Discovery of biologically active fungal metabolites resulting from an international, interdisciplinary research scenario

Marc Stadler
Helmholtz-Centre for Infection Research, Germany

Over the past years, we have been able to build up a sustainable, international network with leading researchers from all over the world to explore systematically the mycobiota of tropical countries for their potential to produce novel chemical entities with potential to combat infectious diseases. In addition, we have targeted rare European species that are difficult to culture.

Over the past 5 years, these activities have resulted in the discovery of over 150 new bioactive metabolites that were published in over 50 original publications. The key to the success of these projects was actually the collaboration of chemists with leading taxonomists and other biodiversity researchers. Most of the new compounds were isolated from new genera and species that were concurrently discovered in the course of taxonomic studies. Some of the new metabolites discovered have substantial potential for application, even though their evaluation is still in a rather early stage and it may take a long time and substantial efforts and additional funding until they even reach preclinical development. The strategy of this approach will be outlined, also including some highlights from our recent research in an international, interdisciplinary scenario.
Cordyceps and cordycipitoid fungi

Xingzhong Liu
Institute of Microbiology, Chinese Academy of Sciences, China

Cordyceps historically comprised over 400 species and some of them are used extensively in traditional Chinese medicine. In the past few decades, the pharmaceutical and cosmetics, health products developed from cordyceps have made great progress of research and development of cordyceps. However, there is different understanding on cordyceps and cordycipitoid fungi between mycologists and Chinese medicine scientists because mycologists emphasize phylogenic relationship while medicinal scientists emphasize medicinal function. Here “cordyceps” is proposed as a common English word to represent the natural or cultivated entities of the fungi on their hosts. The most research progresses are that many new taxa have been discovered in China and other Asia countries and the -omics studies have revealed the mechanisms of evolution and biology of cordycipitoid fungi and their interaction with host insects. In the meanwhile, Chinese cordyceps, a valuable Chinese traditional medicine and national fungus of China, its successful cultivation ex situ by a detailed investigation into the cycle of Thitarodes xiaojinensis infected by Ophiocordyceps sinensis has created a large industry. Breakthroughs in artificial cultivation of Chinese cordyceps will certainly benefit the scientific research, environment protection of Tibet-plateau, human health and social progress.
Current world health issues are referred to frequent appearance of emerging and re-emerging infectious diseases and the increase of multifactorial diseases under increased population of elderly people and changing life-style and environment, in addition to cancer disease, heart disease and cerebrovascular diseases etc.

Traditional Japanese herbal (Kampo) medicines have played important role in the modern medicine of Japan to complement modern western medicines. Kampo medicines have been used as the multi-herbal formula which composed of plant, fungi, animal and mineral-derived traditional herbal medicines. Several important drugs such as ephedrine, quinine, and artemisinin etc. have been discovered from such traditional herbal medicines. Novel immune suppressant, fingolimod has discovered from the constituent of Cordyceps, Isaria sinclaiii, and developed as the drug for multiple sclerosis.

During past over 30 years, we have studied to clarify pharmacological actions and active ingredients of Kampo medicines as the multi-ingredients drugs to confirm the clinical efficacy for the evidence and science-based medicines. We also have found anti-influenza viral, anti-malaria, anti-Trypanosoma parasites, anti-depressive, adjuvant, and anti-cancer substances from the plant and fungi-derived herbal medicines by in vitro and in vivo bioassays. These include not only low molecular weight ingredients but also bioactive polysaccharides. In this lecture, I’d like to introduce these our research findings. Our results indicates that Kampo medicines are very effective to recover complicated symptoms caused by disturbance of the body system such as immunological, endocrine and neural systems, and traditional medicines are also useful resource for the development of Western drug and nutraceutical.
Some of the missing fungi, hidden right in front of us

Jennifer Luangsa-ard
Plant Microbe Interaction Research Team, BIOTEC, Thailand

The BIOTEC culture collection houses 50,000+ isolates and the largest component are insect fungi with 12,000+ isolates, comprising ca. 25% of the collection. The specimens, which are derived from surveys made from natural forests, community forests and agricultural ecosystems in Thailand, are stored in the BIOTEC Bangkok Herbarium. The herbarium consists of 45,000 specimens with insect fungi representing 47% of the collection. Ultimately these fungi are screened for novel metabolites and enzymes in BIOTEC laboratories as well as assessing potential for sources of biological control candidates.

The insect fungi of the collection are mainly the hypocrealean genera contained in three families: Clavicipitaceae, Cordycipitaceae and Ophiocordycipitaceae. Research at BIOTEC has been fundamental in supporting the revision of the group of these hypocrealean insect fungi using molecular phylogenetics. While morphological characterization allows preliminary identification of the family or the genus, species recognition is difficult because of its structural simplicity, lack of distinctive phenotypic variations leading to some species being erroneously classified under one name.

Purpose: To unravel cryptic species in insect fungi

Methods: DNA barcoding using ITS as well as multi-gene analyses comprising the large subunit of the ribosomal DNA (LSU), partial regions of the elongation factor 1-alpha (tef), the largest and second largest subunits of the RNA polymerase genes (rpb1, rpb2) were used. Morphological studies of important phenotypic characters were done in parallel.

Results and conclusions: Initial ITS DNA barcoding shows some isolates belonging to genetically distinct lineages, and multi-gene phylogenies using ITS and LSU rDNA, tef, rpb1, and rpb2 supported these results. Importantly, this work is also linked with traditional morphological based approaches. As a result, many new species and genera have been, and are being, described from Thailand.
Genetic characterization of mating system and population diversity in *Ustilago esculenta*, a smut fungus associated with an oriental vegetable

Wei-Chiang Shen\textsuperscript{1,2)}

\textsuperscript{1}Department of Plant Pathology and Microbiology, National Taiwan University, Taiwan
\textsuperscript{2}Mycological Society of Taiwan

