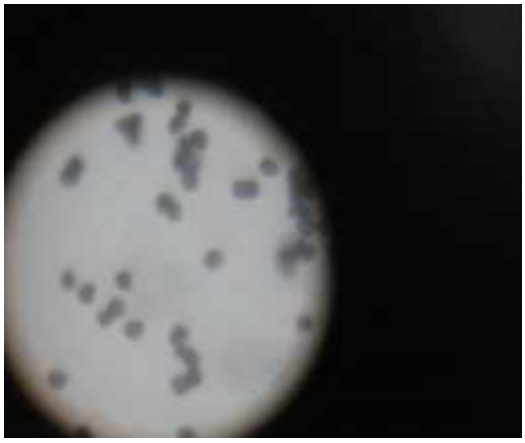

Photo: 16. *Physarum viride*

Photo: 17. *Physarum viride* (Mature)

Photo: 18. *Physarum viride* in natural habitat

Photo: 19. *Hemitrichia serpula*

Photo: 20. *Hemitrichia serpula*

Photo: 21. *Ceratiomyxa fruiticulosa*

Photo: 22. *Didymium iridis*

Photo: 23. *Didymium* flexuosum.

Photo: 24. *Tubifera microsperma*