# THE 8<sup>th</sup> INTERNATIONAL CONGRESS ON THE SYSTEMATICS AND ECOLOGY OF MYXOMYCETES

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Mycological Society of China

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# PREFACE

Dear attendees,

On behalf of the ICSEM8 organizing committee, it is my pleasure to invite you to this exciting congress which will be opened in Changchun, one of the most beautiful Chinese cities on August 12, 2014! It is the first time that the meeting will be held in Asia.

The Congress programme includes traditional plenary sessions, symposia, oral, poster and discussion sessions covering the most crucial topics in myxomycetology.

We have invited many international well-known scientists in slime molds research to introduce the frontier progress of slime molds, as well as their research achievements. I believe that all attendance's visions in slime molds field will be expanded by the valuable discernments and research experiences to be delivered by the speech experts during this conference. I also believe that the deep communications of all top scholars will surely make significant impact on the development of slime molds research in the world.

It will be a magnificent opportunity for us not only to share ideas and experiences but also to deepen the existing friendships among colleagues from all over the worlds.

I look forward to seeing you and wish you will have a wonderful time in Changchun!

Thank you!

Shouhua Feng, Professor, CAS member

SIGTA

Y. Li

Yu Li, Professor, CAE member Chairman of ICSEM8 Organizing Committee



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#### Keynote

# Resource Allocation and Morphogenesis During Fructification in Myxomycetes

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**Abstract:** Sporangial development in the Myxomycetes follows a standard pattern. After the sporangial initial has attained its full size, continuous loss of water leads to the formation of vacuole-like spaces within the protoplasmic mass.

In the Physaraceae, mostly excretion of unwanted material into these spaces gives rise to the capillitium. The stipe is formed by constriction of the outer membrane as the protoplasm moves up, and this space gets filled with refuse matter.

In the Didymiaceae and in the Stemonitales, material is secreted into the spaces the form the capillitium, the process being more refined in the Stemonitales. Thus a part of the resources would go into the making of the capillitium.

It is in the Trichiales that a considerable proportion of the resources is diverted towards the formation of the extensive, beautifully ornamented, hygroscopic capillitium and sometimes even the stipe, and the synthesis of various pigments. Considering the variety of these accessory structures, it seems worthwhile focussing attention on sporangial morphogenesis in this group.

Key words: Resource allocation; sporocarpdevelopment; trust area; Trichiales

#### Keynote



# More than 50 years with Myxomycetes (plasmodial slime molds): highlights and review

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**Abstract:**My first myxomycete collection of *Dictydium cancellatum* is described. Myxomycete morphospecies concepts are discussed and reference sources are given that suggest criteria and options for the recognition of species new to science. Taxonomic assessment of fruiting body variation is given for *Fuligo septica* and spore ornamentation for *Fuligo megaspora*. Variation of fruiting body characters are discussed for spore-to-spore agar cultures of *Badhamia rhytidosperma* and *B. spinisporum*. A suggested protocol for best taxonomic practice is provided that recognizes the impact of environmental parameters on the plasticity of fruiting body characters using *Cribraria intricata* and *Badhamia rugulosa* as examples. The importance is discussed for using type collections, scanning electron microscopy, and *Badhamia ovispora* as an example. Monographic publications are emphasized and examples given that include *Perichaena brevifila* and *P. reticulospora*. Recent publications that describe spore-to-spore agar cultures are given that include assessment of spore ornamentation and commentary on clustered versus free spores.

Key words: best taxonomic practice; Badhamia ovispora; B. rhytidosperma; B. rugulosa;

B. rpinisporum; Cribraria intricata; Fuligo septica; F. megaspora; Perichaena brevifila;
P. reticulospora,; moist chamber cultures; morphospecies concepts; species new to science;
spore-to-spore agar culture.



#### **Myxomycetes Researches in China**

Qi Wang , Yu Li

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**Abstract:** The study of slime mold has begun from the last century 30s and many books and papers including taxonomy, phylogeny and chemistry were published from 1990s in China by Chinese researchers. Myxomycetes and Dictyostelids were investigated throughout the country including northeast plains, southwest plateaus, southeast coastal areas, central areas of China, Hainan island and Taiwan. Forty-three new species of Myxomytetes were described in these years, and three new species, sixteen protocol species and twenty-one new records were found in Dictyostelids. Several books including *Flora Fungorum Sinicorum- Myxomycetes* (*1, II*), *Liceales in China, Trichiales in China* were also published from 2005 to 2007 in China. Until now about 400 species belonged to 6 orders, 11 families, 44 genera have been recorded with their morphological features and distributions. Among them sporophore structures of 178 species with scanning electron microscope were clarified. The book of Dictyostelids will be edited soon. Taxonomic and phylogenetic works of Myxomycetes and Dictyostelids based on various methods, such as clustering analyses with morphological characteristics, chemical compounds obtained by GC-MS and molecular phylogenetic analyses with ITS, SSU, EF1- $\alpha$ , 12S, 16S and COI partial sequences, have been continued in universities and institutes in China.

In addition, the life-cycles of many species were clarified, including *Physarella oblonga*, *P. gyrosum*, *P. tenerum*, *P. compressum*, *P. Septica*, *P. polycephalum*, *P. globuliferum*, *Didymium iridis*, *Arcyria cinerea*, in Myxomycetes and *Dictyostelium candidum*, *D. homostipes*, *D.* globosisporum, *D. magnum*, *D. tenuistipes* in Dictyostelids using improved methods of artificial lab-cultures. **Key words:** Myxomycetes; Dictyostelids; China

#### Keynote



#### Myxomycetology: A challenging and inspirational field

Yu Li

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**Abstract:** Myxomycetes are famous for their interesting life cycle which includes a multinucleate somatic phase known as a plasmodium and a reproductive phase that culminates in the reproduction of stationary sporophores containing walled spores, which makes them very different from all other organisms.

Slime molds have proved to be valuable experimental organisms, not only for mycologists, but also for geneticists, molecular biologists, cytologists, biochemists, biophysicists, and bioinformatists. They are model experimental organisms for studies of evolution, mitotic cycle, the structural physiology and movement of protoplasm, morphogenesis, aging, reproduction, and a variety of other questions that challenge scientists. Especially in 2010, single-celled slime mold was proved that they can construct networks of nutrient channeling tubes strikingly similar to the layout of the Japanese rail system, which was borrowed by the researchers to create a biology-inspired mathematical description of the network formation (Tero et al. 2010). In addition, slime mold was reported to have enviable capability of coping with an unknown dynamic environment and was being developed to control autonomous robots.

Most interestingly, slime molds are one of the few non-human farmers. They can carry bacteria to seed out new food populations, and also carry other non-food bacteria which inhibit the growth of non-farmer *Dictyostelium discoideum* clones that could exploit the farmers' crops. This is the first evidence of some form of cultivation beyond dispersing and seeding their food crops (Brock et al. 2013).

Slime mold are also an important model organism in the genome research. The comparative genomes analyses of *Dictyostelium* species showed that genes involved in primary metabolism, cytoskeletal functions and signal transduction were conserved, while genes involved in secondary metabolism, export, and signal perception underwent large differential gene family expansions. This most likely signifies involvement of the conserved set in core cell and developmental mechanisms, and of the diverged set in niche- and species-specific adaptations for defense and food, mate, and kin selection.

Slime molds are of some other economic importance. They are important node in the food web,



especially as food for insects. Their use as food apparently also extends to humans. About 100 natural compounds including fatty acid, amino acid, alkaloids, naphthoquinone, aromatic compounds, terpenoid, esters and their derivatives were found in slime molds. It shows that the slime molds may be new resources for natural medicinal products. Slime molds usually also produce beautiful and delicate sporophores which have attracted the attention of numerous photographers and artists.

On the other hand, slime mold can also cause trouble in agricultural production. They occur upon the substrates, frames and grounds in mushroom houses, which will decrease the quality and yield of mushrooms. They are also found on many living plants, such as tree nursery, strawberry, grasses, which influence the growth of these plants.

All things considered has caused so much focuses on slime molds and intrigued so many biologists over the years. It is definitely necessary to diverge Myxomycetology as a discipline from biology because slime mold cannot be studied by only following mycological or zoological methods due to their unique life cycle. Myxomycetology will greatly challenge all biologists' brain and will inspire biologists to exploit the mysterious fields to provide remarkable evidences for the evolution of organisms, the mechanisms of cancer cell movement, social dilemmas, biomedicine exploitation .

Key words: Slime molds; biology-inspired; robot control; genome; compound



# Myxomycetes and Protosteloid Amoebae in the Man and Biosphere Reserve at Yangambi (D. R. Congo)

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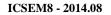
**Abstract:** In 2013 a field survey was undertaken in the Man and Biosphere Reserve at Yangambi (D. R. Congo, Africa) in the framework of the COBIMFO (Congo basin integrated monitoring for forest carbon mitigation and biodiversity) project, financed by the Belgian Science Policy. This reserve was deemed almost unexplored for myxomycetes, apart from 4 records located in the collections of the National Botanic Garden of Belgium, housed at the Botanic Garden Meise. These specimens, collected by J. Louis in 1938-1939 are unfortunately in a deteriorated state.

The fieldwork was performed between October 26 and November 19 in 2013. A total of 280 specimens and 15 photographic records of myxomycetes were taken inside 12 forest plots, 17 of these specimens were collected in various locations in the reserve and on its borders. In addition 63 samples were collected from 3 substrate types - aerial litter, ground litter and aerial bark - intended for agar and moist chamber cultures to record protosteloid amoebae and myxomycetes.

Preliminary results reveal about 100 species of myxomycetes identified from the field collections, as well as the moist chamber cultures and primary isolations plates. From two potential new taxa first recorded during the 2010 Congo River expedition, *Physarum* sp. collected on ground leaf litter and a *Licea*-like taxon that developed in the aerial litter cultures for protosteloid amoebae, ample material is now available to make a full to study. It seems that myxomycetes are most abundant on one particular substrate, fallen leaves of *Bellucia grossularioides*. This introduced tree, an endemic from the neo-tropics, is considered as a pioneer species in woodlands disturbed by human activities. It produces large leaves, up to 40 cm in length and 20 cm in width with a lateral curvature. The fallen leaves serve as large dome shaped moist chambers on the forest floor. Virtually all of the fruiting bodies collected in this particular micro-habitat developed on the underside of the leaves, well protected not only for the frequent heavy rainfall but also for the dehydration caused by sun exposure in between thunderstorms.

The results of the protosteloid amoebae survey are far from complete but indicate a typical species assemblage for tropical rainforests. With 80 % of the aerial litter samples processed, 21 species have been recorded.

Key words: Africa; D.R. Congo; myxomycetes; protosteloid amoebae; survey





# Comparative Diversity of Myxomycetes in Paleotropical (Philippines) and Temperate (USA) Forests

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Abstract: Tropical forests are generally reported to be characterized by higher diversity than temperate forests. Is this indeed the case for myxomycetes? In the present study, we compared the diversity of myxomycetes obtained from moist chamber cultures prepared aerial litter, forest floor litter and woody twigs collected from three lowland tropical forests in the Philippines and three mid-latitude temperate forests in north central Arkansas in the United States. Our results indicated that a higher value for taxonomic diversity was noted for temperate forests (SG ratio = 3.57) than for the tropical forests (SG ratio = 4.0). However, when comparing the different diversity indices, a higher value for species diversity was recorded for the Philippines ( $H_s = 1.43$ ) than for the United States ( $H_s = 1.38$ ), although this pattern contrasted with species richness (i.e.,  $H_G$  was 11.58 for temperate forests as opposed to 10.71 for tropical forests), albeit both types of forests had same value for evenness (E = 0.45). Among the substrata collected, the highest species diversity was noted for woody twigs, regardless of the forest type. A total of 111 species of myxomycetes were recorded in this study.

Key words: Biodiversity; slime molds; temperate myxomycetes; tropical myxomycetes



# Myxomycetes in Forest Patches on Ultramafic and Volcanic Soils: Assessment of Species Diversity and Heavy Metal Biosorption

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Abstract: Ultramafic and volcanic soils are exploited for industrial activities such as mining due to the high metal content of the soil. Thus, it is important that species in these areas are documented before irreversible environmental damage sets in. In this study, aerial and ground leaf litter, dead vines and twigs from forest patches on volcanic and ultramafic soils of Bataan, Pangasinan, and Zambales, Northern Philippines were placed in moist chambers and assessed for diversity and distribution of myxomycetes. From the 77% positive moist chambers for myxomycetes, a total of 33 species from 11 genera were identified. Interestingly, despite the higher heavy metal content, ultramafic forest patches had higher species diversity as compared to volcanic forest patches. Twigs from the ultramafic forest patches had also the highest number of species as compared to other substrates. In this study, eight species were abundant in both the ultramafic and volcanic forest patches, namely, Arcyria cinerea, Diachea leucopodia, Diderma effusum, D. hemisphaericum, Didymium ochroideum, Perichaena chrysosperma, P. corticalis, and Physarum melleum. Collected substrates, fruiting bodies, and plasmodia of selected myxomycetes tested for heavy metal were all positive for chromium and manganese. Interestingly, Cr and Mn contents of tested myxomycetes were equal or higher than that of its leaf substrate. This is the first study to compare diversity and quantify Cr and Mn biosorption of myxomycetes derived from forests on ultramafic and volcanic soils.