*Zizania latifolia* is a perennial aquatic plant that belongs to the family Poaceae. On infection with the smut fungus *Ustilago esculenta*, swollen gall is developed at the basal part of the plant and becomes a favorable vegetable known as “water bamboo, water oat, or *jiaobai*”. Development of edible galls is affected significantly by *U. esculenta*; however, studies related to its genetic features and infection-related processes are limited. To reveal the mating and population features of *U. esculenta*, we have extensively isolated strains from the field materials in Taiwan and Japan. By conducting molecular and genomic studies, we identified three idiomorphs of the mating type locus among collected strains. Screening of meiotic offspring and field strains by multiplex PCR and mating assay, we confirmed the bipolar heterothallic mating system in *U. esculenta*. The MAT-1 locus of *U. esculenta* is 552,895 bp, the largest mating type locus reported so far in fungi, and contains 44% repeated sequences. Sequence comparison revealed that *U. esculenta* MAT-1 shares great gene synteny with other smut fungi and may evolve from a common ancestor with *S. reilianum* due to the chromosomal translocation of P/R and HD loci with an identified intermediate. To further reveal the genetic diversity of *U. esculenta* population, 13 simple sequence repeat markers have been developed to screen for field strains. The results revealed that *U. esculenta* strains are separated into two major clustering groups, i.e. T and MT strains. Additionally, T strains showed higher genetic polymorphism as compared to MT strains. In conclusion, our findings have revealed genetic features of *U. esculenta* population and sexual production and the information will be utilized for commercial breeding and further studies of detailed mechanism of gall formation.
Recent Trends of Mushroom Industry and Mushroom Research in Korea

Geon-Sik Seo, Min-Kyung Kim
Korea National College of Agriculture and Fisheries, Korea

Mushroom cultivation in Korea has begun to 1922 by Shiitake, then, common mushroom (Agaricus bisporus) was started in 1955. Mushroom cultivation in the 1960s and 1970s was the purpose of export. However, since the 1980s, the purpose of mushroom cultivation was converted to domestic consumption. Oyster mushroom, king oyster mushrooms, winter mushroom, common mushroom and shiitake mushrooms are mainly cultivated as edible mushrooms in Korea. In addition, Ganoderma lucidum, Phellinus spp., Agaricus brazei and Cordyceps spp. have been cultivated to medicinal mushrooms. Recently, the functional edible mushroom such as Hypsizygus marmoreus, Auricularia auricula, Pleurotus cornucopiae, Hericium erinaceus and Sparassis crispa are also slightly production. In Korea, mushrooms are produced by mycelial bed-, logs and automatic facilities cultivation. Mushrooms are produced in the form of cultivation of germ, cultivation of logs and automation. Automatic facilities cultivation of mushroom was started in the mid-1980s by the winter mushroom. One of the causes of the King oyster mushroom and Winter mushroom production has increased rapidly, introduction of the liquid spawn which is characterized to cheaper, faster and mass production for spawing compared with sawdust spawn. The emergence of large-scale farms and their improve productivity were show the average annual productivity gains of up to 5% since the 1990s. The cultivation of Shiitake mushrooms has changed from traditional logs cultivation to substrate cultivation using a sawdust medium. Mushroom researches in the 1980s were conducted for the increase productivity, pest control and the development of substrate materials. In the 1990s, genetical relationships, functional component analysis, processing and production cost reduction were mainly studied. In addition, Golden Seed Project for development of mushroom cultivar was started in 2013. Recently, smart farm systems have been introduced in mushroom cultivation.
Fungi are the cause of 1.7 billion human infections. Invasive fungal infections causing 1.6 million death/year, more than malaria and tuberculosis together. They are treatable if diagnosed in a timely matter. The increasing use of antifungals in agriculture/medicine leads to a rise in resistance, making fungal identification a priority at a time where not even a fifth of the 5.2 million species estimated to exist on earth have been described and no accurate species borders are in place. DNA barcoding is a culture independent approach to identification. In 2012, the primary fungal DNA barcode (internal transcribed spacer (ITS1/2) region) was selected, with BOLD containing 154,123 ITS barcodes of 29,177 species. The first quality-controlled database for human/animal pathogenic fungi (ISHAM-ITS database) was established in 2015 (4200 ITS sequences of 645 species). The ITS region is only able to correctly identify 75% of all fungi, making the introduction of the translation elongation factor 1α (TEF1α) as a secondary DNA barcode necessary (2432 TEF1α sequences of 346 species). TEF1α shows less intra-species and higher discriminatory power at inter-species level than the ITS. Next Generation Sequencing (e.g. MinION™ Oxford Nanopore Technologies) allows for a high throughput, real-time, long-read (average 10-15kb) based simultaneous identification of complex samples. To assess the ability of metagenomic sequencing the MinION™ was used to identify the unculturable fungus Pneumocystis jirovecii directly from respiratory specimens and to characterise the associated mycobiome. P. jirovecii was detected in bronchoalveolar lavage and induced sputum samples. False-positive and error-prone reads are major problems for metagenomics, calling for the development of better algorithms and reference databases.
Modern Trichocomaceae taxonomy and its implication for applied research

Robert A. Samson
Westerdijk Fungal Biodiversity Institute, The Netherlands

The Trichocomaceae contains genera which are important for several disciplines of applied research. These genera have a common and worldwide occurrence and they play a beneficial role in biotechnology, fermentation of food and beverages. However, others can be pathogenic for man and animals, spoil food, produce mycotoxins or occur as undesirable organisms in the living environment. After a historic overview the new phylogenetic studies of the family and the important genera are discussed. In the last decade much progress has been made to clarify the taxonomic schemes of the genera and the current state of Aspergillus and Penicillium is presented. The current classification and the species delimitation are based on a polyphasic taxonomic approach. The advantage of this approach is that besides phylogenetic, phenotypic characters also the biochemical properties, such as the production of secondary metabolites including mycotoxins are often revealed. Particularly for the species on food this is a valuable addition. Some examples will be discussed where the polyphasic species concept has a significant input in the assessment of the spoilage of food. For the investigation of food spoilage and the estimation if toxic compounds were produced, the correct taxon identification can contribute to this. Although the species concept in the genera is generally now well-accepted and recognized, current taxonomic delimitation is often based using phylogenetic characters. This means that in some cryptic species the phenotypic and phylogenetic characters are not congruent and that correct morphological identification is problematic. In applied research such as indoor mycology where numerous samples have to be examined in a short time, identification using molecular methods is difficult.
Plenary Symposium

PS-3

Taxonomy of *Ophiostoma breviusculum*-like fungi in Japan

Yuichi Yamaoka
University of Tsukuba, Japan

Purpose: *Ophiostoma breviusculum* (Ophiostomatales, Ascomycota) is a *O. piceae* complex species associated with the bark beetles infesting Japanese larch (*Larix kaempferi*), in Central Honshu, Japan. During a survey of ophiostomatoid fungi, we found another group (*Ophiostoma* sp. B) of the *O. piceae* complex species similar to *O. breviusculum*. Purpose of our study was to determine the taxonomic position of *Ophiostoma* sp. B. In this presentation, we focus on an example of conflicts among the results of different species concepts.

