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# Myxomycetes: The forgotten Fungi like living organisms from India

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### ABSTRACT

*Myxomycetes* represent fungi like organisms which are commonly known as slime molds. Earlier these were included in fungi, but now are included in kingdom Protista. Around 2000 species are reported from all over the world and India has a record of around 200 species, indicating need for further exploration of different ecological niches and habitats. This contribution reviews general account of myxomycetes diversity spectrum, methodology, habitat relationship, ecology and economic importance. Further it adds some information on myxomycetes collected on different substrates in some forest localities of Telangana state which form new additions to this region.

Keywords: Diversity, ecology, economic importance, fossil, habitat, Myxomycetes.

### DEDICATION AND BRIEF BIOGRAPHIC SKETCH OF PROFESSOR K. S. THIND

This manuscript is dedicated to the fond memory of Late Prof. Kartar Singh Thind, (K. S. Thind) (October 30, 1917- December 03, 1991), who was a scientist of repute in botanical sciences in India. He was born on in a Thind (Kamboj) family of village Saidpur, Tehsil Sultanpur Lodhi, District Kapurthala, Panjab, India. An outstanding scientist and educationist, Dr. Thind was M.Sc, Ph.D, F.N.A.Sc, F.N.A. He did his Doctorate in Plant Pathology from the Wisconsin University, USA. Dr. Thind was professor and the Head of the Department of Botany, Panjab University, Chandigarh. He has authored or co-authored numerous research articles on Phytopathology which were published from 1957 till his death (1991) in numerous important national and international science journals like Indian Council of Agricultural Research; Journal of the Indian Botanical Society; Indian Phytopathology; Scientific Research Bulletin of the Panjab University ; Mycologia; Indian Journal of Mycology and Plant Pathology; Transactions of the British Mycological Society;American Journal of Botany; Proceedings of the Indian Academy of Sciences; Kavaka; Bot. Notiser; Czech Mycol; Bull. Natn. Sci. Mus., Tokyo, etc. His name was included among the select Phytopathological Scientists of the world. His field of Specialization was Mycology and Phytopathology. He authored several books including well known Physiology of Fungi which is recognized as authoritative in the field of Phytopathology. He participated in numerous national and international conferences and seminars on Botanical science and read his research papers which were appreciated through out the world. Dr .Thind was Fellow of Indian Academy of Sciences and Member of Indian Phytopathological Society besides many other associations. In 1979, he received PANCHANAN MAHESHWARI MEDAL of the Indian Botanical Society. He served as President of IPS, IBS, etc. Late Prof. K.S. Thind did monumental work on Aphyllophorales of India which received international recognition. His work on myxomycetes in 1977 had been source of inspiration for young workers in mycology.

### **INTRODUCTION**

Myxomycota are referred to as slime molds. These are presently classified in the Kingdom Protista. Slime molds were thought to be fungi (=Kingdom Mycetae) as they produce spores in sporangia, a characteristic feature of some fungi. The assimilative stage in this organism is similar to that of an amoeba, called myxamoeba. The myxamoeba is a uninucleate, haploid cell not enclosed by a rigid cell wall. It ingests its food by means of phagocytosis. In this process of ingestion, the food particles, usually bacteria become surrounded by the pseudopodia of the myxamoeba then get engulfed. They are surrounded by a membrane or food vacuole. The hydrolytic enzymes that are secreted digest the food. The assimilative stages in fungi are mycelium and single cell, both of which are surrounded by a rigid cell wall and obtain their food by means of absorption for these reasons the mycologists recognized slime molds as separate group. However, this group has been studied in mycology as a matter of tradition and not because they are thought to be related to true fungi.

#### **MYXOMYCETES AND THEIR LIFE CYCLE**

Approximately 2000 species are reported and all are found on moist soil, decaying wood, and dung. Most of the species are found throughout the world. *Physarum polycephalum* and *Didymium iridis* are being used as examples to represent the myxomycete life cycle. These are selected since much literature exists on these species and their life cycles are well known.