Key words: Chromium; manganese; heavy metal; slime molds; ultramafic soil; volcanic soil



# Looking at the Diversity of Myxomycetes in the Limestone Forests of Puerto Princesa Subterranean River National Park in Palawan, Southern Philippines

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Abstract: The Island of Palawan is considered as the last frontier of biodiversity in the Philippines. Its Puerto Princesa Subterranean River National Park has been recently awarded as one of the world's seven wonders of nature. This brings more tourists to witness its aesthetic value and enchanting beauty. However, ecotourism can also threaten an ecosystem. Documenting its biodiversity is therefore an urgent task. In this study, we assessed the diversity of myxomycetes in limestone forest habitats within Puerto Princesa Subterranean River National Park. A total of 740 moist chambers were prepared from aerial (AL) and ground (GL) leaf litter, dried inflorescences (IF), grass litter (GR), and woody twigs (TW) collected within the area. Seventy percent of the moist chambers yielded myxomycetes. Characterization of the collected specimens resulted in the identification of 33 species belonging to 16 genera and 5 taxonomic orders. Highest number of species was recorded among woody twigs (27 species) followed by aerial (21 species) and ground (15 species) leaf litter. The most abundant species included the cosmopolitan species Arcyria cinerea and Stemonitis fusca. Twenty eight species were recorded as rare. Comparing their diversity, highest species diversity ( $H_s$ =4.99) and species richness  $(H_G=0.44)$  was again noted in woody twigs. Our research study is the first extensive assessment of myxomycete diversity in the province of Palawan.

Key words: Diodiversity; karst forest; slime molds; tropical myxomycetes



#### More Additions to the Checklist of African Myxomycetes

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Abstract: During compilation of the first checklist of African myxomycetes in 2009, a number of myxomycetes records for some countries were not reported. These records were either in (i) literature not easily accessible; (ii) specimens identified but not reported or uploaded onto the Global Biodiversity Information Facility (GBIF) database or (iii) collections in museums that are not identified. To update the checklist of African myxomycetes, the first author visited Botanic Garden at Meise (Belgium) and together with the co-author scrutinized collections of myxomycetes that were collected in the Africa in the past. The herbarium of the Botanic Garden in Meise holds an important collection of myxomycetes obtained from Africa in last century and estimated to be 1094 specimens. A significant percentage of these collections belongs to Ghent University. During this study a total of 600 specimens were checked, names verified or identified. This work was carried out in December 2012 and August 2013. The first author's stay in Belgium was supported by Belgian National Focal Point to the Global Taxonomic Initiative. Our findings show that countries with significant number of specimens were Democratic Republic of the Congo, Rwanda and Burundi. Others countries with a substantial number were Zambia, Malawi and Morocco. Some collections were obtained in the early 1900s though most were collected between 1970 and 1990, primarily from field collections with only a few from the laboratory method of moisture chamber. Additional data will be included from recent field surveys in Kenya by the first author and in the Democratic Republic of the Congo by the co-author. Once compilation of species data is complete, it will be possible to (i) say with more certainty the number of species collected in each of the studied countries or territories in Africa, (ii) produce an updated checklist of African myxomycetes, and (iii) compile a monograph of African myxomycetes in the series of Fungus Flora of Tropical Africa published by the Botanic Garden Meise.

Key words: Africa; collections; checklist; distribution; myxomycetes; tropics



# Digitalization of the Types from the N.E. Nannenga-Bremekamp Myxomycetes Collection xx

Myriam De Haan, Ann Bogaerts

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Abstract: The myxomycetes collection of N.E. Nannenga-Bremekamp (1916-1996) was transferred by legacy to the Botanic Garden Meise (formerly known as the National Botanic Garden of Belgium) in 1997. This collection contains about 17.000 specimens from all parts of the world including 264 type specimens. Because of its importance this material is often used for scientific study and therefore send on loan to different institutes worldwide. During the transport specimens can be lost or damaged, and scientific studies mostly need destructive sampling which reduces the amount of material available, certainly when only a few fruiting bodies exist. To solve these problems, herbaria have initiated digitalization projects to help the preservation of important collections and type specimens. In the case of the Nannenga-Bremekamps collection a first digitalization initiative was made by the publication of SEM photographs taken from a selection of type specimens in the series Icones Mycologiae 1982-1986. Only 9 types from the Nannenga-Bremekamp collection were treated, e.g. detailed descriptions were given, but only electron micrographs featured in this publication. A second digitalization project was the release of a CD-ROM in 2002 with her original drawings and notes of nearly all of the specimens, including the types. More recently in 2013 a personal initiative was started by systematically taking macro-, micrographs and/or SEM images of all type specimens of myxomycetes housed in the Botanic Garden Meise.

The digitalization of a type specimens in this case means not only the imaging and databasing of the type but also the re-examing of the exsiccatum which can lead to new insights in the morphology of the concerning taxon. One example, *Licea bulbosa* Nann.-Bremek. & Y.Yamam., is presented.

Key words: Digitalization; myxomycetes



#### Myxomycetes Growing on Epiphytic Bryophytes: an Opportunity

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**Abstract:** Previous studies of myxomycetes growing on bryophytes have proven that only a few species can be called bryophyllous. Mosses provide an opportunity for air-born spores of myxomycetes to germinate, feed and develop in the moist conditions near the base of the thalli, and to produce fruiting bodies in the dryer environment in the top layers of the mosses.

The opportunity arose to investigate which species of myxomycetes complete their life cycle on epiphytic bryophytes in Flanders (Belgium, Western Europe). Although gaining in importance, this microhabitat has never before been systematically explored in this region. With changing climate conditions the quantity of mosses in general and more specific those growing on trees has been, and is still, steadily increasing for the last 20 years in Western Europe. The study was conducted in 16 woodlands located in four provinces of Flanders. Trunks of living trees baring epiphytic mosses were examined at 1.5 to 2 m height and specimens of myxomycetes growing among the moss thalli were collected. For comparison collections from other substrates in the same locality were also recorded. Ten species of epiphytic mosses were recorded on twelve tree species. A total of 67 specimens representing 38 taxa of myxomycetes were collected. Six of these specimens could only be identified to genus level. One of the taxa is most likely new to science. The ratio between species of mosses, trees and myxomycetes was examined.

Key words: Belgium; epiphytic bryophytes; Europe; microhabitat; myxomycetes



# Some Ecological Aspects of Nivicolous Myxomycetes of the Khibiny Mts.(Kola Peninsula,Russia)

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Abstract: Nivicolous myxomycetes of the Khibiny Mountains were surveyed within a vegetation gradient from the subalpine crooked-stem birch-rowan forest to dwarf shrub communities in the arctic mountain tundra during June 2012 and 2013. Both surveys yielded in total 666 specimens representing 34 taxa (27 species, 4 varieties and 3 forms) from 8 genera and 4 families. Among them 7 are new for Russia, 30 are new for this region and 28 are new records for Fennoscandia. Most of the species (56 %) were classified as rare (frequency of occurrence below 0.5% of the total number of records); only eight species were abundant (exceeding 3% of all records). Figures of the Chao1 estimator computed from a species accumulation curve showed that our sampling effort was sufficient to recover all of the most common species in the whole studied area (80% of the expected species number; Chao1 =  $42.3 \pm 6.9$ ), as well as in the subalpine crooked-stem birch forest (74%; Chao1 =  $38.1\pm9.0$ ). However, we did not manage to sample exhaustively the alpine mountain tundra (50%; Chao  $1 = 40.3 \pm 20.2$ ). In addition, the sample coverage factor (Turing's factor) estimates the completeness of this survey as 71%, the total number of expected species is 48. Species richness and diversity is rather high in subalpine crooked-stem birch-rowan forest (28 taxa, H = 2.16) whereas alpine mountain tundra harbored a more depleted myxomycete assemblage (20 taxa, H = 2.02). A comparison to the most studied nivicolous myxomycete biota of the Teberda State Biosphere Reserve (Northwest Caucasus) based on the Sørensen similarity index Cs demonstrates a rather high similarity with myxomycete biota of the Khibiny Mts. (Cs = 0.81, 27 species shared). This survey is the most northern of a large-scale study of nivicolous myxomycete diversity throughout Eurasia.

Key words: Amoebozoa; arctic and alpine ecosystems; diversity; ecology; Myxogastria; slime mold; species inventory

This research was supported by two grants from the Russian Foundation for Basic Research (13-04-00839 A, 12-04-33018 mol a ved).



#### Nivicolous Species of *Diderma* spp.: Morphology vs. Genetics

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**Abstract:** Nivicolous species from the genus *Diderma* form a morphological complex with many transitional forms, therefore it is difficult to delimit species within this apparent morphological continuum. To elucidate their taxonomic status an in-depth morphological analyses using non-metric scaling (NMS) based on the characters of sporocarp and spore ornamentation was carried out. Morphological data were compared with the genotypes obtained from partial sequences of 18S rRNA (SSU) and EF1alpha. The collections of the focal species (*D. alpinum*, *D. fallax*, *D. globosum* var. *europaeum*, *D. meyerae*, *D. microcarpum* and *D. niveum*) were obtained from Russia (Khibiny Mts., Kola Peninsula; the Teberda State Biosphere Reserve, Northwestern Caucasus; the Valamo Island, Ladoga Lake; Vaskelovo, Leningrad oblast), Central Europe (French Alps and German Alps) and Kazakhstan (Ily-Alatau ridge, around Almaty), representing a total of 544 specimens. During this study we gained 105 sequences of the SSU gene and 57 of EF1alpha gene and compared the resulting dendrograms. A Mantel test was conducted in order to estimate the possible correlations in the different genotypes geographical distribution.

Key words: Amoebozoa; nivicolous myxomycetes; slime molds; taxonomy; molecular phylogeny

This research was supported by grants from the Russian Foundation for Basic Research (13-04-00839 A, 12-04-33018 mol a ved).



# Nivicolous Myxomycetes in Agar Culture: First Results and Remaining Problems

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**Abstract:** A total of 63 specimens of nivicolous myxomycetes representing 10 taxa (9 species and 1 variety) from 5 genera and 3 families were tested for their ability to germinate on agar plates at room temperature, using bacteria associated with the spores as food source. Germination occurred in 28 specimens representing 6 species. In some samples microplasmodia started to appear at room temperature, but in most samples further developmental stages were observed only when cultures were kept at +2 °C. Two species (*Lepidoderma chailletii* and *Physarum nivale*) developed microplasmodia and even larger plasmodia, but we did not manage to induce sporulation. Partial sequences of the SSU gene obtained from cultured amoebae of two specimens from *Lepidoderma chailletii* and one from *Physarum nivale* were identical to those obtained from the spores of the specimens of origin, and comparison with GenBank sequences proved species identity. Some data about tolerance of *Lepidoderma chailletii* amoebae to low temperatures were obtained that can be helpful to understand better the ecology of nivicolous myxomycetes.

Key words: Amoebozoa; agar culture; germination; Myxogastria;nivicolous myxomycetes;

temperature tolerance

This research was supported by grants from the Russian Foundation for Basic Research (13-04-00839 A, 14-04-01408 A).



#### **Passportication for Myxomycetes Conservation**

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**Abstract:** Observation of myxomycetes conservation activity was made. Propose the introduction of "Environmental safety passports of species" for improving of conservation policy.

First steps for conservation of myxomycetes was the creation of a myxomycetes reserve by Bruce Ing in Wales near the town of Mold, in a small town park (UK). Martin Schnittler analised 413 myxomycetes species from Germany by special conservation categories. Yuri Novozhilov proposed to include 21 endangered species of myxomycetes in the Red Book of Nature of Leningradskaya oblast in Russia, then Alexander Lebedev recommended including in the Red Book of Tver' oblast in Russia 10 species of rare myxomycetes. Preliminary analyses of threat were made by author for 278 myxomycetes species of Ukraine. Species considered as endangered included 12 myxomycetes species, with 22 mainly nivicolous species being assessed as vulnerable. Detail evaluations were made for the biggest genus of myxomycetes, *Physarum*. Irina Dudka is preparing a proposal for the next edition of Red Book of Ukraine where myxomycetes were included. The IUCN Specialist Group promoting Conservation of Myxomycetes is beginning to prepare a foundation on which future conservation policy for Myxomycetes can be developed. The first myxomycete Diacheopsis metallica was published in Red List Species on the Edge of Survival. Evaluation of conservation status for 10 species of nivicolous myxomycetes and 10 species of order Trichiales was made. The information base included specimens, databases, bibliographic sources and field observations. Using the program "Geocat" (geocat.kew.org) estimates were made of extent of occurrence and occupancy. For each species population trend and threats were analyzed, and evaluation using IUCN criteria took place. For improving of conservation policy the introduction of "Environmental safety passports of species" for species of myxomycetes has been proposed to make. This certificate include of dates about morphology, metabolism, life cycle, geographical distribution of species and influence of abiotic and biotic factors with estimation of risks. As result of this scientific document will be analysis of threats and conservation recommendation for myxomycetes.