Methods: Colony appearance, microscopic morphology, molecular phylogenetic analysis of the sequences of internal transcribed spacer (ITS) region of rDNA and β-tubulin genes (βT), and mating compatibility tests were conducted using isolates of *Ophiostoma* sp. B and *O. breviusculum*.

Results and conclusions: *Ophiostoma* sp. B differed in colony appearance from *O. breviusculum*, but was indistinguishable in microscopic morphology and molecular phylogeny using βT gene as well as ITS. The mating compatibility test, showed two incompatible groups (Group 1 and Group 2) within *O. breviusculum*. They were indistinguishable based on colony appearance, microscopic morphology and molecular phylogeny. However, *Ophiostoma* sp. B was able to mate with Group 2, but not with Group 1. The biological species concept is one of the most popular species concepts. In the *O. piceae* complex, a mating compatibility test is used sometimes. However, the ability to mate is different among species and isolates. Therefore, the results of the mating compatibility test should not be too highly trusted. We recommend the use of morphology and molecular phylogeny rather than mating compatibility to distinguish between species of the *O. piceae* complex, because species delimitation by these two factors coincides.
Ash die back: Lessons and perspectives from the "ground zero"

Tsuyoshi Hosoya
National Museum of Nature and Science, Japan

Purpose: Ash dieback is a disease of European Fraxinus trees caused by Hymenoscyphus fraxineus. The disease rapidly spread, and now covers almost entire distribution of European ash trees. Interestingly, the pathogen was first isolated in Japan and identified as Lambertella albida in 1993, so Japan is "ground zero" of this fungus. In the present talk, I review the history and discuss future perspectives of this disease.

Methods: The history of the identity of the pathogen was reviewed. Genome analysis was implemented.

Results: The fungus was first isolated and described in 2006 in Europe as Chalara fraxinea, and later connected with its teleomorph Hymenoscyphus pseudoalbidus. Due to the “1F=1N” principle, H. fraxineus was later proposed. The identity of Japanese isolates with European isolates was confirmed in 2014. Based on the molecular phylogenetic analysis incorporating Lambertella, Hymenoscyphus and their allies, the taxonomic placement to Hymenoscyphus was confirmed. Population genetic studies revealed that the genetic diversity including SNPs was far greater in Japan compared to Europe, and the EU population was assumed to be founded by the two diverse individuals from Asia. Using the species specific primers, the endophytic phase of the fungus was recently revealed. Based on the genome analysis, more than thousand genes of effector candidates have been recognized.

Conclusions: The review of H. fraxineus also provokes interest in consideration of consequences of artificial transfer in Anthropogenic era, and extinction of H. albida the original habitants in the same habitat in Europe.
Purpose: The effects of the Last Glacial Maximum in the Japanese Archipelago have been extensively assessed for tree species. However, the effect of this environmental event on the populations of saprophytic fungi is still poorly understood. This study aimed to examine genetic diversity, past environmental variables, and past demographic fluctuations of Dasyscyphella longistipitata and Fagus crenata to understand the historical events that shaped the current distribution of the genetic diversity of D. longistipitata in a space-time context.

Methods: 274 D. longistipitata isolates from 14 localities were genotyped using ITS and Beta tubulin sequences to estimate the genetic diversity and structure. Distribution modeling analyses were performed to evaluate the influence of past environmental changes in the distribution of D. longistipitata and F. crenata. Finally, the past demographic trends and dispersion routes were estimated using coalescent-based analytical tools.

Results and conclusions: The analysis displayed high levels of genetic diversity of D. longistipitata with low structure. The distribution models displayed past environmental changes that lead to shifts in habitat suitability. Also, the estimated effective-size in both species suggested a historical demographic growth while dispersion routes corresponded to areas of environmental suitability. The results resembled the strict relationship between D. longistipitata and F. crenata.
Unveiling deep-sea fungal diversity, ecology and potential exploitation: examples from Mexican oceans

Patricia Velez
Universidad Nacional Autonoma de Mexico, Mexico

Purpose: Fungi seem to be successful deep-sea colonizers, with remarkable abilities to adapt to adverse conditions. However, deep-sea fungal communities remain largely unexplored, particularly in the American waters. Herein, we explored deep-sea ecosystems in the Gulf of California and Gulf of Mexico, approaching deep-sea fungal diversity, ecological patterns and potential utilization.

Methods: Fungal isolates were obtained from both littorals using classical culture-based techniques. Preliminary taxonomic diversity estimates were obtained for the Gulf of California through Illumina metabarcoding. In addition, fungal isolates from the Gulf of Mexico were explored for their potential use as candidate species in oil bioremediation, analyzing by differential transcriptomics the genetic basis of hydrocarbons breakdown.

Results and conclusions: In view of their adaptations to bear environmental stress, high abundance, and diversity of ecological strategies, fungi might represent valuable genetic resources for exploitation. Our results on fungal ecological patterns in the recently discovered hydrothermal vents system from Pescadero Basin (including carbonate and sulfide chimney structures) suggest a remarkable fungal diversity. Moreover, we identified fungal isolates with the ability to tolerate and use hydrocarbons as the sole carbon source, presenting evidence from differential expression analyses. Overall, our findings highlight the importance of integrating culture-dependent and independent approaches in deep-sea fungal exploration. These efforts contribute to a better understanding of the deep-sea ecosystem dynamics, and the utilization of deep-sea derived fungal taxa as valuable genetic resources.
Fungal-bacterial interactions in an oxygen minimum zone and a deep-sea hydrothermal system of Mexico

Abril Hernandez-Monroy\textsuperscript{1,2), Laura Espinosa Asuar\textsuperscript{2), Valeria Souza\textsuperscript{2), Luis Soto\textsuperscript{3), Diana L. Salcedo\textsuperscript{3), Patricia Velez\textsuperscript{1)}}

\textsuperscript{1)Instituto de Biologia, Universidad Nacional Autonoma de Mexico, Mexico
\textsuperscript{2)Instituto de Ecologia, Universidad Nacional Autonoma de Mexico
\textsuperscript{3)Instituto de Limnologia y Ciencias del Mar, Universidad Nacional Autonoma de Mexico

Purpose:} Oxygen minimum zones and hydrothermal systems are deep-sea unique and extreme ecosystems where temperature, pressure, and nutrients play a key role modeling microbial communities. In these extreme environments microbial ecological interactions are fundamental for life, though these remain poorly studied. Therefore, in order to elucidate some of the microbial interactions, we evaluated long-term interactions among bacteria and fungi by using a set of growth indexes.