The spores of slime molds are globose, uninucelate, haploid and spore surface may be smooth, spiny to reticulate. Spores of *Physarum polycephalum* and *Didymium iridis* are spiny. The spore wall is made up of cellulose and is only one of two stages where a cell wall is formed. The other stage that forms a cell wall is the microcyst, on germination; the spore breaks open and releases a single, uninucleate myxamoeba, which moves by amoeboid motion and ingest food, through phagocytosis. Later it feeds and grows, reproduces asexually by mitosis and cytokinesis. This stage may proliferate for any lengths of time provided nutrients are available and the environment is favorable in the presence of free water. Myxamoeba get differentiated into flagellated swarm cells. Although two flagella are present, one of them is long, anteriorly directed and the second one is very short. During unfavorable conditions the protoplast of the myxamoeba or swarm cell can form microcyst, which offers protection.

- 1. Typical spore of myxomycetous organisms is a haploid, globose, uninucelate structure. In the case of *Physarum polycehalum*, the surface is spiny.
- 2. Spore germination occurs by the cracking of the spore wall and releasing a single myxamoeba in *P. polycephalum*.
- 3. Myxamoeba is usually the assimilative stages that ingest food by phagocytosis, but during sexual reproduction myxamoeba also function as gametes (isogametes).
- 4. When free water is available, myxamoeba can become flagellated and swim through the water.
- 5. During conditions unfavorable for myxamoeba growth the cells may round up and form the resistant microcyst stage.

After some time, when a critical number of swarm cells or myxamoebae are formed, sexual reproduction occurs and these vegetative stages function as gametes. In P. polycephalum, the swarm cells act as gametes and are derived from a common population of myxamoebae (the different mating strains are designated as a1, a2, a3 undergo syngamy. Once the compatible strains come into contact with one another and fusion occurs to form the zygote and then undergo numerous mitotic divisions to form the large, multinucleate plasmodium. This is referred to as the cellular slime mold because the plasmodium state of the life cycle is not composed of many cells. It is a single, multinucleate cell and there is also an assimilative state that consumes food by phagocytosis. However, the plasmodium is a diploid structure and is much larger. In *P. polycephalum*, it is a bright yellow, slimy structure while in *Didymium* iridis the plasmodium is colorless. Under unfavorable conditions, the plasmodium forms a protective, brittle layer and become dormant. This dormant stage is termed a sclerotium and is composed of smaller multinucleate cells called macrocysts under favorable conditions. Each macrocyst can give rise to a new plasmodium.

- 1. The *Physarum polycephalum* has a bright yellow plasmodium. The plasmodium results from syngamy of two compatible, myxamoebae, followed by numerous mitotic divisions.
- 2. Plasmodium of *Didymium irdis* is colorless.
- 3. When conditions become unfavorable, a plasmodium can become dormant; forming a resistant stage that is darker, yellowish-orange colored called sclerotium.
- 4. Sclerotium is actually composed of smaller units called macrocysts. The number of nuclei in each macrocyst is variable.

The plasmodium migrates and feeds for a period of time before being converted to numerous sporangia. In *Physarum polycephalum*, the exhaustion of food leads to formation of sporangia. The plasmodium stage persists for some time. Light appears to be another stimulus to fruiting in this species.

The sporangium in *Didymium iridis*, is light blue, globose, produced on a yellowish stripe while in *Physarum polycephalum* the sporangium is dark gray to almost black, lobed and is produced on a yellowish stipe. The fragile, outer layer of the sporangium is the peridium, which may be persistent or degenerate by the time the sporangium is ready to disperse its spores.

In *Didymium iridis*, the light blue powdery appearance of sporangium is due to calcium carbonate crystal present on the peridium surface.

*Physarum polycephalum* sporangium has numerous lobes. Calcium carbonate is also present on the peridium surface of this species, but is not obvious.

During formation of sporangium, the plasmodium becomes denser and forms a thick sheet called the hypothallus. The protoplasm of the plasmodium then becomes knotted into discrete nodules, representing sporangial primordia. The nodules elongate and as development continues the basal portion becomes the stalk, decreases in diameter while the upper portion becomes the sporangium proper and develops the finger-like projection characteristic of *P. polycephalum*. In *Didymium iridis*, the sporangium would become a single, globose sac.