Key words: Myxomycetes; conservation; passports of species



# Higher Myxomycete Diversity in Mountainous Vegetation than Agricultural Plantation?

-An Evidence from Mt. Kanlaon National Park, Negros Occidental, Philippines

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Abstract: Higher floral and faunal biodiversity is expected in multi-species-covered mountainous forests than in mono-typic agricultural plantations. Is this also true for slime molds? This study comparatively evaluated the occurrence, diversity, and community assemblages of myxomycetes between agricultural and mountain forest areas, here represented by sugar cane plantations and Mt. Kanlaon, respectively. A total of 23 species of myxomycetes were collected and identified in Mt. Kanlaon National Park in Negros Occidental, Central Philippines. Morphological characterization identified these as belonging to the genera *Arcyria, Ceratiomyxa, Collaria, Craeterium, Cribraria, Diderma, Didymium, Hemitrichia, Lamproderma, Physarum, Stemonitis,* and *Trichia.* In contrast, only one species of myxomycetes, i.e. *Arcyria cinerea*, was recorded in the sugar cane plantations, indicating that mountain forests have a higher taxonomic and species diversity. This research is the first study to report the myxomycetes from Negros Occidental.

Key words: Agricultural plantation; biodiversity assessment; mountain forests; slime molds; species list

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# A Look at the Diversity of Myxomycetes in the Mountain and Coastal Forests of Puerto Galera, Oriental Mindoro

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**Abstract:** Myxomycetes are commonly associated with decaying plant materials in all types of terrestrial ecosystems. In the Philippines, these organisms have been reported in island and lowland forests. However, many areas in the country remain unexplored for myxomycetes. In this research study, the diversity of myxomycetes in the coastal, community and mountain forests of Puerto Galera in Oriental Mindoro was assessed and compared. Field specimens were collected directly from the forest sites while moist chambers were setup from twigs, dead vines, and ground and aerial leaf litter. A total of 42 species were recorded in the study. These belong to 15 genera, namely *Arcyria, Ceratiomyxa, Collaria, Comatricha, Cribraria, Didymium, Diachea, Diderma, Echinostelium Hemitrichia, Lamproderma, Lycogala, Perichaena, Physarum,* and *Stemonitis*. Mountain and coastal forests also had the same species diversity index while a lower value was reported for community forest. *Arcyria cinerea* was found to be the most abundant among the myxomycetes recorded. This study is the first extensive report on myxomycetes in Puerto Galera and in the island of Mindoro.

Key words: Species occurrence; slime molds; species listing; tropical forests



# Myxomycete Diversity and Ecology in Tropical Forests of Southern Vietnam: First Results and Perspectives

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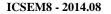
Abstract: Myxomycete assemblages were surveyed in October-December 2010-2013 in lowland tropical forests of Cat Tien National Park, (CTNP, 11°21'-11°48' N, 107°10'-107°34' E) and Vinh Cuu Nature Reserve (VCNR, 11 °-11 30' N, 106 54'-107 °13' E) and in mixed montane tropical forests and tropical cloud forests of Bi Dup-Nui Ba Nature Reserve (BDNB), centered in the Bi Dup Mountain massive (12°08' N, 108°40' E), belonging to the Da Lat Plateau (Lam Dong Province) of southern Vietnam. We present data on biodiversity of myxomycetes in southern Vietnam with special emphasis on vegetation types and the associated substrates, and a compare our data with results from other surveys in tropical Southeast Asia. Specific objectives were (i) to characterize the assemblage of commonly occurring species by assessing their abundances and (ii) to obtain data on the distribution of myxomycetes along an elevational gradient. This study is based on both field collections and those from moist chamber cultures prepared with ground litter, aerial litter and bark of living trees and liana. Within a vegetation gradient reaching from deciduous monsoon tropical lowland forests in CTNP and VCNR (elevations 70-85 m a.s.l.) to mixed montane tropical forests and coniferous mountain tropical forests (elevations 1400-1600) and cloudy tropical forests (elevations 1650-1750 m) in BDNB, myxomycete assemblages were surveyed. A total of 1477 records representing 135 species of myxomycetes in 22 genera were considered; including 494 field records (79 species) and 982 records (94 species) observed in 1513 moist chamber cultures of various decaying plant material. The majority of species (110) were classified as rare (frequency of occurrence below 0.5% of the total of 1477 records); only nine species were found to be abundant (exceeding 3% of all records). We report 134 species the first time for Vietnam. Three of these are species new to science. An evaluation of these data by a species accumulation curve estimated that between 75 to 83 percent of the species richness had been recorded. Shannon diversity and species richness reached maximum values in the deciduous



monsoon tropical lowland forests, whereas coniferous mountain tropical forests and tropical cloud forests had the most depauperate but most specific myxomycete assemblages. The assemblages associated with ground and aerial litter are most diverse, but the one associated with bark of coniferous trees is the most distinctive. Assemblages associated with the monsoon tropical forests of the southeastern Vietnam displayed a high level of similarity to those of other tropical regions for which data exist.

**Key words:** Amoebozoa; diversity; ecology; Myxogastria; slime mold; species inventory; Southeast Asia; tropical forest

This research was supported by grants from the Russian Foundation for Basic Research (RFBR 13-04-00839 A) and by the project «Ecolan-1.2» from the Russian Academy of Science and Russian-Vietnamese Tropical Centre.





# Four Years in the Caucasus – Observations on the Ecology of Nivicolous Myxomycetes

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Abstract: We report the results of four years surveying abundance and habitat requirements of nivicolous myxomycetes at the northwestern Greater Caucasus ridge. An elevational transect spanning 3.66 km from 1,700 to 3,000 m a.s.l. was established at the summit Malaya Khatipara situated within the Teberda State Biosphere reserve. Revisiting this transect every year between 2010 and 2013, we recorded 1177 fructifications of nivicolous myxomycetes, with 700 of these determined to 44 taxa. Fructifications developed usually at the margin of a snow field or in its close vicinity. Abundance of myxomycetes varied extremely between years, ranging from nearly zero to hundreds of fructifications. At sites with known myxomycete occurrences we established 16 data loggers in the years 2011 and 2012, measuring relative humidity and temperature at the soil surface. Together with weather data recorded on the nearby Klukhor pass, these data explain the observed extreme fluctuations in myxomycete abundance. If the uppermost soil layer freezes before snow cover, freezing temperatures may be preserved until snow melts, which is indicated by a phase of constant temperature (0 °C). If snow falls before frost, the insulating features of the snow allow for temperatures around 0 °C for most of the winter. In addition, both weather data and the data logger revealed a deep frost before the first snow falls in 2011. Temperature minima exceeding -10 °C at the soil surface most likely caused a nearly complete failure of myxomycete fruiting at the next spring. Although the overall snow cover was much lower in 2012, frost occurred only after the first snow falls, and myxomycetes fruited again at higher elevations. Our data show that frost / snow events in the previous autumn are critical for the temperature regime under the snow, determining together with the absolute duration of snow cover the time period suitable for amoebal growth. At sunny days, a steep temperature gradient in soil temperatures develops with the retreating snow, which may be the reason that myxomycetes fruit often immediately after snow melt. These assumptions are supported by amoebal cultures germinated from spores, which grew well over several months at temperatures between 1 and 4  $\,^{\circ}$ C but did not survive frost below -10  $\,^{\circ}$ C.

Key words: Amoebal growth; data logger; frost; undersnow microbial communities



#### Myxomycetes of Vyatka River Valley

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**Abstract:** Results of researches of a myxomycetes specific variety in natural and urban ecosystems of a Vyatka river basin in a subzone of a southern taiga of the European North-East are generalized. Researches are spent by a route method, as well as by method of moist chambers. The revealed species of myxomycetes were photographed under field conditions with Canon EOS 5D Mark II having adaptations for macro shooting. Identification was spent by means of microscope Leica DM 2500, according to Yu.K. Novozhilov's (1993) qualifier, G. Nojbert's 3-volume guide (1993, 1995, 2000) and the Internet resource http://www.discoverlife.org/.

In a large forest located in Kirov suburb, 14 species of myxomycetes, concerning 5 orders and 5 families were revealed. The majority of the found out species are cosmopolitans, widespread in territory of Russia. The species found out in a large forest belongs to families *Physaraceae: Physarum viride* (Bull.) Pers., *Ph. nutans* Pers., *Badhamia macrocarpa* (Ces.) Rost., *Fuligo septica* (L.) Wigg., *Leocarpus fragilis* (Dicks.) Rost.; *Arcyriaceae: Arcyria denudata* (L.) Wettst., *A. cinerea* (Bull.) Pers., *A. pomiformis* (Leers) Rost., and *Trichiaceae: Trichia decipiens* (Pers.) Macbr., *T. favoginea* (Batsch) Pers., *T. varia* (Pers ex J. F. Gmel) Pers. Along with listed, species *Stemonitis fusca* Roth. and *Lycogala epidendrum* (L.) Fr. were constantly found out on foozles and trunks of tumbled down trees. In the summer, after rains, on trunks of the tumbled down trees and foozles, plentiful growth of *Ceratiomyxa fruticulosa* (Mull) Macbr. was observed. Higher, than in the summer, a specific variety of myxomycetes is noted in the fall.

A specific variety of myxomycetes in parks of a Kirov city has appeared essentially lower, than in suburban wood phytocenoses. It was possible to find out representatives of two species only: *L. epidendrum* (L.) Fr. (order *Liceales*) and *Mucilago crustacea* F. H. Wigg (order *Physarales*). Both species are noted on foozles.

In the route researches spent in woods of the State natural reserve "Nurgush" (southwest suburb of Srednevjatsky lowland on the average a watercourse of Vyatka river) 20 species of myxomycetes, concerning 5 orders and 7 families are revealed. Xylobiontic substrate complex was characterized by the greatest variety – 85 % of all found out species concerning mainly to families *Arcyriaceae* (23.5 %).



Nine species of myxomycetes are revealed in epiphyte and covering substrate complexes of territory of the Reserve. Representatives of families *Physaraceae* (44.4 % of total number of species), *Stemonitidaceae* (22.2 %), *Trichiaceae* (11.2 %), *Arcyriaceae* (11.2 %), and *Didymiaceae* (11.0 %) dominated. The wide circulation of the species belonging to family *Physaraceae* in all substrate complexes indicates ecological plasticity of many representatives of this taxon.

About 160 samples of rotten wood and a bark of the trees collected in territory of Reserve had been analyzed by a method of moist chambers. As a result 13 addition species has been revealed which have not been found out during route researches.

As a whole 33 species of myxomycetes of various substrate complexes (epiphytic, xylobiontic, and covering) are revealed in the surveyed especially protected territory of Reserve "Nurgush".

**Key words:** Myxomycetes; southern taiga subzone; European North-East; species diversity; substrate complex; natural ecosystem; urban ecosystem



# Myxomycete Diversity and Distribution in the Mountain Valley of Kamikochi in the Northern Japan Alps

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**Abstract:** Most myxomycetes are distributed in coarse woody debris in forests and play an important role in the detritus food chain and in the process of material recycling. However, the species diversity and the ecological characteristics in a primitive forest are little known in Japan. The present study investigated myxomycete species diversity and distribution on logs in a natural conservation forest of the Kamikochi that is located in Central Mountain National Park of Japan, situated in a subalpine great valley, altitude 1500-1600m, of the Northern Japan Alps, Central Japan.

The forest vegetation consists of deciduous broad leaf trees, i.e. *Ulmus davidiana* var. *japonica*, *Pterocarya rhoifolia* and *Populous suaveolens*, in a belt along the Azusa river and coniferous forest that is located consecutively on slope of the mountains, i.e. growing *Tuga diversifolia* and *Abies veitchii*. Fruiting bodies of myxomycetes were surveyed on the decaying logs of those different tree types in the forest floor throughout 2011-2013 field seasons.

Myxomycete species appeared on both types of deciduous broad leaf logs and coniferous logs, associating with the decay state in normal distribution with moderately peaked stage. Ninety-two species (with varieties treated as species) from 1579 samples in total were recorded and reached 90 % of estimated species richness. Species richness was 61 species in summer and 63 species in autumn. The broadleaf logs yielded 60 species and the coniferous logs were 68 species.  $\beta$ -diversity of myxomycetes was higher between summer and autumn, i.e. value of 0.640 in the Sørensen dissimilarity indices, than between the different tree types of broadleaf logs and coniferous logs, i.e. that of 0.540. Seasonality of species occurrence was recognized on 17 species in summer and 15 species for coniferous wood. The myxomycete assemblages were ordinated using non-metric multi-dimensional scaling (NMDS) in order to compare both woods types in different season. The first NMDS-axis expressed seasonal distribution and the second axis corresponded to the difference of woods types. The assemblages indicated no remarkable difference among woods types in summer time but separated clearly into distinctive groups of deciduous woods and coniferous woods in autumn.