Methods: We isolated and identified fungi and bacteria from sediments collected in an oxygen minimum zone, infiltrations and hydrothermal vents in the Pescadero Basin (Gulf of California) at Western Mexico. Cross-kingdom interaction bioassays were implemented and documented with a select group of organisms for up to six months.

Results: Competition and cooperation patterns were detected, with competition and antagonism as the prevailing interactions. This was expected considering ecological theories such as the Hutchinson niche and the Red Queen. Microbial morphological changes and interaction types may be related to fluctuations in resource availability. Remarkably, the establishment of peculiar cross-kingdom interactions was detected. These interactions were characterized by morphological modifications, where fungi and bacteria merged growing in close proximity, forming digitiform projections.

Conclusion: The tested microorganisms dominantly established antagonistic cross-kingdom interactions. Morphological modifications in some key interactions may represent a prokaryotic adaptive trait to colonize challenging environments. This study sheds light into the network of interactions co-occurring into deep-sea unique ecosystems.
Lifecycle and ecology of *Pyrenopeziza protrusa* (Helotiales, Dermateaceae sensu lato) in Japan

Hiyori Itagaki¹), Hosoya Tsuyoshi²)
¹Graduate School of Science, The University of Tokyo, Japan
²National Museum of Nature and Science, Japan

**Purpose:** To clarify the versatility of the saprophytic fungi with high host selectivity, we focused on *Pyrenopeziza protrusa*, which forms apothecia solely on the fallen leaves of *Magnolia obovata*.

**Methods:** The fungal specimens were collected across Japan, and the lifecycle and ecology was studied in Tsukuba botanical garden in detail. The intraspecific phylogenetic analysis and molecular detection of the fungal DNA from the plant by PCR and RT-PCR with the primers developed for the present study were carried out. Apothecial development was observed.

**Results:** *Pyrenopeziza protrusa* showed relatively high genetic diversity in ITS-5.8S rDNA, but showed no genetic structure among local populations. The fungus was well defined by the barcoding region and probably forms a single population covering wide areas in Japan.

The DNA concentration of *P. protrusa* showed drastic increase in the fresh leaves just before the defoliation and kept high quantity in fallen leaves. The fungus was isolated from fresh leaves only in October, while it was more frequently isolated from fallen leaves throughout the year. From these results, this fungus presumably switches the mode of life from endophytic to saprophytic when the leaf senesce.

Because *P. protrusa* was detected from the roots of the seedlings, it probably shows systemic infection. The apothecia occur underneath the epidermal layer of the plant tissue and then penetrate though the epidermis when matured. The microconidia of *P. protrusa* were discovered next to the immature apothecia.

**Conclusion:** This study elucidated the versatility of the fungus previously known only as saprophyte.
Linking fungal wood decay functions to forest dynamics

Yu Fukasawa
Tohoku University, Japan

**Purpose:** The decay process of deadwood is crucial for biodiversity in forest ecosystems. Wood decay types, traditionally categorized into white, brown, and soft rots, are the consequences of fungal decay activities and strongly affect biotic communities inhabiting deadwood, including tree seedlings. Given that fungal community is affected by climatic conditions, it is important to evaluate the occurrence patterns of the decay types along a geographical range to understand forest dynamics in wide spatial scale.

**Methods and Results:** Field surveys in 30 sites in Japan and 15 sites in Europe revealed a clear latitudinal gradient in decay type distribution, which is significantly associated with climate condition (temperature, precipitation, and their seasonality). Fungal community variations in deadwood detected by DNA metabarcording are also explained by those climate conditions. Incubation experiments using fungal strains obtained from pine deadwood showed that hyphal growth rates of brown rot fungi were significantly higher than that of white rot fungi in warm conditions (25-35°C) whereas no difference was detected in cooler conditions, suggesting that activity of brown rot fungi is more prominent in the warmer lower-latitude areas than in the cooler higher-latitude areas in pine log decomposition in Japan. I also examined the effects of wood decay type on seedlings growing on pine and spruce logs and found that responses to brown rotted wood was considerably different among tree species.

**Conclusions:** These results suggested that wood decay function of fungal communities could affect seedling regeneration and forest dynamics, reflecting biogeography of wood decay fungi.
Wood decomposition is a process dominated by fungi. These fungi access and metabolize wood carbohydrates using varied approaches. "White rot" type fungi use peroxidase enzymes to catalyze oxidative reactions that target and remove lignin, before hydrolyzing carbohydrates to release monomer sugars. "Brown rot" type fungi, on the other hand, use reactive oxygen species (ROS) to loosen lignocellulose and enable more selective extraction of sugars embedded in lignin. As genomes have become increasingly available, however, a new "Grey rot" type has been discussed among fungi that blur the line between brown and white rot. Some fungi, for example, degrade lignin and produce white rot-like residues without having any genes to code for lignin-targeting peroxidases.

This presentation will explore the genomes of these unique fungi, along with the wood physiochemical patterns produced along this spectrum of decay types. Then, this information will be contextualized in lumber pest management, biomass processing, and ecology. With wood harboring 80% of Earth’s aboveground biomass carbon, these variable fates for lignin at the hands of a relatively select group of fungi have enormous potential to shift.
Marker gene profiling toward larger sample size and lower biases using MGISEQ platform

Zewei Song
BGI-Research, China

Inference community composition from marker genes has come to the age of flourish, which researcher without much molecular background can use it as a common tool. The future for the usage of marker genes, is however, facing various challenges. The science community need next generation marker gene profiling to be accurate, fast, and at a lower cost so that it can fit into a large-scale research accommodating the increasing global collaborations. Here we present an alternative platform for marker gene profiling using the MGISEQ platform. By using short primers, and a new sequencing chemistry called CPas and pattern array, we have achieved a much higher throughput of marker gene profiling at the same cost as the current options. We have benchmarked our solution with the current option using 1,276 soil samples from three 20 ha permanent forest plots. We concluded with layout of near future plan including a multi-PCR solution for library prep and a new algorithm for utilizing multiple regions.
Meta-multi omics analyses of wood decay in nature

Chiaki Hori
Research Faculty of Engineering, Hokkaido University, Japan

**Purpose:** Wood decay fungi play a key role in depolymerizing, degrading and mineralizing the major components of woody cell walls, including cellulose, hemicellulose and lignin. Only a fraction of the species has been isolated in pure culture. Moreover, the laboratory conditions employed with model white rot and brown rot fungi for cell wall degrading enzymes (CAZymes) fail to mimic natural decay processes. To identify key enzymes and further understand lignocellulose deconstruction, we have examined the metatranscriptome and metaproteome of extensively decayed lodgepole pine (*Pinus contorta*).