On the completion of movement of protoplasm into the sporangium, the stalk becomes more constricted and is without protoplasm. Spore formation along with the formation of cell walls takes place around the diploid nuclei, the nucleus in each spore undergoes meiosis to produce four haploid nuclei, and of which three degenerate. Only one myxamoebae results from each spore. The interior of the sporangium is of branched, thread like capillitium. The capillitium arises from coalescence of vacuoles, which contain various materials from the protoplasm, especially calcium carbonate (CaCO<sub>3</sub>), in *Physarum polycephalum* and related species.

However, there are variations in sporangial types and structures. *Diachea leucopodia* is another species with sporangia that have stipes. *Trichia favoginea* is a species that produces sessile sporangia. *Lycogala epidendrum* is the example of a species that produces aethalia. An aethalium resembles a sessile sporangium but is much larger. The larger size is thought to have evolved from many smaller sporangia that have fused. *Fuligo septica* also produces aethalia, they are reported to be the largest known aethalium.

*Hemitrichia serpula* produces plasmodiocarps. The spores and capillitium of this sporangium type retains the shape of the plasmodial stage. *Stemonitis* sp. is the example of a stipitate sporangium in which the peridium disintegrates at maturity thereby leaving the capillitium and spores exposed. This species also differs in that stipe development continues with in the sporangium. The extension of the stipe is the

### columella.

Capillitium (pl.=capillitia) are filamentous structures that usually develop with sporangia. They are thought to function in the retention of spores in the sporangia thus allowing gradual dispersal of spores over a long period of time. Some spores are always retained that are dispersed later, possibly during favorable period. Capillitia are often ornamented and have been used in defining some taxa in *Myxomycetes*.

### **DIVERSITY SPECTRUM**

Myxomycetes represent a small group of eukaryotic organisms encompassing more than 60 genera and around 2000 species distributed in different parts of the world (Keller and Everhart, 2008). From India the estimate being 500 species representing 60 genera. Many mycologists from India have attempted to describe myxomycetes (Agnihothrudu, 1956, 1958, 1959, 1961, 1968; Bhide et al., 1987; Bilgrami et al., 1979; Butler and Bisby revised by Vasudeva, 1960; Dhillon, 1976, 1977a, b; Ghosh and Datta, 1962a, b, c, 1963; Gilbert, 1928; Indira, 1968, a, b, 1975; Kar, 1964; Lakhanpal and Mukerji, 1981; Manoharachary et al., 2012; Manoharachary and Rajithasri, 2015; Mukerji and Juneja, 1975; Mundkar, 1938; Nanir, 1979; Pathak and Ghosh, 1962; Patwardhan and Joshi, 1975; Ramakrishnan and Subramanian, 1952; Ranade, 1978; Ranade et al., 2012; Rangaswami et al., 1970; Sarbhoy et al., 1975, 1980; Sekhon, 1978, 1979; Singh and Puspavathy, 1965; Tembhurne and Nanir, 2011a, b; Thind and Rehill, 1957; Thind and Sehgal, 1960, 1964; Thind and Manocha, 1963; Thind, 1977; Tilak and Rao, 1968).

Check list of myxomycetous organisms from India published by Ranade *et al.* (2012) clearly indicates that the myxomycetes have not been reported from Arunachal Pradesh, Meghalaya, Nagaland, Manipur, Tripura, Mizoram, Telangana and Andhra Pradesh. They have also provided information of 373 species belonging to myxomycetes. Recently Manoharachary *et al.* (2012) and Manoharachary and Rajithasri (2015) have reported 35 myxomycetes from Andhra Pradesh and Telangana states of India. In the present paper around 30 myxomycetes which form new additions to Telangana state are given in **Table 1**. All the above studies indicate that there is a hidden wealth of myxomycetes colonizing diversified habitats in India and there is a need to explore this group to enrich our biodiversity status.

# METHODOLOGY

Slime molds can be collected from dead and decaying leaves, plant parts like twigs, dead wood, bark, litter, etc. Meticulous care has to be taken during transportation from field to laboratory. The collected specimens have to be preserved in small vials of 2.5 to 4 cm and have to be placed in card board boxes. Later samples are dried and treated with potassium chloride for dehydration, keeping them in desiccators (Davis, 1965). Hoyer's medium (Distilled water 50 ml, Arabic gum 30mg, Chloral hydrate 200mg, Glycerin 20 gr.).