Species of Trichiales appeared dominantly on deciduous wood and species of Stemonitales emerged abundantly in coniferous woods in autumn.

Distribution pattern on decayed wood state was different between summer and autumn. Most species separately occurred on hard wood and/or softer wood in summer, i.e. *Cribraria species* occurred on softer wood, contrary to *Physarum*. On the other hand intermediated decaying wood intensively yielded many species in autumn, especially abundant was the occurrence of *Trichia decipiens* and *Lamproderma columbinum*.

The present study demonstrated that several species seasonally use particular decayed states of wood and have substrate specificity for wood types. Thus the primitive forest in a natural conservation area of Kamikoch in central Japan furnishes myxomycete species diversity that depends on the different vegetation types in a forest.

Key words: Coniferous wood; Deciduous broadleaf wood; Natural conservation; Primitive forest;

Seasonality; Substrate preference

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# PART I Ecology, Biogeography, Biodiversity

### **Diversity of Myxomycetes Inhabiting Wood in Tibet**

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Abstract: Tibet, the world's highest plateau, is located in the southwest of China averaging over 4000m above sea level. Until now only 75 species of Myxomycetes were recorded in Tibet. Because of variable vegetation and environmental condition diversity of myxomycetesshould be suspected to be richer in this area. Therefore, field survey was carried out in 2012 and 2013, more than 500 specimens were collected. Based on these collections species composition (diversity) was analyzed together with their geographic distributions and substratum types. Sixty-nine species of wood-inhabitingmyxomycetes were collected during survey of woodlands in Tibet. These species belonged to 20 genera, 7 families, 5 orders. Forty-eight species, most of Trichiales and Liceales, collected in broad-leaved and coniferous forests located from1400m to 4700m above sea level in Nyingchi, where rich plant species were observed in mountains and valleys because of abundant rainfall. Not only richness of species diversity, but also species composition of this area was different from Qamdo, where large area was occupied with grassland and alpine meadows. Species of Physarales and Stemonitales are mainly distributed in Qamdo. Copmaring species collected from deadwood, bark from dead and living trees, leaf litter and moss. Fifty-six species occured on the xylem of logs like Abies, Pinus and Quercus. Thirteen species were observed on the bark. A few samples could collect from various substrata except wood, such as Lycogala epidendrum, Arcyria incarnate and Craterium minutum. Therefore, it was suggested that most species have specificity of substrata in their habitats.

Key words: Myxomycetes; species composition; geographic distributions; substratum types



### Myxomycetes of Mahe Island in the Seychelles

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**Abstract:** The first study of the myxomycete biota of Mahe Island in the Seychelles was carried out in October 2011. Twenty-eight species of myxomycetes were identified from field collections, and 21 species were recovered from moist chambers cultures prepared with the bark of living lianas. In total, 43 species were recorded.

The first study of the myxomycete biota was carried during six days (9 to 14 October 2011) in nine different localities on Mahe Island, the largest island of the Seychelles archipelago, located in the western Indian Ocean. From 100 specimens collected in the field, 28 species of myxomycetes were collected and identified by Alain Michaud and Tetyana Kryvomaz. In addition, samples of the bark from living lianas were collected for preparation of moist chamber cultures. Steve Stephenson recorded 21 species from 97 specimens of myxomycetes recovered from these moist chambers cultures. In total, 43 species were recorded, and seven of these (Arcyria cinerea, Physarum compressum, Ph. crateriforme, Ph. lakhanpalii, Ph. melleum, Fuligo cinereum, and Didymium nigripes) were recorded as both field collections and collections from moist chamber cultures. The highest frequency of occurrence in the field was noted for *Physarum lakhanpalii* (10 specimens), whereas the most common species in moist chambers were Physarum compressum (21), Collaria arcyrionema and Perichaena dictyonema (both represented by14 specimens). The vegetation of the Seychelles archipelago is marked by nearly 2000 species of tropical plants and some myxomycetes were found on dead leaves. These were Diachea bulbillosa, D. leucopodia, Diderma effusum, Physarum bogoriense, Ph. compressum, Ph. hongkongense, Ph. melleum, and Ph. mutabile. The most common substrata for Diderma effusum, D. chondrioderma, Perichaena corticalis and Physarum lakhanpalii were the wood and bark of living coconut palm trees, and Perichaena quadrata was found on decayed palm wood. Arcyria cinerea, A. insignis, Cribraria intricata, Lycogala epidendrum, Physarum bogoriense, and Ph. crateriforme were collected from the dead wood of various kinds of trees, whereas Diderma chondrioderma was associated with mosses.

Key words: Myxomycetes; tropics; island biogeography



#### **Quantitative Taxonomy?**

—An Approach for Automated Analysis of Spore Ornamentation from SEM Images

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**Abstract:** Within the last decade an increasing number of myxomycete species was described as new to science, often based on subtle morphological details derived from SEM micrographs. Indeed, first case studies let expect that most of these descriptions will turn out to be justified by molecular results. However, combined morphological and molecular studies showed that eye catching characters like presence or absence of a stalk (*Lamproderma/Diacheopsis*) or a capillitium (*Alwisia bombarda/A. morula*) may be misleading, whereas spore ornamentation seems to be rather more reliable. For comparisons of morphological and molecular data, a quantitative analysis of spore ornamentation would be desirable. Beside obvious figures like size and height of ornaments, we propose the following parameters to be used in quantitative investigations:

- 1. *coverage*: proportion of surface covered by ornamentations,
- 2. *density*: number of ornaments per surface area (density),
- 3. a) size and b) shape: mean values for all analyzed ornaments,
- 4. *evenness*: how evenly ornaments are spread over the spore surface,
- 5. degree of *reticulation*: to which degrees single ornaments are connected to a reticulum.

Image processing algorithms implemented in the freeware software ImageJ (<u>http://imagej.nih.gov/ij/</u>, NIH, Bethesda, Maryland) can be applied to derive estimations of these parameters.

On the example of the genus *Meriderma* we show that contrast-rich SEM images of spores, especially if prepared with the critical point drying method, are well suited for an automatized approach. This allows to separate ornaments and remaining spore surface by differences in pixel saturation. The resulting binary images can be analysed with three basic algorithms: measuring area/shape of ornaments; counting contiguous and artificially separated ornaments (like warts connected to chains) or the meshes in-between them, and Dirichlet tessellation to estimate how evenly these ornaments are distributed. Finally, we present results of an analysis for the genus *Meriderma*.

Key words: Spores; critical point drying method; multivariate analysis



# Taxonomy, Phylogeny, and Morphological Evolution of the *Polysphondylium pallidum – P. album* Complex (Dictyosteliomycetes)

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**Abstract:** The taxonomy of Dictyostelid cellular slime molds (DCSMs) is confusing because their morphology is very simple. The genus *Polysphondylium* is phylogenetically problematic since the type species, *P. violaceum* is known to be phylogenetically separated from the most common members of the genus, the *P. pallidum* and their related species. Furthermore, a phylogenetic group including *P. pallidum* and its allied species, *P. album* (the *P. pallidum–P. album* complex) defined by a phylogenetic tree based on small subunit ribosomal DNA (SSU rDNA) has been also taxonomically confused at species level. And then, *P. pallidum* and *P. album* were redefined recently(Kawakami and Hagiwara 2008).

In this study, in order to taxonomically reevaluate this complex and the genus *Polysphondylium* and to infer phylogeny and morphological evolution of this complex, morphological, mating, and molecular phylogenetic analyses were carried out.

Firstly, based on the redefinition of *P. pallidum* and *P. album*, other described species and several isolates were systematically compared, resulting in the finding of 8 new taxa (morphospecies, tentatively named *P.* sp. 1–8). Morphological differences were clearly shown among 12 described species and 8 new morphospecies belonging to this complex, but *P. tikaliensis* and *P. colligatum* were morphologically similar mainly in having numerous nodes and small spores. In all the new morphospecies, macrocyst formation was observed. Mating systems of *P.* sp. 1, 3-7 were heterothallic and had two mating types. *P.* sp. 2 had three mating types exceptionally. On the other hand, *P.* sp. 8 produced macrocysts by itself, namely, this taxon was homothallic. By interspecies mating between all the species, a small number of macrocysts were found on some combinations. However, *P. anisocaule*, *P. pseudocandidum*, and *P.* sp. 5 formed a large number of macrocysts on each combination and were morphologically similar in forming the violaceum-type aggregation.

Next, the *P. pallidum–P. album* complex was examined phylogenetically at species level. The partial nucleotide sequences of D1/D2 region of large subunit ribosomal DNA (LSU rDNA) were determined for a total of 53 strains of 14 species (13 described species of *Polysphondylium* and *D. gloeosporum*) and 8 new morphospecies of this complex. Phylogenetic tree based on these sequences showed that all the species were shown to be monophyletic. In addition, *P. anisocaule*, *P.* 



*pseudocandidum*, and *P*. sp. 5 formed a monophyletic clade. D1/D2 sequences of the morphologically similar species, *P. tikaliensis* and *P. colligatum* were the same and thus two species are considered to be same species. In addition, it is possible that three species, *P. anisocaule*, *P. pseudocandidum*, and *P.* sp. 5 belong to same one. Morphological, mating, and phylogenetic analyses revealed that 9 described species and 7 new morphospecies are distinct species. The *P. pallidum–P. album* complex was morphologically different from the type species, *P. violaceum* besides phylogenetically. Therefore, I propose that this complex is transferred to a new genus, *Oxysphondylium*.

Finally, in order to construct more resolved tree and discuss about morphological evolution on the *P. pallidum–P. album* complex, phylogenetic analyses based on SSU plus LSU rDNA sequences were carried out. The phylogenetic tree revealed the presence of two major clades within the *P. pallidum–P. album* complex. The species with larger whorl index, considerably large number of nodes, and elongation of terminal segments were detected on both the clades. Therefore, these characteristics were considered to have arisen by parallel evolution. Only *D. gloeosporum* does not have whorled branches. Therefore, it is considered that the whorls secondarily diminished during the divergence of *D. gloeosporum*. As a result of the analysis of reconstruction of ancestral states, it was suggested that the common ancestor of the *P. pallidum–P. album* complex had low whorl index, small number of nodes, and unelongated terminal segments, and aggregation of the mucoroides-type.

Key words: Taxonomy; phylogeny; evolution; Polysphondylium; cellular slime molds



### Dictyostelids from Jilin Province, China

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**Abstract:** Dictyostelid cellular slime molds (dictyostelids) are microscopic organisms that occur in the soil and leaf litter of fields and forests soils as well as also being associated with animal dung. Dictyostelids feed mostly on bacteria. They are difficult to observe and collect in the field because they are microscopic. Dictyostelids are ideal organisms suitable for investigating problems in genetics, cytology and developmental biology because of their unique macroscopic characteristics and simple life cycles.

Jilin Province situated in the middle of Northeast China and located between 122 °131 °E and 41 °46 °N which belongs to the monsoon climate of medium latitudes. The primary objectives of the present study were to find more dictyostelids from Jilin Province and compared with known species. Eleven species of dictyostelid cellular slime molds (dictyostelids) in two genera were isolated from soil samples collected from Jilin Province in China. They are four new records for China *Dictyostelium longosporum*, *D. multistipes*, *D. gracile* and *Polysphondylium tenuissimum*, *D. clavatum* and *D. brefeldianum* only isolated from Taiwan province, and another three known species *Dictyostelium giganteum*, *Dictyostelium mucoroides* and *Polysphondylium violaceum*.

Key words: Dictyostelid cellular slime molds; Dictyostelium; Polysphondylium; taxonomy

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### Dictydiaethalium dictyosporangium sp. Nov. from China

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**Abstract:** *Dictydiaethalium dictyosporangium* is described as a new taxon being characterized by branched pseudocapillitium and spores marked with big rigs, sometimes formed incomplete banded-reticulate (about 10–12 µm in diam.) based on the bark surface of the dead log. Sporophores a pseudoaethalium, at maturity simulating an aethalium, effused, depressed, spreading over 22 mm, olivaceous to gray olivaceous, pulvinate, irregular in outline, more or less circular, extending up to 2.2 cm, up to 1.1 cm thick. Hypothallus shining, membranous, abundantly develop and surrounding the pseudoaethalium. PERIDIUM single, membranous, translucent, slender and evanescent at the base, olivaceous brown in transmitted light, smooth, persistent. Clumella absent. Cpillitium absent. Pseudocapillitium filiform, flat, thick on one side, 3–5 µm wide, smooth expect the thickened part which bears a row of warts, branched and anastomosed, running down to the base of pseudoaethalia, yellowish green to olivaceous green, pale yellow by transmitted light. SPORES free, bright yellowish green in mass, pale yellow to colourless by transmitted light, 10–12 µm diam., marked with long rigs, sometimes formed incomplete banded-reticulate. Holotype collected from Henan Province in China was deposited in the Herbarium of Mycological Institute of Jilin Agricultural University (HMJAU), Changchun, China.