**Methods:** Three samples of decayed lodgepole pine were collected in the two different locations, U.S. Forest Service's Tenderfoot Creek Experimental Forest (TCEF) and Gibbon's Pass on the Bitterroot National Forest (GP). Poly RNA and proteins were extracted and applied to HiSeq and LC-MS/MS, respectively.

**Results and conclusions:** This study suggested that lodgepole pine decay mainly involves the combined activities of functionally diverse enzymes from various wood decay fungal species. The known synergistic actions of conventional endo- and exo-cellobiohydrolases in vitro were detected in the environment. More recently, LPMO has been shown to boost the performance of these hydrolases, which worked in the environment as well. We also found genes most closely related to other fungi, slime molds and protists as well as several bacteria many of which have been associated with decomposition. The enzymatic machinery and interactions among these species merit further investigation.
A novel single-stranded RNA virus displays a circular genomic form in the phytopathogenic fungus *Sclerotinia sclerotiorum*

Jichun Jia¹², Fan Mu¹², Jiasen Cheng¹², Yanping Fu², Daohong Jiang¹², Jiatao Xie¹²

¹State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, China
²Hubei Key Laboratory of Plant Pathology, College of Plant Science and Technology, Huazhong Agricultural University, China

Mycoviruses are ubiquitously found in all major taxa of fungi, including phytopathogenic fungus *Sclerotinia sclerotiorum*. *S. sclerotiorum* is a cosmopolitan fungal pathogen and causes sclerotinia stem rot in many important crops including rapeseed plant (Brassica napus). Previously, we identified two mycoviruses, SsReV1 and SsBRV1, from a hypovirulent strain SCH941 of *S. sclerotiorum*. Here, we discovered another novel +ssRNA virus, tentatively named *Sclerotinia sclerotiorum* yado-kari virus 1 (SsYkV1), from strain SCH941 via metatranscriptomics sequencing technology. The full genome of SsYkV1 was obtained and it has an unsegmented positive-sense RNA genome of 5256 nucleotides excluding the poly(A) tails. SsYkV1 possesses a single open reading frame that encodes a single polyprotein of 1372 amino acid (aa), which showed 51.69% identities to Rosellinia necatrix yado-kari virus 2 (RnYkV2). Phylogenetic analysis based on the conserved RNA-dependent RNA polymerase showed that SsYkV1 is phylogenetically related to RnYkV1, RnYkV2, Fusarium poae mycovirus 2. These related mycoviruses were grouped an independent cluster belonging to a newly proposed family Yadokariviridae. Mapping of sequencing reads to the virus genome revealed the existence of bridge reads encompassing the 3’ and 5’ terminus, suggesting that SsYkV1 can form circular, which was confirmed by inverse RT-PCR. In summary, we reported a new member of the Yadokariviridae with circular genomes, but its function in virus replication and maintenance are still unknown.
A novel double-stranded RNA mycovirus isolated from *Trichoderma harzianum*

Beilei Wu, Xiliang Jiang, Chenchen Liu
Institute of Plant Protection, Chinese Academy of Agricultural Sciences, China

**Purpose:** Trichoderma spp. is used extensively in agriculture as a biological control agent to prevent soilborne plant diseases. In recent years, mycoviruses from fungi have attracted increasing attention due to their effects on their hosts, but Trichoderma mycoviruses have not been the subject of extensive study. We sought to discover novel mycoviruses from Trichoderma spp. and determine the effects of the biocontrol function of Trichoderma spp.

**Methods:** Mycoviruses were screened by dsRNA extraction and metagenomics. RT-PCR, 5' RACE, and 3' RACE were used to obtain the genome sequence. MEGA software was used to classify the new mycovirus. The effects of the mycovirus on biological properties of the host strain 525 were evaluated using cucumber plants and Fusarium oxysporum f. sp. cucumerinum.

**Results:** A novel mycovirus, Trichoderma harzianum mycovirus 1 (ThMV1) (accession number MH155602), was discovered from the Trichoderma harzianum strain 525, a soil-borne fungus collected from Inner Mongolia, China. The mycovirus had double-strand RNA (dsRNA) with a complete genome sequence of 3,160 base pairs and two open reading frames (ORFs) on the negative strand. Phylogenetic analysis indicates it belongs to an unclassified family of dsRNA mycoviruses. The removal of ThMV1 from host strain 525 reduced host biomass production and improved the biocontrol capability of the host on Fusarium oxysporum f. sp. Cucumerinum. At same time, the presence of ThMV1 functioned to improve the growth of the cucumber.

**Conclusion:** ThMV1 is a new unclassified mycovirus found in *T. harzianum*. It not only affected the phenotype of the host strain, but also reduced its biocontrol function, which sheds light on the interaction between the mycovirus and Trichoderma spp.

**Keywords:** Mycovirus, Trichoderma harzianum, dsRNA, Trichoderma harzianum mycovirus 1
A symptomless hypovirus facilitates stable infection of a coinfecting reovirus in chestnut blight fungus likely through suppression of RNA silencing

Annisa Aulia1,2, Ida Bagus Andika1, Hideki Kondo1, Bradley I. Hillman3, Nobuhiro Suzuki1
1) Institute of Plant Science and Resources, Okayama University, Japan
2) Graduate School of Environmental and Life Science, Okayama University, Japan
3) Plant Biology and Pathology, Rutgers University, USA

Purpose: Mixed infections of fungal hosts are common, and various types of virus/virus interactions are observed. Herewith we show another interesting virus/virus interaction occurring in a destructive plant pathogenic fungus, the chestnut blight fungus (Cryphonectria parasitica).

Methods: Field-collected US strain C18 of C. parasitica, earlier reported to be infected by a double-stranded RNA virus, mycoreovirus 2 (MyRV2), was used. Next-generation sequencing techniques and conventional molecular and genetic protocols were employed.