The collected material has to be classified as per sporangial type (aethalium, pseudoaethalium, plasmodiocarp, sporangium, stipitate or sessile) followed by spore colour,

Sr. No.	Species	Habitat	Place	Accession No.
1.	Ceratomyxa fruticulosa (Mull.) Maubr	Wood	Bhadrachalam	OUFH 900
2.	Ceratomyxa hydnoides (Jaco.) D.Kurtz	Bark	Bhadrachalam	OUFH 901
3.	Enteridium lycoperdon (Mull.) Farr.	Wood	Kothagudem	OUFH 902
4.	Lycogala epidendrum (L.) Fries	Wood	Mannanure	OUFH 903
5.	Cribraria atrofusca Martin & love	Fallen	Narsapur	OUFH 904
	Joy	leaves	-	
6.	Cribraria aurantiaca Schrad	Litter	Narsapur	OUFH 905
7.	Cribraria elegans Berk. & Curt.	Wood	Narsapur	OUFH 906
8.	Dictydium cancellatum (Batsch) Machr.	wood	Anathagiri Hills	OUFH 907
9.	Arcyria cinera (Bull.) Pers.	Wood	Nizamabad	OUFH 908
10.	Arcyria glauca A. Lister	Wood	Nizamabad	OUFH 909
11.	Arcyria incarnate (Pers.) Pers.	wood	Hyderabad	OUFH 910
12.	Arcyra telocarpa (Cooke) Martin & Alexopoulos	Dead wood	Hyderabad	OUFH 911
13.	Hemitrichia calyculata (Speg.) Farr.	Dead wood	Hyderabad	OUFH 912
14.	Hemitrichia clavata (Pers.) Rost.	Wood	Bhadrachalam	OUFH 913
15.	Trichia botrytis (J.F.Gmel) Pers.	Dead wood	Ananthagiri	OUFH 914
16.	Physarum melleum (Berk & Br.) Mosses	Wood	Pakala	OUFH 915
17.	Physarum pezizoideum (Jungh.) Rev.e Lag.	On a fungi	Kothagudem	OUFH 916
18.	Physarella oblonga Berk. & Cart.	Dead wood	Kothagudem	OUFH 917
19.	Diachea leucopodia (Bull.) Rost.	Wood	Yellandu	OUFH 918
20.	Diderma effusum (Schw.) Morgw	Bark	Yellandu	OUFH 919
21.	Didymium floccosum Martin, Thind & Rchill	Dried leaves	Kothagudem	OUFH 920
22.	Comatricha elegana (Racib). G.Lister	Wood	Achampeta	OUFH 921
23.	Comatricha laxa Rost.	Bark	Mahaboobnagar	OUFH 922
24.	Comatricha subceesopitosa Peck	Wood	Nalgonda	OUFH 923
25.	Stemonitis oxifera (Bull.) Macbr.	Wood	Adilabad	OUFH 924
26.	Stemonitis fusca Rath.	Bark	Adilabad	OUFH 925
27.	Stemonitis mussooriensis Martin	Wood	Ananthagiri	OUFH 926
28.	Stemonitis smithii Macbr.	Bark	Nizamabad	OUFH 927
29.	Stemonitis uvifera Macbr	Wood	Mahabubnagar	OUFH 928
30.	Stemonitis virgiiensis Rw.	Dead wood	Mahabubnagar	OUFH 929
Note: OUFH= Osmania University Fungal Herbarium.				