Key words: Myxomycetes; taxonomy; Reticulariceae



### A New Record Species of Polysphondylium from China

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Abstract: The dictyostelids (cellular slime molds), were first described by Brefeld, have been known for almost a century and a half. Three genera were included in Dictyosteliomycetes, namely Dictyostelium, Polysphondylium and Actyostelium. Up to the present, 21 species of Polysphondylium have been described in the world. Ten species of *Polysphondylium* have been reported from China before this study. In 2012, forest soils were collected for dictyostelids from Mao mountain, Jiangsu Province, which situated in eastern China and belongs to subtropical climate. Spruces and oaks are the predominant vegetation types in Mao Mountain. A new record species of Polysphondylia, Polysphondylium colligatum Vadell & Cavender' was isolated from the soils. The taxonomic system of Raper & Hagiwara were used. Sorocarps, sorophores, sori, spores, cell-aggregations and pseudoplasmodia were observed. This new record species is characterized by the high frequency coremiform sorocarps, pigmentation of the sorophores and unconsolidated polar granules. Sorogens rise up synchronously and becoming clustered, which is similar to *D. polycephalum* Raper. However, the latter species without whorl branches when matured. It was worth noting that, the branches keep their verticillated pattern in this strain. However, in the original report, the branches often lose their regularity and become crowded. We suggested that this difference is likely to be caused by environment.

Key words: Dictyostelids; Polysphondylium colligatum; taxonomy



# Revision of the North American *Lamproderma* (Myxomycetes) Collections from the Donald T. Kowalski's Herbarium

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Abstract: Donald T. Kowalski was one of the influential scientists who studied myxomycetes in the North American mountains. His monographic paper published in Mycologia in 1970 (Kowalski D.T. 1970) remains an important source of information on species diversity of the genus Lamproderma in the USA. However, after more than 40 years of taxonomic progress in myxomycetes, this work needs a reappraisal. A revision of original collections is also needed to approach the species concepts applied by Kowalski. The present study aims at the taxonomical revision of Lamproderma specimens collected by Kowalski and cited in his paper in order to clarify the diversity of species in the light of current knowledge on the genus. Out of 116 specimens cited by Kowalski (1970), 95 were examined under dissection microscope, light microscope and scanning electron microscope (21 further specimens have not yet been loaned despite several requests sent to the UC Herbarium where the collections are most likely deposited). Results of the taxonomical revision revealed that: (i) the current treatment of some species, e.g. Lamproderma scintillans did not change with respect to that presented by Kowalski; (ii) Kowalski's interpretation of some species was clearly different from the original species concept, e.g. treatment of L. fuscatum (Ronikier A., Lado C., Meyer M., Wrigley de Basanta D. 2010); (iii) interpretation of most species changed because of progress in the taxonomy of the genus, e.g. Lamproderma carestiae; (iv) some collections are heterogenous and contain more than one species. As a result of the taxonomical revision of the available herbarium collections 21 species were recognized. They belong to five genera: Comatricha, Diacheopsis, Enerthenema, Lamproderma and Meriderma.

Key words: Amoebozoa; eumycetozoa; SEM; stemonitales; taxonomy

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#### Molecular phylogeny of some myxomycetes taxa

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**Abstract:** Myxomycetes are most diverse fungi. Their life cycle includes a multinucleate somatic phase known as a plasmodium and a reproductive phase producing sporophores with walled spores, which makes them very different from all other organisms. The molecular phylogeny of the taxa in Myxomycetes has been studied based on the sequences analysis of SSU rDNA and EF1A genes. But there are only limited species used in these analysis. So it is very necessary to they can not resolve evolutionary

In order to exam the phylogenetic relationship among the groups, COI and EF1A gene were amplified and sequenced. The sequences were initially aligned using the Clustal X package. The alignments were manually edited using MEGA 6. Phylogenetic trees were obtained from the data by the Maximum-Parsimony method using the heuristic search option in the program PAUP\* 4.0b8. This search was repeated 100 times with different random starting points, using the stepwise addition option to increase the likelihood of finding the most parsimonious tree. Transversions and transitions were treated with equal weight. All sites were treated as unordered, with gaps treated as missing data. The branch-swapping algorithm was TBR, the MULPARS option was in effect, and zero length branches were collapsed. The strength of the internal branches from the resulting trees was tested by bootstrap analysis using 1000 replications. The results showed that the four orders of myxomycetes formed three clades, Stemonitales forming a clade, Liceales and Trichales forming a clade, Physarales forming a clade. In Physarales clade, plasmodiocarp and aethalia groups were more closed than sessile sporocarp groups, which showed that the type of fruiting bodies of slime molds may indicate some phylogenetic meaningful during the morphogenesis of slime mold fruit body.

#### Key words: slime mold; COI; EF1A; sporocarp

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# The Phylogeny of Slime Moulds (Mycetozoa): from One Gene to the Whole Genome

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**Abstract:** Over the past three decades, the sequencing technologies have been developed rapidly, from first-generation Sanger DNA sequencing to the current fast and cost-effective next-generation sequencing (NGS). By using these sequencing technologies, more and more complete genomes have been sequenced, which has pushed phylogenetic analysis into a new era, in particular shed light on the complicated evolutionary history of slime molds.

The evolutionary history of a collection of organisms is usually represented by a phylogeny in the form of a tree. Phylogenies can be constructed in many ways. For example, some methods are at the sequence level, constructing phylogeny trees based on sequencing similarities; some methods are beyond the sequence level, they use features across the whole genome; and et al. Each method has its advantages and bias, they all more or less help us to understand the history of life better and may leave some problems unsolved.

In this article, we review the recent advances in phylogeny construction, in particular the phylogenetic approaches have been applied to uncover the evolutionary history of slime molds, and discuss possible developments towards a comprehensive reconstruction of slime molds phylogeny in the future.

Key words: phylogeny; slime moulds (Mycetozoa); next-generation sequencing

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# Bioinformatics Analysis of Conserved Motifs of Actins from Dictyostelium discoideum

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**Abstract:** *Dictyostelium discoideum* is widely used as a model organism in slime mold. Its predation process is closely related to the polymerization of actins which is an abundant and highly conserved globular protein. The complete genomes of *D.discoideum* have been sequenced and there are 32 actin all over its genome, including five copies clustered on chromosome 1, 13 copies on chromosome 2,12 copies on chromosome 5, and single copies on chromosomes 3, 4. To investigate the characteristics of actins from *D.discoideum*, the conserved motifs of 32 actins from *D.discoideum* were analyzed by bioinformatics analysis. Three conserved motifs, motif1, motif2, motif3, were found from the 32 actins sequences; three conserved motifs, Motif1, Motif2, Motif3 were found from actin17 with its 21 best hit genes of other organisms actins by using MEME SUITE analyzing method. Three motifs, motif1, Motif1, Motif1, Motif2 were newly found in this study, and were located on the crystal structure of Profilin–actin–VASP<sub>202-244</sub>. which suggested that motif1, Motif1 and Motif2 may play important roles in the evolution of conserved motifs.

Key Words: Slime mold; actin; motif; bioinformatics

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# Amplification and Sequencing of EF-1α Region from Didymium squamulosum

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**Abstract:** *Didymium squamulosum*, Didymiaceae, Physarales, Myxomycetes, is a widely distributed species throughout the world. Its fruiting bodies are mainly sessile or stalked, and sometimes sessile sporangia melt into a short plasmodiocarp. It is one of the most common species in China, but there is no reports about its molecular phylogeny based on the analysis of elongation factor gene. Up to now there is only one EF-1 $\alpha$  gene sequence of *D. squamulosum* published on GenBank. EF-1 $\alpha$  region from *D. squamulosum* was amplified and sequenced in this paper by using primers designed in our laboratory. DNA was extracted from 3–5 adjacent sporophores by using 5% Chelex-100. Semi-nested PCR was used to amplify DNA fragment. Thirty-five cycles were conducted in a TC-512 Thermo Cycler: 94 °C for 30s, 52 °C for 1 min, 72 °C for 1min30s, with a final extension at 72 °C for 10 min. PCR products were separated on 1.0% agarose gels, stained with ethidium bromide and viewed under UV light. The length of EF-1 $\alpha$  fragment 944bp. The highest identity was 87% with that of *Physarum polycephalum* by blasting on NCBI. The result of this study not only enriched EF-1 $\alpha$  gene sequences, but also provided an evidence for the phylogeny of myxomycetes.

Key words: Myxomycetes; Elongation factor; Molecular phylogeny; Didymium

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# Nuclear DNA Contents of 4 Orders of Myxomycetes Collected in Jilin, China

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Abstract: With special life cycle living as both protozoa and fungi, myxomycete was included into protozoa phylogenetically. However, their phylogenetic position and evolution are still not clarified in the morphologically different groups. Nuclear DNA content is significant in many fields of research including ecology, taxonomy and evolution, that it has been proved by many tests in plants and animals. In order to elucidate the relationships among the myxomycetes based on DNA content variation, combining with the morphological characteristics and living environment, morphological classification relationships were recognized. Thirty samples belonged to 4 orders for the measurements of nuclear DNA content were mainly collected from Jilin province in China. Nuclear DNA content can be measured quickly and simply by flow cytometry (BD Accuri® C6) using the base unspecific intercalating fluorochrome propidium iodide (PI) with Saccharomyces cerevisiae as the primary internal standard. There was a small range of DNA fluorescence absorption value and spore size in dark-spore myxomycetes as Physarales and Stemonitales, by contrast, a bigger range of DNA content compared with genus in light-spore myxomycetes including Liceales and Trichiales was estimated by fluorescence absorption value. The intraspecific DNA contents were within limits, that was closed to spore size which were small discrepancies among the samples from different places like Lycogala epidendrum collected from Jilin province, Sichuan province and Tibet. It was suggested that DNA content was related with the differentiation of spore size. And DNA content may be contributed to interspecific morphological divergence within genus.

Key words: Myxomycetes; DNA content; flow cytometry; Lycogala epidendrum



# Analysis on the Internal Transcribed Spacers Structures and Phylogenetics of Physarida

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**Abstract:** Physarida is the biggest order in the class Myxogastria. It mainly includes two families: Physaridae and Didymiidae. In order to explore the effects of internal transcribed spacers region of ribosomal DNA (rDNA ITS) on the phylogenetic analysis of Myxogastria, we analyzed the primary structure and secondary structure of ITS, and the phylogenetic relationships in Physarida.

The universal primers PHYS4 and PHYS5 were used to amplify and sequence the rDNA ITS sequences from eight species of five genera in the order Physarida. The obtained sequences were combined with known sequences in GenBank to construct a phylogenetic tree using both Maximum Likelihood (ML) and Bayesian Inference (BI) methods. The varieties for the rDNA ITS of different Physarida species were observed both on the base composition and length. The range of length are 777-1445 bp, G + C mol % are between 53.4 and 61.9%. Physarida and Stemonitida clustered for two distinct branches. On the branch of Physarida, Physaridae and Didymiidae were divided into two separate branches respectively. The results supported those taxonomic viewpoints which make a distinction between the two families that based on capillitium with or without calcareous granules in the morphology. The samples of *Didymium squamulosum* from different geographic regions are composed of three branches, which again confirmed that this morphospecies is consist of a biological species complex with different geographical origins, reproductive incompatibility and genetic variations.

The RNA secondary structures of ITS were calculated and drawn using the program RNA structure. The results showed that the ITS1 secondary structure in most tested Physarida species are difficult, which has only 24 bp sequences are conservative. The base composition are A(G)C(U)C CGC(U)ACUGGUGAACCUGCGGGU(C). There is a common stable helix structure in most tested Physarida species. Covariation happened in three positions that maintain the complementary base pairing. The stable helix structure plays an important role in the processing of mature rRNA. The 5.8S secondary structures in different species were similar to each other, which are composed of four helixes with two main types. The ITS2 secondary structure was constructed based on the 5.8S rRNA-28S rRNA interaction. A closed multibranch loop and at least four major helixes were



predicted with the helix IV being relatively conservative. The ITS2 secondary structure of Physarida species can form a core around with several regions in pairs, and is a closed multibranch loop. At least four major helixes were predicted with all tested Physarida species. Among them the helix IV being relatively conservative, and there are no branch ring which will influence the stability of secondary structure. The varieties of length in different species are also smaller. The secondary structures of ITS regions were more conserved than their nucleotide sequences. Therefore, the further analysis of ITS structures will benefit to the understanding of the relationships between the ITS structure and molecular evolution of Physarida species.

Key words: Physarida; rDNA ITS; Molecular evolution; sequence; secondary structure

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#### What an Intron May Tell

— An Analysis of Two Markers in *Meriderma* spp. (Stemonitales)

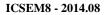
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Abstract: Molecular phylogenies revealed the genus *Meriderma*, traditionally known as the *Lamproderma atrosporum* group, to be a basal clade within dark-spored myxomycetes. Recently, the pioneering studies of Poulain, Meyer & Bozonnet led to the differentiation of several taxa, which encouraged us to start a molecular investigation, including specimens from the French and German Alps, the Caucasus, and the Rocky Mountains. As to expect, studying partial SSU sequences we found more than 50 genotypes within ca. 200 investigated specimens. Even more interesting was the first part of the protein elongation factor EF1a we sequenced as a second marker. In this gene, the protein-coding sequence is interrupted by an extremely variable spliceosomal intron.