Results and Discussion: Next-generation sequencing has revealed co-infection of C18 by a positive-strand RNA virus, hypovirus 4 (CHV4). The current analyses showed interesting commensal interactions between the two viruses. CHV4 facilitated the stable infection and enhanced vertical transmission of MyRV2, which was readily lost during subculturing and showed reduced vertical transmission in single infections. Deletion of a key antiviral RNA silencing gene, dcl2, in isolate C18 increased stability of MyRV2 in single infections. The ability of CHV4 to facilitate stable infection with MyRV2 appears to be associated with the inhibitory effect of CHV4 on RNA silencing via compromising the induction of transcriptional up-regulation of dcl2. These results suggest that natural infection of isolate C18 by MyRV2 in the field was facilitated by CHV4 co-infection.
Viruses are ubiquitous parasites of cellular life on Earth. Unprecedented fungal virus diversity has also been characterized over the past decade. The influences of virus to host are various. Mycoviruses impairing the virulence of phytopathogenic fungi catch the attention of phytopathologists. Partitiviruses usually mediate persistent and cryptic infections of their hosts, especially for plant partitiviruses. Previously, we identified a typical partitivirus, Sclerotinia sclerotiorum partitivirus 1 (SsPV1), in strain WF-1 of Sclerotinia sclerotiorum, and confirmed that this partitivirus confers hypovirulence on its host. Botrytis cinerea is phylogenetically close to S. sclerotiorum. Herein, 

**Purpose:** we investigated the effect of SsPV1 replication in strain B05.10 of B. cinerea.

**Methods:** The purified SsPV1 virion was successfully introduced into virus-free strain B05.10 by PEG-mediated transformation.

**Results and conclusions:** Virus infection suppressed hyphal growth and boosted hyphal branching frequency. SsPV1 also causes hypovirulence in strain B05.10 as that was observed in S. sclerotiorum, which may relate to delay infection cushion development. Although there is no influence of SsPV1 to conidiation of B05.10 strain, SsPV1-infecting B05.10 strain produces more and smaller sclerotia on PDA medium.

**Methods and results:** Gene expression analysis of SsPV1 infecting hyphal growth phase was analyzed and we found that two clusters of melanin related genes and boticinic acid genes are significantly downregulated due to SsPV1 infection.

**Conclusions:** Altogether, our research implied some new clues to understand the mechanism by which SsPV1 causes host hypovirulence.
Artificial and natural plant viroid infections in phytopathogenic fungi

Ruiling Bian¹, Shuang Wei¹, Ida Bagus Andika², Erbo Niu¹, Hideki Kondo³, Liying Sun¹

¹State Key Laboratory of Crop Stress Biology for Arid Areas and College of Plant Protection, Northwest A&F University, China
²College of Plant Health and Medicine, Qingdao Agricultural University, China
³Institute of Plant Science and Resources (IPSR), Okayama University, Japan

Purpose: To investigate the infection of viroids in fungi.

Methods: Protoplast inoculation and fungal inoculation.

Results: We showed plant viroid infections in three filamentous plant pathogenic fungi, Cryphonectria parasitica, Valsa mali, and Fusarium graminearum. By transfection of fungal spheroplasts with viroid RNA transcripts, each of the three, iresine 1 viroid, hop stunt viroid (HSVd) and avocado sunblotch viroid (ASVBd) can stably replicate in at least one of those fungi. HSVd infection severely debilitates the growth of V. mali but not that of the other two fungi, while in F. graminearum and C. parasitica, with deletion of dicer-like genes (dcl), the primary components of the RNA-silencing pathway, HSVd and ASVBd accumulation, respectively, increases. Under laboratory conditions, we further demonstrate that HSVd can be bidirectionally transferred between F. graminearum and plants during infection. To investigate whether in natural environment fungi are infected with viroid, we extensively screened for viroid infections in various fungal species isolated from plants infected with viroids. The results indicate that around 36% of fungal isolates are infected with viroids.

Conclusion: Our results demonstrated horizontal transfer of viroids between plants and fungus, and also suggest that fungi could possibly serve as the reservoir or biological vector of viroids in nature.
Ectomycorrhizal fungal communities of secondary *Tristaniopsis* forests in Indonesia

Helbert Helbert¹, Maman Turjaman², Kazuhide Nara¹

¹Dept. of Natural Environmental Studies, The University of Tokyo, Japan  
²Forestry Research and Development Agency (FORDA), Ministry of Forestry, Indonesia

**Purpose:** Primary tropical rainforests in Southeast Asia are mostly ectomycorrhizal (ECM) ecosystems due to the dominance of Dipterocarpaceae trees, but it usually turns to arbuscular mycorrhizal ecosystems after disturbance. *Tristaniopsis* (Myrtaceae) is a rare potentially ECM host group that can colonize disturbed sites, while we know nothing about ECM fungal communities of *Tristaniopsis* under these conditions. Here, we investigated ECM fungal communities of secondary *Tristaniopsis* forests in Bangka and Palangkaraya, Indonesia.

**Methods:** In total, we collected 250 soil samples (of dimensions 5 x 5 x 10 cm), from which ECM tips were morphotyped and subjected to molecular analyses to identify both ECM fungal (ITS region) and host species (rbcL gene).

**Results:** By applying 97% ITS similarity threshold, 127 ECM fungal species were identified from a total of 1641 ECM tips. Thelephoraceae (26 species), Russulaceae (26 species), and Boletaceae (13 species) was the most species-rich families. In term of frequency, Thelephoraceae, Russulaceae and Gloniaceae was present in 120, 106 and 40 soil samples, respectively. ECM fungal communities were marginally different between Bangka and Palangkaraya (R²=0.24, p=0.047) and weakly structured by geographic distance (R²=0.32, p=0.052). *Tristaniopsis* and coexisting *Shorea* trees in Palangkaraya shared many common ECM fungal species and the fungal communities were not significantly different between these host groups (p=0.206). Some ECM fungi found in this study were also recorded from Dipterocarpaceae forests in Borneo, although none were shared with other biogeographical regions beyond the Sundaland.

**Conclusions:** These findings suggest that secondary *Tristaniopsis* forests could function as refugia of ECM fungi inhabiting undisturbed dipterocarp forests, which have been quickly disappearing.