Table 1. List of Myxomycetes collected from Telangana

presence and distribution of lime and structure cum presence or absence of capillititum. Mount the sporangium in a drop of water and observe for peridium type, capillation structure, colour and also presence or absence of columella. The presence of line can be evaluated by watching for effervescence after adding a drop of 1% HCl. Morphotaxonomic characters of myxomycete specimen and their identification can be done using Lister (1924) and Martin and Alexopoulos (1969). Spore germination has to be observed in a watch glass containing 0.5 ml sterile tween 80 solution (1:1000). Separate spores after ten minutes and 2ml of 2% carrot decoction is added. Spore germination is observed by hanging drop technique in moist chamber at 25°C. After an interval of 24 hours spore germination and swarm cells have to be observed. Carrot agar, oat agar and water agar are used to grow some myxomycetes. All cultures have to be maintained at 25+2°C in humid chamber and under light intensity of 5 lux stock cultures are to be maintained in 250 ml Erlenmeyer flask having carrot or oat medium. Duplicate cultures are to be maintained in test tube slants. Revival and storage is done by drying cultures on filter paper, recovering them from stored dry specimen or from fruit body. Plasmodia require warmer environment besides temperature, relative humidity, moisture, pH and other. (Camp, 1936; Carlile, 1971; Howard, 1931; Venkatramani et al., 1977).

### HABITAT OR SUBSTRATE RELATIONSHIP

Myxomycetous organisms are adapted to many ecological

niches. Coprophilous species grow on dung and are isolated through moist chamber technique. Several species are recorded on droppings from birds. Around 114 species are reported on coprophilous habitat and the examples include *Licea alexopouli*, *Kelleromyces fimicola* and *Trichia brunnea* (Uno Ellason, 2013). Stephenson *et al.* (1993) and Stephenson and Stempen (1994) have studied myxomycetes associated with plant parts, coffee plantation and woody plants of some forests in south India.

Ground sites attract many myxomycetes and substrates are more diversified. Myxomycetous organisms like Arcyria versicolor Phill. on decaying tea twigs, Badhamia capsulifera (Bull.) Berk. on living mosses, Ceratomyxa fruticulosa (Muller) Macbr. on decaying wood, Comatricha typhoides (Bull.) Rostaf. on rotting bark, Lycogala conicum Pers. on dead wood, Diderma alpinum (Meyl.) Meyl. on grass leaves, Didymium delhianum Lakhanpal & Mukerji, Physcorum diderma Rostaf. on bark, Reticularia splendens Morgan on bark. Stemonitis webberi Rex. on dead wood and many other species have been reported to colonize different plant parts. Aquatic habitats may also harbour different life cycle stage like swarm cell, amoeboid myxamoebae and plasmodia of Diderma difforme, Physarum cinereum, Fuligo cinerea and few others. Water film on the surface of bark, on decaying wood, leaves or submerged leaves form practical sites for myxomycete colonization (Tamayama and Keller, 2013).

**Ecology:** The ecology and seasonal occurrence have to be recorded regularly while studying myxomycetes. Everhart and Keller (2008) have stated that little is known about the phenology of myxomycetes. Further the researcher has to describe the habitat, seasonality of fruiting, survival state, fruiting morphology along with date of collection and meteorological factors / ecological factors prevailing. Myxomycetes occur mostly in large numbers on diversified habitats at altitudes above 500 msi, a temperature range between 15-20°C and a low (0-250 mm/month) or medium (250-500 mm) rainfall. These conditions favour the growth and sporulation. The calcareous species were more tolerant of the tropical climate than non calcareous ones. Calcareous species preferred dead leaves. pH seems to be a decisive factor.

**Fossil record:** Protozoa are known as preserved records in amber (Frederick and Corliss, 1990) but myxomycete preservation is rare. The fruiting bodies of *Stemonitis splendens* Rostaf. in Baltic amber has been reported in Tertiary and Eocene approximately 35 to 40 million years ago (Domke, 1952). Further *Arcyria sulcata* Dorfelt & Schmidt was reported from fossilized Baltic amber in early Tertiary. *Protophysarum balticum* Dorfelt & Schmidt was described as a new taxon in Baltic amber from the Tertiary without latin description besides the preservation of some plasmodial stages (Dorfelt and Schmidt, 2006, Waggoner and Poinar, 1993). Thus there is little or no information available on fossilized myxomycetes.