More than three thirds of the 70 specimens investigated for this marker were heterozygous in the intron section; substitution but especially deletions complicated sequencing. A major SSU genotype of *M. aggregatum* shows a unique intron which is nearly inaccessible for sequencing, consisting of a stretch of >14 subsequent C followed by a dinucleotide SSR with the motif CA. In contrast, genotypes of specimens assigned to *M. spinulisporum*, *M. echinulatum*, and *M. carestiae* showed sequences rich in T, which are typical for splicesomal introns.

The high proportion of specimens with heterozygosities in the intron points to frequent sexual reproduction. However, in several cases we found the same heterozygosity pattern in specimens collected at different locations, even at different mountain ranges. This lets dispersal via microcysts and/or amoebae appear to be unlikely: according to textbook (sexual) life cycle these propagules should be haploid, thus transport only one allele. An alternative explanation would be to assume an optional asexual life cycle with diploid amoebal stages and spores, which could transport both alleles. The occasional observation of "macrosporic" forms in several taxa could be a hint on the existence of such an apomictic life cycle, which was postulated already by earlier studies with cultivated members of the Physarales.

Key words: 18S rRNA gene; intron; protein elongation factore EF1a





# The Genus *Alwisia* (Myxomycetes) Revalidated, with Three Species New to Science

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**Abstract:** Based on morphological investigations and 18S rRNA phylogeny, we revalidate the formerly monotypic genus *Alwisia*. The monotypic genus *Alwisia* Berk. & Broome (Reticulariaceae), described in 1873, was a few decades later united with *Tubifera*, and the new combination *Tubifera bombarda* (Berk. & Broome) G.W. Martin was proposed for its single species. However, 18S rRNA sequences revealed that *T. bombarda* forms a separate clade within the family Reticulariaceae which should be recognized as a separate genus under its original name *Alwisia*.

The same locus of 18S rRNA gene was studied in several unidentified collections from Costa Rica, Australia and Tasmania. Obtained sequences appeared to be related, but yet different from *A. bombarda*, and seem to be identical within the specimens of one morphotype. Studied collections differ from *A. bombarda* in morphological characters as well. Therefore we described them as three new species within the revalidated genus.

All new species have subspherical sporothecae, against fusiform ones in *A. bombarda*. Among them, *Alwisia morula* is characterized by erect and branched individual stalks, while *Alwisia repens* has procumbent stalks and iridescent peridium. Both *A. morula* and *A. repens* completely lack a capillitium, while the third new species, *Alwisia lloydiae*, possesses a tubular capillitium ornamented with globular warts.

Capillitial structures of *Alwisia bombarda* and *A. lloydiae* occur inside separate sporocarps with intact walls. Therefore they do not represent remnants of confluent peridia and thus do not correspond to the definition of a pseudocapillitium, but should be seen as a true capillitium. Capillitial threads in *Alwisia* resemble those found in the genera *Dianema* and *Lycogala*, thus providing a new argument for a close relationship between Reticulariaceae and Dianemataceae and also for considering the



tubular threads of Lycogala as a true capillitium.

Key words: 18S rRNA gene; Tubifera bombarda; Alwisia bombarda; A.Morula; A. Repens;

A.lloydiae



#### New Insights into the Tubifera ferruginosa - Complex

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**Abstract:** A phylogeny based on partial 18S rRNA gene sequences revealed that *Tubifera ferruginosa* (Batsch) J.F. Gmel. is actually a complex of seven different species, showing a high degree of sequence dissimilarity especially in the variable helices. Among them, *T. applanata* Leontyev & Fefelov and *T. dudkae* ad int. (comb. nov. pro *Reticularia dudkae* Leontyev & G. Moreno) were recently described and now confirmed by species-specific 18S rRNA sequences which are identical for specimens from different geographic origins.

Within *T. ferruginosa* sensu strictu we recognize ssp. *ferruginosa* ad int. and ssp. *acutissima* ad int., differing constantly by hemispherical to obtusely conical vs. acute conical sporothecae with subulate apices, respectively.

The remaining taxa are described as new to science; their 18S rRNA phylogeny corresponds to morphological characters like the structure of sporothecal tips, color of immature fructifications and ultrastructure of the inner peridial surface. *T. montana* ad int. develops from orange-red plasmodia the sporothecae with strongly accreted tips and peridia iridescing by golden to pinkish hues; spores are considerably larger then in *T. ferruginosa*.

The large pseudoaethalia (3–12 cm long) of *T. magna* ad int. have flattened sporothecal tips resembling those of *T. applanata*. However, in *T. applanata* immature fructifications are flesh-colored to salmon, and sporothecal tips are isodiametric and roughly hexagonal, while *T. magna* appears with pink colors, and its sporothecal tips are elongate and variable in shape.

*T. pseudomicrosperma* ad int. differs from *T. ferruginosa* by its thick, black hypothallus and *beige peridium, and from T. microsperma* by much larger pseudoaethalia, prostrate hypothallus and even smaller spores. Its peridium shows small rimmed craters indistinguishable in phase contrast LM, while in *T. microsperma* these craters are larger and clearly visible.

*T. corymbosa* ad int. possesses small spherical sporothecae at the base of the pseudoaethalium resembling those of *T. dimorphotheca*, but lacks the prominent hypothallic stalk of the latter species and has a peridium, iridescent in blue and green tints, with *a metallic, silvery or golden luster on the sporothecal tips*.



All taxa of the *T. ferruginosa*-complex lack a capillitium; structures described by Nannenga-Bremekamp (1961, 1991) with LM and SEM were found to be fungal hyphae feeding on spores.

Key words: 18S rRNA gene; species differentiation; capillitium



# Application of 3D Imaging of Light and Electron Microscopy in Studying Myxomycetes

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Abstract: Developing and mature myxomycete fruiting bodies have complicated structures which usually too thick and opaque to observe the inside under light microscope, and too big to understand the arrangement of their complex by transmission electron microscope. To understand the structure inside of myxomycetes, I combine the conventional serial sectioning technique and the computer-based Three-Dimensional (3D) imaging technique.

3D imaging is an effective technique to define the structure of organisms, and the use of computers to aid in the reconstruction and segmentation of the large data files of the imaging is now widely available to biological researchers. We have several high-end electron microscopes to acquire the raw date of 3D image nowadays, still general-use computers are not up to the task to reconstruct and segment of the entire structure of myxomycetes in electron microscopic level, since its big and complex structure generates too large data files to process.

Here, I present some application examples of 3D imaging for myxomycete research, acquired its data set by the conventional serial sectioning technique, reconstructed and segmented by a general-use computer. The correlation between 2D and 3D imaging of light and electron microscopy links the overview and orientation of the complex structure inside the myxomycetes, and detailed localization and correlation of subcellular structures. Although the 3D imaging is a technically demanding and time-consuming technique, it has the potential to provide new insights about morphogenesis and morphological characteristics of myxomycetes.

Key words: Three-Dimensional imaging; Serial Sections; Reconstruction; Segmentation; TEM; LM

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# An Accurate Method to Measure Velocity of Protoplasm Streaming in Myxomycetes Plasmodium

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**Abstract:** In myxomycetes, the more or less rhythmical reversal in the direction of protoplasmic flow within the veins of a plasmodium is a well-known phenomenon. The protoplasmic flow is typical cyclosis. In general, it is difficulty to measure the velocity of cyclosis. But, larger phaneroplasmodium make it possible under the optical microscopy. In recording a streaming video of myxomycete phaneroplasmodium, determine clear vacuoles through the channel, do a series of video capture, determine the circumference of the vacuole and center in the screenshot, overlapping do screenshots, the trajectories of bubbles can be obtained. By displaying the center distance of adjacent bubbles, be able to get the total distance, finally get the velocity of the vacuoles in a period of time. The less time of the video capture get the more accurate velocity. Determine the average flow velocity of the number of vacuoles can represent the flow rate of the plasma flow in this section.

Key words: Myxomycetes; phaneroplasmodium; protoplasm streaming; vesicles



# A Comparative Study on the Developmental Processes of the Family Physaridae in the Pure Culture

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Abstract: Myxogastria (slime molds) are unique eukaryotic microorganisms with both some characteristics of fungi and amoebae. Different development processes have been found in different species of Myxogastria. In this paper, a comparative study on the developmental processes of the family Physaridae was conducted. Four myxogastrian species in the family Physaridae were purely cultured, and their life cycle were observed. They were Physarum flavicomum, Physarum melleum, Physarum nutans and Physarella oblonga. Sterile culture techniques concluding hanging drop culture and oats-agar culture, and some observation technologies for microstructures were used in order to study and compare the differences of the life history of Physaridae. The results showed that spores of these 4 myxogastrian species germination all by V-shape split, then produced one or more myxamoebae. Spores germination was observed within 2 d after inoculation, but germination time was slightly different among four Physaridae species in the same condition. Spores of Physarum flavicomum took up to 5 h for germination to occur, however spores of Physarum nutans took up to 1 d, spores of Physarum melleum took up to 36 h, spores of Physarella oblonga took up 2 d for germination to occur. A typical V-shape split was observed in the spores of *Physarella oblonga* and Physarum flavicomum, the length of the crack accounts for almost 2/3 of the spore's diameter, however only a narrow gap was formed in the spores of *Physarum melleum* and *Physarum nutans*. The myxamoeba of Physarum melleum and Physarum melleum changed into a swarm cell with 2 flagella, whereas in the same conditions swarm cell was not found in Physarum flavicomum and *Physarum nutans.* The experiment observation that several young plasmodia of *Physarum melleum* were found on the oat agar in 9 d after spore inoculation, however young plasmodia of Physarella oblonga and Physarum flavicomum were observed in 12 d after spore inoculation. It required for more time to observe young plasmodia of *Physarum nutans*, which spend about 16 d. Young plasmodia of Physarella oblonga and Physarum nutans required about in 10 d to grow all over the plate, whereas young plasmodia of Physarum melleum and Physarum flavicomum needed in 18 d to maturity after inoculation. It is proposed that the plasmodia of different myxogastrian species had different growth rate. Plasmodia of these 4 myxogastrian species had obvious differences in colour and veins. These 4



myxogastrian species could produce mature fruiting bodies on oat agar medium, which sporulation all needed light stimulation. The plasmodia of *Physarella oblonga* and *Physarum melleum* needed 2–3 d to produce young fruiting bodies. Furthermore, the plasmodia of *Physarum flavicomum* transformed to young fruiting bodies in 7 d. Whereas to *Physarum nutans*, the time during the maturity of plasmodia to sporulation needed 13 d in the same culture condition.

Key words: Myxogastria; spores germination; plasmodia; sporulation

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### Some Hypotheses about Lepidoderma

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Abstract: Slime molds' sporangia are not the result of a predetermined assembly plan that ensures morphological consistency, as in multicellular organisms, but rather the result of a series of coordinated processes.

Some of these processes may be affected by environmental conditions causing variations that can make sometimes impossible the correct determinations of the species or even the genus according to macroscopic characteristics.

I will propose some simple interpretative keys which may be useful in determining the species of the genus Lepidoderma.

This work is based on the direct observation of the phenomenon of recrystallization of the lime that can affect many Physarales and on some simple assumptions on the external structure of the peridium.

Key words: Lepidoderma; recrystallization; external structure of the peridium.



# Distribution and the Food Resource Preference of Protostelids in Sugadaira Highlands, Nagano, Japan

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Abstract: In Japan, the biodiversity of true slime molds and dictyostelids are well investigated, on the other hand, such a study on protostelids are extremely deficient. There is not yet any comprehensive monograph of Japanese protostelids (except a few partial records of some species: 4 spp. in Moore et al. 1995). It has been known that some species of protostelids, for example *Protostelium mycophagum*, have the preference of food resource. But their preferences have not been precisely examined from ecological or physiological view point. Then, in order to start the floristic survey of Japanese protostelids at first, we try to clarify the relationship between the distribution in micro-scale and the preference for food resources of protostelids, using baiting method. The study was performed in Sugadaira Montane Research Center, Sugadaira Highlands (c. 35 ha, c. 1360 m alt.), Nagano, Japan in temperate climate zone. As baits, sterilized 3 kinds of substrates (2 mm squared cut culm of Miscanthus sinensis, bark of Pinus densiflora and bark of Quercus crispula) were placed on the litter of 4 sites of defferent vegetation (M. sinensis grassland, P. densiflora forest, Quercus forest, Daimyoujin-no-taki waterfall in Q. crispula forest in Sugadaira Montane Research Center). After 3 weeks samples were recovered, brought back to the laboratory and soaked in sterilized distilled water. Eight pieces of each sample were inoculated onto wMYA (weak Malt extract and Yeast extract Agar) plates and observed by light microscope with long focus lens for 2 weeks. We counted the number of the pieces on which protostelids appeared, and calculated their frequencies. As a crude culture, the spore was isorated from recognized fruiting body and cultured with 2 isolates of bacteria obtained from each site by dilution plate method. For judging whether the preferences against food resources (certain isolates of bacteria) are exist or not, the presence of spore germination and the formation of fructification under cultures were checked. These processes were performed 2 times in August and October. Protostelid fruiting bodies appeared on 30 baits from among 196 pieces. Four isolates of two-membered cultures were established among 30 crude cultures. Two isolates of them were identified as Protostelium arachisporum, one was identified as Schizoplasmodiopsis vulgare based on their morphological characteristics and life cycles. The other one isolates could not be unidentified. P. arachisporum and S. vulgare were newly recorded from Japan. The total frequency of protostelids was highest on *M. sinensis* grassland. But they do not always prefer the isolate of bacteria from *M.* 



*sinensis* grassland was, namely the preference to the food could not clearly detected. Thus, in *M. sinensis* grassland, high frequency of protostelids may be caused not by the preference of the food resources but by the environmental factor such as instability of the temperature and humidity. In general, the frequency of protostelids may be higher, when the baits are placed on the site where the substrates does not originally exist.