**Keywords:** Myrtaceae; Refugia; Sundaland; Geographical barrier.

Keita Okada, Yosuke Matsuda
Graduate School of Bioresources, Mie University, Japan

**Purpose:** Endangered Japanese Douglas-fir, *Pseudotsuga japonica*, relies on root associating ectomycorrhizal fungi for its nutrient absorption. Among the fungi, an ectomycorrhizal fungus, *Rhizopogon togasawariana*, was detected only from Japanese Douglas-fir seedlings grown in soils under both in vitro and in situ. This infers that spores rather than hyphae of the species can play an important role for the growth and survival of the host tree. The goal of this study was to clarify spatial distribution of the soil inocula of *R. togasawariana* in Japanese Douglas-fir forests. To achieve this, we developed specific primer pairs for *R. togasawariana*, and examined soil samples collected in the field to detect the fungus.

**Methods:** We established three study sites at where Japanese Douglas-fir populations were remained. In each site, soil samples were collected at the border between Japanese Douglas-fir forests and Japanese cedar (*Cryptomeria japonica*) and/or Japanese cypress (*Chamaecyparis obtusa*) plantations. Specific primer pairs for *R. togasawariana* focusing on the ITS region were designed based on its holotype sequence, and their effectiveness was assessed by using *R. togasawariana* colonized roots. Candidate specific primer pairs were applied for genomic DNA extracted directly from the soils. Successfully amplified PCR products were sequenced and compared by the BLAST search to estimate the identity of fungal species.

**Results and Conclusions:** In total, 80 pairs of primers were designed in silico, of which 5 pairs were specific and successfully amplified derived from in vitro *R. togasawariana* roots. Based on the obtained data, we discuss the availability for the primer pairs to detect the fungal inocula and possible distribution pattern of this fungus.
High mycorrhizal preference between two co-occurred mycoheterotrophic plants in a subalpine cold temperate forest

Wan-Rou Lin\textsuperscript{1)}, Ren-Cheng Liu\textsuperscript{2)}, Pi-Han Wang\textsuperscript{2)}
\textsuperscript{1}Bioresource Collection and Research Center, Food Industry Research and Development Institute, Taiwan
\textsuperscript{2}Department of Life Science, Tunghai University, Taiwan

Mycoheterotrophic plants (MHPs) lack chlorophyll and relied on their mycorrhizal fungi for carbon and nutrient supply. The mycorrhizal fungi can have profound effects on the distribution of MHPs and might play an important role in plant speciation. In this study, the diversity of ectomycorrhizal fungi (EMF) associated with two coexisting MHPs, \textit{M. humile var. humile} (Mhh) and \textit{var. glaberrimum} (Mhg) was investigated by next-generation sequencing. Eighteen species of EMF belonging to Amanitaceae (1), Russulaceae (15), Sebacinaceae (2) were found. There were 13 and 10 EMF species associated with Mhh and Mhg, respectively. Some EMF were only associated with one MHP. For example, \textit{Russula densifolia} and \textit{R. xerampelina} were only associated with Mhh, whereas \textit{Sebacina dimitica} and \textit{Lactarius sp.} were only found in roots of Mhg. \textit{Amanita liquii}, \textit{R. peckii}, \textit{R. alnetorum}, Russulaceae sp., and \textit{Lactarius badiosanguineus} were associated with both \textit{Monotropastrum} spp., however, they were differentially abundant between roots of Mhh and Mhg. The reads of \textit{A. liquii}, \textit{R. peckii} and \textit{R. alnetorum} associated with Mhh and Mhg were 2,932/5, 15,830/618, and 12,486/18, respectively. They were more abundant in Mhh. Russulaceae sp. (20/18,214), and \textit{Lactarius badiosanguineus} (7/15,497) were more abundant in Mhg. The specific EMF might facilitate sympatric speciation in sister MHP species.
Diversity of Arbuscular Mycorrhizal Fungal Spores in Jeju Island, Korea

Hyeok Park¹, Kang-Hyeon Ka², Ahn-Heum Eom¹)

¹) Department of Biology Education, Korea National University of Education, Korea
²) Special Forest Products Division, National Institute of Forest Science, Korea

Arbuscular mycorrhizal fungi (AMF) are one of the most important symbionts in ecosystem. They exist as asexual spores in rhizosphere until they meet the plant root. When they meet the plant root, hypha from AMF spores infect into the root and form the arbuscular mycorrhiza. AMF provide better nutrient absorptivity to host plant, enhance tolerance against plant root pathogen, and improve resistance against the stress by heavy metal or salinity. Due to their benefits for plants, AMF have importance in plant and soil ecosystem. Jeju island is the largest island in Korea, about 88 miles from the mainland of Korea. Jeju island shows different vegetation from the mainland, so there are needed studies about mycorrhizal fungi in Jeju island. In this study, we confirmed AMF spore distribution and diversity of AMF species in soils of Jeju island. We sampled the rhizospheres from 12 sites in Jeju island, and sampled rhizospheres were trap-cultured with host plants (Sorghum bicolor) in greenhouse. AMF spores were extracted from cultured soils using wet sieving method. AMF spores were identified by morphological characteristics and molecular analysis. As a result, we identified 20 AMF species from 12 genera. Acaulospora sp. JJ18046 showed similar morphological characteristics to Acaulospora scrobiculata, but result of molecular analysis indicated that DNA sequence of the spore is similar to Acaulospora spinosa, or compose another clade. Therefore, we considered the strain JJ18046 as the novel AMF species candidate.
Colonization of Arbuscular Mycorrhizal Fungi along an Altitudinal Gradient at the Ibukiyama Mountain

Linda Yustikasari, Yosuke Matsuda
Graduate School of Bioresources, Mie University, Japan

Purpose: Arbuscular mycorrhizal (AM) fungi associations are one of the most widespread interactions between plants and fungi in forest ecosystems. AM fungi colonize fine roots forming two distinctive morphologies, Arum and/or Paris types. Understanding the determinant factors regulating the occurrence frequency of the two types in the natural gradient is a fundamental knowledge in order to predict the functional relationship of AM associations. Since climatic parameters and soil properties change along altitudinal gradients in mountains, AM colonization might have different patterns accordingly. This study aimed to clarify the AM association along the altitudinal gradient. For this purpose, we determined the colonization rate of AM fungi and both of its types at the Ibukiyama mountain.

Method: Root and soil samples were collected from 5 different altitudes at Ibukiyama. Three plant species were selected based on their altitudinal distributions: Cryptomeria japonica (200, 500, and 800 m), Acer japonicum (500 m), and Chrysanthemum japonicum (1,350 m). A total of 15 root samples from each altitude were examined microscopically to identify AM fungal colonization using the magnified intersect technique.