# TAXONOMY

*Myxomycetes* have to be classified as per sporangial type (Aethalium, Pseudoaethalium, Plasmodiocarp, Sporangium,



Note: All Figures (1, 2, 3, 4, 5 & 6) in 100X

- Figs No. 1. Arcyria cinerea (Bull.) Pers.
  - 2. Cribaria elegans Berk. & Curt.
  - 3. Dictydium cancellatum (Batsch) Machr
  - 4. Hemitrichia calyculata (Speg.) Farr.
  - 5. Physarum pezizoideum (Jungh.) Rev.e Lag.
  - 6. Trichia botrytis (J.F.Gmel) Pers. 100X

stipitate or sessile). Classification of higher taxa is based on spore colour, presence and distribution of lime, presence or absence of capillitium. The specialist has to note the colour, shape and other gross characters. Spore germination also forms a parameter in the identification of myxomycetes. Myxomycetous organisms are identified using monographs of Thind (1977) Lakhanpal and Mukerji (1981), Martin and Alexopoulos (1969), Lister (1925) and several taxonomic contributions. Ranade *et al.*, (2012) have presented a checklist of myxomycetous organisms from India. Classical taxonomy was based on morphotaxonomic criteria and the modern approach is based on DNA sequencing, DNA barcoding, etc. The molecular approaches and derived phylogenetic relationships will highlight taxonomic aspects of myxomycetes.

DNA analysis will pave the way for understanding population diversity and their differentiation. Species concept must include information on morphological differences, biogeographical patterns, habitat analysis and seasonal variation. Good monographic studies and taxonomic practices lay foundation for myxomycete systematics. All the above in-depth studies will help in understanding phylogeny, biogeographic patterns of distribution and restriction of species to habitats having specialized ecological characteristics when myxomycologists collaborate then the answer will be revealed to "what a myxomycete species is" (Keller and Everhart, 2008).

**Taxonomic practices- protocol:** Field collections and careful observation of substrates along with their seasonal occurrence, variations, quantitative occurrence and quality of collected material along with a record of environmental conditions form the important criteria. Formation of fruiting bodies and their observation will help in accurate identification of species. However multiple collections in

relation to multiple location will help in proper identification of taxa (Keller, 2012).

**Type specimens:** The literature on myxomycetes indicates that species new to science that lack sufficient collections and of specimens leads to many problems. Therefore type specimen selected by the author after observing many specimens is an important aspect which cannot be neglected. Therefore,

- 1. Prepare very good species description
- 2. Healthy type specimen with adequate fruiting bodies
- 3. The specimen has to serve to compare closely related species.

The selection and the study of the type specimen cannot be over emphasized. Melbourne nomenclature code of fungi has to be followed for describing myxomycete taxa.

# **IMPORTANCE OF MYXOMYCETES**

Myxomycetous organisms are considered as true slime molds and are currently classified as myxogastrids in the super class Amoebozoa. Myxomycetes are used in laboratory for teaching and experimentation. Slime molds are referred and portrayed in folklore literature and in the science fiction movies to depict the horror scare tales. Some species of these organisms are often found as weak plant pathogens also. But no penetration of host plant tissues and symptoms are observed hence cannot be considered as plant pathogens. Myxomycetes exhibit number of ecological survival strategies. Myxomycetes like Physarum and Plasmodia have been used in aging research. The plasmodium of Physarium polycephalum has been used in the space programmes by Americans, Germans and Russians. The first amorphous biological robot using the plasmodium of Physarum polycephalum as a motive source has been designed to operate the computer circuitry, directionally move light weight floating objects on water and perform computations. These are also used as human food source eg. Plasmodium of *Fuligo septica* and possibly help in biotic pollution indexing. These organisms are used in simple laboratory demonstration such as spore germination, spore release and culturing under moist chamber. The novel compounds isolated from fruiting bodies and plasmodia of different myxomycetes like Arcyriaflavin A and B Cribrarione B, Fuligoic acid, Lycogarubins, Polycephalin B and C, Pubiferal A and B exhibit biological activity that function as antibiotics, antimicrobials and cytotoxic compounds on cancer cells. Myxomycete spores are also aeroallergens causing rhinitis, asthma and other allergic diseases. Ground pollution may be remediated by myxomycetes fruiting bodies. They also concentrate highly toxic elements like Cadmium, etc. The environmental pollution decreases the myxomyctes species richness. Interestingly there are some myxomycetes like Diachea arboricola H. Keller & M. Skrabal that exhibits rainbow colours in the sporangial wall (Keller and Everhart, 2010).

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