Key words: Prototelids; food resource preference



# Studied on Isozyme in Different Ontogenetic Stages of Didymium iridis

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**Abstract:** The spore to spore culture of *Didymium iridis* were completed in our lab. And details of its life cycle are provided (Including from spores, myxoamoeba, zygote, young plasmoidium, mature plasmoidium, young fruiting bodies to mature fruiting bodies). In this paper using the fourth different ontogenetic stages of *Didymium iridis* (mature plasmoidium, split phase of plasmoidium, the young fruiting bodies and mature plasmoidium) as material to extract the isozyme, and using PAGE (olyacrylamide gel electrophoresis) to analyse, the results show that zymogram bands of the mature plasmoidium were according with the split phase of plasmoidium, and zymogram bands of the young fruiting bodies were according with the mature fruiting bodies. But there was distinctive bands between the mature plasmoidium, the split phase of plasmoidium and young fruiting bodies, mature fruiting bodies. So, isozyme variation in different ontogenetic stages could be used as indicator of physiology andgenetic identification of *Didymium iridis*.

Key words: Isozyme; ontogenetic stage; myxomycetes; life cycle



# Fatty Acids Detection and Its Application in Taxonomy of Six Dictyostelid Cellular Slime Molds

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**Abstract:** Fatty acids of six species of Dictyostelid Cellular Slime Molds (Dictyostelids) were detected by using gas chromatography and the polymorphism was analyzed in order to investigate the relationship between the polymorphism of fatty acid and taxonomies of Dictyostelids in existence. Six species of Dictyostelids are *Polysphondylium candidum*, *Dictyostelium discoideum*, *D. implicatum*, *D. globisporum*, *D. clavatum*, *D. tenue*. The fatty acids from these Dictyostelids were analyzed by cluster analysis and find out the characteristics of fatty acids. The results showed that the polymorphism of fatty acids of 6 species of Dictyostelids was evident based on fatty acid distribution, these Dictyostelids could be separated into two groups: group I and group II. Group I was *Polysphondylium candidum*, group II were the other species of *Dictyostelium*. And 38 fatty acids from these Dictyostelids were also analyzed by cluster analysis, they could be separated into three groups: group I, group II and group III. Group I showed that frequency of occurrence of fatty acids was high, group II was middle, and group III was low. Fatty acid might become a new index for taxonomy of Dictyostelids.

Key words: Dictyostelium; Polysphondylium; Cluster analysis; Gas Chromatogram

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# Foraging Behaviors of Phaneroplasmodia in 6 Species of Myxomycetes to Three Types of Food Sources

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**Abstract:** Foraging behaviors of phaneroplasmodia of *Didymium megalosporum*, *D. squamulosum*, *Physarum melleum*, *Physarella oblonga*, *Badhamia gracillis* and *Fuligo septica* to three types of food sources (cereals, mushrooms and vegetables) were examined and compared under the same condition within 48 hours. They had two foraging phase: explorative and exploitative growth reported in *P. polycephalum*. During explorative growth, their time of vigorous growth, migrating direction, contacting pattern and distribution mode were not related to new food sources. But, phaneroplasmodia of *D. megalosporum*, *D. squamulosum* and *P. melleum* were more vigorous than those of *P. oblonga*, *B. gracillis* and *F. septica*. During exploitative growth, their degree of biomass increase and resuming exploration were related to new food sources. Oat, *Lentinus edodes* and *Auricularia auricula* were favorable for their nutritional requirements, especially *L. edodes* could be useful for their culture.

Key words: Physarales; foraging strategies; explorative growth; exploitative growth; nutritional status



# Ultrastructure Observations on the Sporulation of *Physarum compressum*

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**Abstract:** Cultured the plasmodia of *Physarum compressum* with sterile oat powder and water on the substrate of 2% water-agar, and chose the plasmodia turning to be sporangia at different stages to be surrounded by epoxide resin, than cut into ultrathin section, and observed by TEM.

The observation results showed that the inner structures of proto-sporangia were denser than the plasmodia growing normally. The plasmodia were cleavaged into small spheres which were proto-spores, following the proto-sporangia developed with plasmodia injected and the color changed from white to dark gradually. There was an electrondense layer between two close spheres and two electron-lucent layers at the opposite sides of the electrondense layer. When the color of sporangia getting black,, we can see that the electrondense layer became two layers, each layer became the outer spore wall with concave-convex parts which adapted to each other, and these were the ornamentations of the matured spores. And except for two layers, there was another thin electrondense layer speculated as capilitia. So, some electondense layers didn't divided into two layers but three layers, and the middle layer would became capillitia after spores formed. It was extremely different from the traditional viewpoint that capillitium formed before spore wall. The results should be tested and verified by many other species, than can be used in the taxonomy and systematics of Myxomycetes. **Key words**: *Physarum compressum*; sporulation; ultrastructure



# First Report of Sporangia of Two Myxomycetes (Stemonaria longa, Stemonitis spledens) Collected from Shiitake Cultivation

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**Abstract:** The shiitake mushroom, *Lentinula edodes*, is widely cultivated and managed throughout China. Henan province is one of the highest yield of shiitake in China. Myxomycetes are a small, relatively homogeneous group of eukaryotic organisms and common inhabitants of decaying plant material found throughout the world. They are particularly abundant in forested regions where decaying logs, stumps, and dead leaves furnish a plentiful supply of potential substrates. Specimens of Mycomycetes collected from *Lentinulla edodes* cultivation in Henan, China were examined. Two species of myxomycetes, *Stemonaria longa* and *Stemonitis splendens* were identified based on morphological characters. The specimens were deposited in the Herbarium of Mycological Institute of Jilin Agricultural University (HMJAU), Changchun, China. The specimens were collected from *shiitake* cultivation in Henan Province, China in October, 2013. Among two species, occurrence of *S. longa* has become the most serious problem. This is the first report that Mycomyctes caused mushroom cultivate disease.

Key words: Amoebozoa; morphogenesis; SEM; taxonomy



#### A Preliminary GC-MS Study of Four Species of Physarales

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**Abstract:** In this study, Ultrasonic extraction and Saxhlet extraction of the fruiting bodies of four species of myxomycete and plasmodium of one species with petroleum ether were analyzed by GC-MS. The extractions of liposoluble constituents were identified from the plasmodium of *Diderma chondrioderma* and the fruiting bodies of *D. chondrioderma*, *Diderma crustaceum*, *Craterium leucocephalum* and *Physarum cinereum*.

The results showed the compound structure patterns of the fruiting body and plasmodium of *D*. *chondrioderma* were mainly classified as aliphatic acids, aliphatic hydrocarbons, esters, higher aliphatic alcohol, higher aliphatic ketone and sterol, etc. The compounds of the fruiting body of *D*. *crustaceum* were mainly aliphatic acids, aliphatic hydrocarbons, esters, higher aliphatic alcohol, higher aliphatic acids, aliphatic hydrocarbons, esters, higher aliphatic alcohol, higher aliphatic acids, aliphatic hydrocarbons, esters, higher aliphatic alcohol, higher aliphatic acids, aliphatic hydrocarbons, esters, higher aliphatic alcohol, higher aliphatic acids, aliphatic hydrocarbons, esters, higher aliphatic alcohol, etc. The compounds of fruiting body of *P. cinereum* were mainly aliphatic hydrocarbons, phenols, steroids, etc.

The results showed that main chemical types of the compound were generally same in different species of myxomycetes which belonged to the same order. The molecular structure of these compounds was relatively simple. Both the structure and type of aliphatic acids were typical aliphatic acids that once found in marine algae and plankton. These aliphatic acids particularly closed to similar aliphatic acids found in amoeba. For example 9-hexadecenoic acid, n-hexadecanoic acid, etc. This showed the evolution status of myxomycete to some extent. Also confirmed the closer relationships between myxomycete and amoeba.

The analysis results of fruiting body of *D.chondrioderma* were compared with that of plasmodium. It showed that more than 50% chemical constituents of them were the same. This might related to that they were the different life stages of the same myxomycete. It also showed that constituents of fruiting body were richer than plasmodium's from the spectrogram. This might mean a more complex physiological and biochemical process in the fruiting body.

The results showed there were several similar even the same compounds in the same species, genera and families respectively, such as high content of 5-methyl-2-pyrrolidinone in the same order of Physarales. If it was a characteristic compound, we also need a lot of experimental data. Comparing



the analysis results of the spectrogram of *D. chondrioderma* with that of *D. crustaceum* and *C. leucocephalum*. It showed that the proportion of the similar even the same compounds in the same genera is higher than that in different genera. The results showed that the species which had the similar genetic relationship had more similar GC-MS spectrogram. Indicated their similar physiological and biochemical characteristics. So the chemical characteristics of myxomycete might be able to provide an important evidence of the study of genetic relationship of myxomycetes.

Key words: D.chondrioderma; D.crustaceum; C. leucocephalum; P. cinereum; fruiting body; plasmodium; GC-MS

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# Nuclear Observations of Physarum melleum

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**Abstract:** Nuclear condition and its behavior are very important to understand life cycle of Myxomycetes. Nuclear conditions of each step of life cycle were observed by Carl Zeiss Confocal Laser Scanning Microscope (LSM710) and its behavior was analyzed. Spores of *Physarum melleum* were used as materials as a first step of life cycle, and myxamoebae and plasmodia were cultured on media. For staining nuclei DAPI was used and its fluorescence was observed with argon laser under the designated conditions. Fluorescence images were acquired in the sequential mode and processed using the software ZEN2012. **Key words:** Nuclear; life cycle; confocal Laser Scanning; fluorescence



# Liposoluble Constituents Comparison from Five Species of Myxomycetes

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**Abstract:** Myxomycetes is a group of eukaryotic organism with special life cycles and produces a number of metabolites reported biological activities. In this study, we developed a method used to process the sporophores of myxomycete before extracting with petroleum ether. Liposoluble constituents from sporophores of 5 species, *Diderma crustaceum*, *Lycogala epidendrum*, *Stemonitis splendens*, *S. flavogenita* and *Arcyria obvelata* were exacted and analysed by GC-MS,.

The results demonstrated that the molecular structure of most liposoluble constituents from the sporophores of species belonged to different order was simple. Most of the liposoluble constituents were esters, phenols, aliphatic hydrocarbons, aliphatic acids, higher aliphatic alcohol, higher aliphatic aldehyde and aliphatic ketone and sterol. With closed relationships among the species, *S. flavogenita* and *S. splendens* had similar comopounds, such as alkanes, tetradecanal, oleic acids and unsaturated aliphatic acids. That also indicated the different liposoluble constituents among the orders. *A.obvelata* which belonged to Trichiales had a unique compound named ethyl citrate, which was not found in other samples belonged to Liceales, Physarales and Stemonitales.

In addition, more compounds of *S. splendens* were obtained by the modified method, such as oleic acid, stigmasterol. It indicated that the new method could increase the efficiency of spore wall broken and petroleum ether extraction.

Key words: Slime mould; sporophore; liposoluble constituent



# Description the Amoeboid Movement of Myxamoebae in Several Myxomycetes Species

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**Abstract:** In nature, myxomycete spores probably germinate and issue one or more myxamoebae. Myxamoebae is different from diverse slime molds, mainly different is the presence or absence of transparent ectoplasm and regularity or irregularity of the shape. Transparent ectoplasm may be responsible for the pattern of amoeboid movement. It is difficult to form typical pseudopodium for myxamoeba with thick ectoplasm, and exhibits gliding motility with slow speed. The others with less ectoplasm will form lobopodium or filopodium and exhibit flowing motility and creeping motility. In order to describe accurately the motility pattern of myxamoebae, In this paper, the myxamoebae of *Hemitrichia calyculata* and *H. clavata* (Trichiales); *Physarum melleum* and *P. globuliferum* (Physarales); *Stemonitis flavogenita* (Stemonitales) as the experimental material, with the help of graphing software, to paint trajectory and calculate movement velocity, draw the instantaneous perimeter of myxamoeba which show the change speed of pseudopodia. All above can visualize the movement characteristic of myxamoeba.

Key words: Myxamoeba; transparent ectoplasm; pseudopodium; amoeboid movement





# Isolates of the Entomopathogenic Fungus *Metarhizium Anisopliae* and Some Characterization of ITS Local Strain G09/22

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**Abstract:** It is being main aim to reduce the usage of chemical substances for plant protection, in Mongolian agricultural sector, by isolating and of course determining local strain that can fit in condition of our country specifically to control harmful pests that are having negative impact on the environment especially in the forest and pasture plants.

Thus, research work concentrated on isolating local strain of entomopathogenic fungi Metarhizium anisopliae and to determine its biological activity. Chronically suffered grasshoppers and moth larvae have been used as a research material and pure culture of Metarhizium anisopliae have been isolated in laboratory condition as well as the biological activation of local strain have been experimented on gypsy moth larvae (*Ocneria dispar Linn*) in the forest and grasshoppers (*Eclipophleps bogdanovi Serg.Tarb*) in pasture. Isolated strain of M.anisopliae has been identified by PCR using ITSMetarhizium-F and ITS4-R primers. In laboratory experiment local strain G09/22 of M.anisopliae at 2.5x10<sup>7-9</sup> spores/ml resulted in 85-90% mortality in grasshoppers. Therefore, isolated local strain of M.anisopliae is demonstrated to be an efficient biocontrol agent that can be used to control insect pests in plant protection.

Key words: Metarhizium anisopliae; local strain; grasshopper; moth larvae; PCR

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# Species diversity of myxomycetes on different decay stages of coarse woody debris in laurel forest of warm temperate western Japan

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Abstract: The distribution of myxomycete species on coarse woody debris (CWD) associating with decay state was little investigated in laurel forests of warm-temperate ecosystem in the world. Present study carried out entire year surveys during 2006-2008 and revealed seventy species on decayed evergreen logs from total 1079 samples. Myxomycetes occurred on various decaying stage of wood which contained sufficient moisture. Most species of 81 % in total species occurred on moderately decayed wood with the highest species diversity. Dominant species were four species, i.e. Ceratiomyxa fruticulosa (O.F. Müll.) T. Macbr., Lycogala epidendrum (L.) Fr., Physarum viride (Bull.) Pers. and Hemitrichia calyculata (Speg.) M.L. Farr. The 42 species recorded with eight or more samples were arranged in order of succession index corresponding to the stage of decay. Species of Physarales characteristically dominated on hard wood, while species of Trichiales did on decayed softer wood. According to decay stage dominant species were distinctive, most species had preference to moderately decay stage of wood, while several species occurred on hard and/or softer decayed wood. *Physarum viride* (Bull.) Pers. occurred on hard wood, C. fruticulosa on moderately decayed wood, L. epidendrum, Hemitrichia calyculata (Speg.) M.L. Farr, Arcyria denudata (L.) Wettst., and Arcyria cinerea (Bull.) Pers. on decayed softer wood, and Cribraria tenella Schrad. on brittle decayed wood. Consequently the myxomycete assemblages were the lowest similarity between the different stages of decaying progression, hard wood and brittle decayed wood. It was quantitatively revealed that the myxomycetes intently inhabit with the highest diversity on the moderately decayed CWD of the angiospermous evergreen trees.

Key words: Moisture; dominant species; Physarales

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Address: No. 2888, Xincheng Street, Jilin Agricultural University, Changchun, China. P.C.: 130118 E-mail address: zhuhe311@sina.com In the past 40 years, an excellent team on Myxomycetology has formed in China. And many outstanding students were trained and got the doctoral and/or master degree in the team. Some of their theses are listed in the following table.

No.	Name	Degree	Year	Supervisor	The titles of Doctor and Master theses
	V., L	Master	1981	Chung-Hwang	Taxonomic Studies on the Family
1	Yu Li			Chow	Cribrariaceae of China
2	Yu Li	Doctor	2000	Makoto Kakishima, Hiromitsu Hagiwara	Liceales in China
2	Ronglin Bai	Master	1981	Chung-Hwang	A Study on Some Species of
3				Chow	Acrasiomycetes
4	Zonglin Liu	Master	1981	Chung-Hwang	Notes on Myxomycetes from Jilin
4				Chow	Province
5	Yuefeng	Master	1983	Chung-Hwang	Study on the Moist Chamber Cultures of
J	Zhao			Chow	Myxomycetes
		Master	1988	Ziquan Wang, Yu Li	Taxonomic Studies on 4 Genera of
					Family Trichiaceae of China and
6	Qi Wang			TUE	Establishing of Genus Ditrichia
		Doctor	1994	Jinkai Bai,	Systematics Studies on Taxonomy of the
		Doctor		Yu Li	Order Trichiales from China
	Chuonalin	Master	1990	Ziquan Wang,	Study on Arcyria and Its Two Related
7	Shuanglin Chen			Yu Li	Genera in China
,	Chen	Doctor	1996	Jinkai Bai,	Systematics Studies on Taxonomy of the
				Yu Li	Genus Physarum from China
		Master Doctor	1997	Yu Li	Studies on the Use of PCR in Phylogeny
					of Myxomycetes
8	Shuyan Liu		2000	Yu Li	Sequencing of 19S rDNA from Some
					Main Taxa of Myxomycetes for
					Molecular Systematic Studies
	Liping Shi	i Doctor	2003	Yu Li	Studies on Life Cycle of Some
9					Myxomycetes in Physarales and
					Trichiales
	Xiaoli Wang <sup>1</sup>	Doctor	2004	Yu Li	Micro-ultrastructure Studies on
10					Phaneroplasmodium and Nuclear
					Matrix of Myxomycetes
	Xiujuan Bai	Postdoctor	2003	Yu Li	DNA Molecular Systematic Analysis of
11					Main Slime Mold Population
40	Shicui Jiang	Postdoctor	2014	Yu Li	Studied on Biology in Different
12					Ontogenetic Stages of Didymium iridis
10	Jingzhi Pan	Doctor	2010	Qi Wang	Ontogeny and Molecular Systematics of
13					Main Taxa in Myxomycetes

A list of the Post-doctor, Doctor and Master theses from the Chinese Myxomycetology Team

14		Doctor	2010	Yu Li	Studies on Taxonomy and Molecular
	Pu Liu				Phylogeny of Dictyostelid Cellular Slime
					Molds
15					Development Characteristics of
	Xiaoshu	Doctor	2011	Qi Wang	Vegetative Stage and Phylogenetic
	Chen		2011		Relationship of Major Species of
					Myxomycetes
	Dongxia Lv		2012	Yu Li	Studies on Microstructure and
16		Doctor			Development Regularity in Different Life
					Cycle Stage of Several Myxomycetes
			2004	Qi Wang	Mycological and Chemical Proof of
		Master			Systematic Taxonomy on Trichiales
	_				Myxomycetes
17	He Zhu				Studies on Ontogeny and Chemical
		D	2012	0. 11	Constituents of Myxomycetes from
		Doctor	2012	Qi Wang	Representative Regions in Northern
					China
				Qi Wang	Studies on Phylogenetic Relationships
	<b>T</b> 7				among Representative Groups of Four
18	Xiaoxia	Doctor	2013		Orders in Myxomycetes Based on Living
	Song				Habit, Morphogenesis and Molecular
					Characteristics
	Qisha Liu	Doctor	2014	Shuanglin Chen	Studies on Molecular Systematics and
19					Species Diversity of Myxomycetes from
					Conifer
	Qun Dai	Doctor	2014	Shuanglin Chen	The Research of Species Diversity and
20					Flora about Myxomycetes in Qinling
	Wenchen Gao	Master	1994	Yu Li	Systematics of Didymiaceae
21					Myxomycetes in Northeast China
	Juntao Cui	Master	1994	Yu Li	Systematics of Dictyostelids in Northeast
22					China
	Baojun Shi	Master	1999	Yu Li	Systematics of Stemonitaceae
23					Myxomycetes in Northeast China
24	Xinyu Li	Master	2002		The Study of Moist Chamber Culture on
				Yu Li	Myxomycetes
25	Le Yang	Master	2004		The Biodiversity of the Myxomycetes of
				Tolgor Bau	the Changbai Mountains in Jilin Province
	Yabo Zhang	Master	2006		Studies on the Molecular Systematics of
26				Shuyan Liu	Physaraceae and Trichiales
27	Meiqin Xu	Master	2006		A Preliminary Study on Corticolous
				Shuanglin Chen	Myxomycetes from Conifer
28	Ping Chen	Master	2006	Shuanglin Chen	
					Studies on Taxonomy and Floristics of
					Myxomycetes in Yunnan-Guizhou
					Altiplano

29	Yanshuang Li	Master	2007	Yu Li	Culture and Microstructure and Ultrastructure Study on <i>Fuligo septica</i> and <i>Physarum compressum</i>
30	Xiaolan He	Master	2008	Yu Li	Preliminary Taxonomic Studies on Dictyosteliaceae in China
31	Xue Wang	Master	2008	Yu Li	The Preliminary Studies on the Comparative Genomics of <i>Dictyostelium</i> <i>discoideum</i>
32	Chenguang Liu	Master	2008	Shuyan Liu	Studies on the Amplification and Molecular Phylogenetic Analysis of Elongation Factor 1A in Myxomycetes
33	Jiage Song	Master	2010	Shuyan Liu	The Molecular Systematics of Some Myxomycetes of Physarales Base on Three Gene Sequences
34	Fujie Liu	Master	2010	Qi Wang	The Study on Moist Chamber Culture of Myxomycetes and Liquid Culture of Several Plasmodium
35	Huiqin Yao	Master	2010	Shuanglin Chen	The Species Diversity Research of Myxomycetes in East China Hilly Ground
36	Shuo Gu	Master	2011	Qi Wang	Study on Culture, Biological Characteristics and Phagocytosis to Bacteria of Plasmodia in Major Species of Myxomycetes
37	Ming Li	Master	2011	Yu Li	Studies on Myxomycetes Diversity of Liaoning Province
38	Xu Zhang	Master	2011	Qi Wang	DNA Molecular Systematic Analysis of Some Species in Physarales and Stemonitales
39	Jinyue Sun	Master	2011	Yu Li	Study on the Life Cycle of Important Taxa in Dictyosteliaceae
40	Xiaoli Wang <sup>2</sup>	Master	2011	Xiaoli Wang <sup>1</sup>	Study on Microstructure and Ultrastructure about Each Stage of Life Cycle of <i>Physarum Mellrum</i>
41	Ying An	Master	2012	Yu Li	Preliminary Studies on Fatty-acid Detection and Its Application in Taxonomy of Dictyostelid Cellular Slime Molds
42	Shu Li	Master	2012	Qi Wang	Gene expression in Ontogenetic Stages and Phylogenetic Relationship of Representative Species of Myxomycetes
43	Haiyan Yuan	Master	2012	Yu Li	Studies on Taxonomy of Dictyostelid Cellular Slime Molds in the Southwest of China

					The Evaluation of Barcode Gene for
44	Ao Zhang	Master	2012	Shuyan Liu	Molecular Identification of Fuligo and
					Related Genera
45	Xiaomin Zhu	Master	2012	Shuyan Liu	The Molecular Phylogeny of Fuligo
45					Based on EF1A gene sequences
	Yisi Ma	Master	2012	Qi Wang	Nutrient Absorption Regularity and
46					Elemental Analysis of The Different
					Growth Stages of Myxomycetes
	Ning Jiang	Master	2013	Qi Wang	Study on Chemical Ingredients and
47					Activities of Major Representative
					Species of Physarale Myxomycetes
		Master		Yu Li	Studies on Distribution of Dictyostelid
48	Yuzhuo Ren		2013		Cellular Slime Molds in the Tropic and
					Frigid Zone of China
	Chao Li	Master	2013	Yu Li	Studies on Taxonomy of Dictyostelid
49					Cellular Slime Molds in Henan and
					Hunan Province
-0	Ling Fang	Master	2013	Shuanglin Chen	A Preliminary Study on Actin Gene and
50					β-tubulin of Myxomycetes
51	Fengyun Zhao	Master	2014	Shuyan Liu	Molecular Phylogeny of Myxomycetes
	Wan Wang	Master	2014	Qi Wang	Research on Biological Activity and
52					Phylogeny from Certain Myxomycetes of
					Trichiales and Physarales
F.2	Mingjun	N	2014	Yu Li	Studies on Taxonomy of Dictyostelid
53	Zhao	Master			Cellular Slime Molds of Eastern China
F 4	Tianpeng	Master	2014	Shuanglin Chen	Species Diversity of Myxomycetes in
54	Song				Huanglianshan Reserve Yunnan
	Mingquan Guo	Mastar	2014	Shuanglin Chen	Species Diversity of Myxomycetes in
55		Master			Daweishan Reserve Yunnan
	Qian Li	Master	2014	Shuanglin Chen	ITS Structure Analysis and
56					Significance in Systematics of
					Myxomycetes