Results and conclusions: AM fungal colonization rates were significantly and positively correlated with the altitude. Both Arum and Paris types were observed in all plant species examined at each altitude. However, Arum type, but not for Paris type, showed a significant positive correlation with the altitude. These results indicate that environmental changes along the elevation regulated the distribution of plant communities which in turn reflected the occurrence of either Arum or Paris types.
The effect of symbiotic association between AMF and maize on GHG emission from agriculture soil and its microbial metabolism study

Heng Gui1), Kai Yan2), Zeng Hu2), Jianchu Xu1), Peter E. Mortimer1)
1)Kunming Institute of Botany, Chinese Academy of Sciences, China
2)Yunnan Agricultural University, China

Purpose: As a widely distributed fungi in the soil, arbuscular mycorrhizal fungi (AMF) can affect carbon and nitrogen metabolism in the soil through their large network of mycelium. But the research on the relationship between AMF in the soil and the flux of greenhouse gases and related regulatory mechanisms is relatively lacking.

Methods: In this research, we investigated the effect of AMF on GHG especially N₂O and CH₄ from an agricultural soil. Our experimental set-up included a dual microcosm unit with two treatments: Maize inoculated with AMF (AM) and uninoculated (NM). GHG flux was measured using the statistic chamber methods and destructive soil sampling was carried out at different times. Real-time PCR and Illumina sequencing was applied to test the key genes abundance related to N₂O and CH₄ dynamics and soil microbial community changes due to the AMF inoculation.

Results: After 3-month observation, we found that N₂O flux from the soil was affected by the AMF inoculation at 1st month. Furthermore, we also found that the gene abundances of several key genes like nirK and nosZ related to N₂O dynamics in the soil were affected by AMF inoculation. The correlation analysis showed the potential link between AMF inoculation and the N related gene which could explain the GHG emission affected by AMF present in the soil.

Conclusions: This study provided insights into the importance of AMF in controlling the GHG emission from agricultural soils, although AMF are not believed to play direct roles in GHG emission.
Environmental DNA metabarcoding reveals the most comprehensive phylogenetic diversity and invisible lineages of early diverged wood-decomposers

Takashi Shirouzu¹, Shunsuke Matsuoka², Nobuaki Nagata³, Masayuki Ushio⁴, Kentaro Hosaka⁵, Hideyuki Doi²

¹Graduate School of Bioresources, Mie University, Japan
²Graduate School of Simulation Studies, University of Hyogo, Japan
³Collection Center, National Museum of Nature and Science, Japan
⁴Hakubi Center, Kyoto University, Japan
⁵Department of Botany, National Museum of Nature and Science, Japan

Purpose: We aimed to conduct the most comprehensive diversity survey of early diverged wood-decaying basidiomycetes, Dacrymycetes, in cool-temperate forests. The diversity of Dacrymycetes has not been thoroughly investigated, despite their significant functions as brown rotters. To obtain a more reliable phylogenetic hypothesis for the early-evolution of wood-decomposers, we conducted a comprehensive diversity survey of Dacrymycetes using a combined approach comprising a fruiting body survey, culture isolation, and eDNA analysis.

Methods: The study site was a mixed forest of Pinus densiflora and broad-leaved trees at an elevation of ca. 1,300 m at the Sugadaira Research Station (N 36.521, E 138.350), University of Tsukuba, Nagano, Japan. At this site, we surveyed Dacrymycetes diversity for 3 years by fruiting body collection, culture isolation using a dilution-to-extinction method, and eDNA analysis with DNA metabarcoding.

Results and Conclusions: The 3-year investigation revealed a total of 28 OTUs, of which 10 were collected as fruiting bodies, 10 were isolated as cultured mycelia, and 27 were detected as eDNA sequences. The eDNA metabarcoding revealed various lineages across the Dacrymycetes phylogeny, including previously undiscovered early branches that could not be obtained as fruiting bodies. The fruiting body survey and culture isolation could uncover only half of the OTUs estimated from eDNA metabarcoding data, suggesting that a large number of invisible lineages are latent in the environment. This complemented survey approach allowed us to detect previously invisible early branches. These findings contribute to our understanding of the evolutionary history of mushroom-forming fungi.
The influential factors affecting fungal diversity in forests

Young Woon Lim\textsuperscript{1)}, Seung-Yoon Oh\textsuperscript{1)}, Ki Hyeong Park\textsuperscript{1)}, Shinnam Yoo\textsuperscript{1)}, Chang Sun Kim\textsuperscript{2)}

\textsuperscript{1)}School of Biological Sciences, Seoul National University, Republic of Korea
\textsuperscript{2)}Forest Biodiversity Division, Korea National Arboretum, Republic of Korea

**Purpose:** Fungi play a fundamental role on plant physiology and nutrient cycles in forest ecosystems. To understand their function in ecosystems, it is prerequisite to know how many and what kind of fungal species exist in the forest. Fungal diversity can be affected by various environmental and geographical factors. Recent advancement of sequencing technology has accelerated the discovery of the fungi; however, the research of the influential factors affecting that the fungal diversity and communities has progressed rather slowly. The main objective of this study is to investigate fungal diversity in the local forests and factors that affect the diversity and communities.

**Methods:** We attempted to detect guild pattern from ten different tree species for five years (2014-2018) in Mt. Jeombong National Park. Bulk soil samples were collected seasonally for each tree species with three replicates. DNA extracted from bulk soils was sequenced with Illumina MiSeq using ITS region to determine fungal community; we also measured soil chemical properties.

**Results:** Our results were compared with environmental factors, geographical factors, and source factors. Those factors significantly influenced both symbiotrophic and saprotrophic fungal communities while the type of factors and the degree of influence varied between these communities. Within the same tree species, the symbiotrophic communities were more influenced by geographical distance, whereas the saprotrophic communities were more affected by sources. Finally, we evaluated the effect of survey methods on the fungal diversity and found that only small proportion of species was shared between different methods.

**Conclusion:** The results show that symbiotrophic and saprotrophic fungal communities show different responses to abiotic and biotic factors. Therefore, different ecological drivers and investigation methods should be considered to uncover fungal diversity in forests.
Abundant and diverse fungal microbiota inhabit the white female and brown cyst of cereal cyst nematode

Jianyang Hu\textsuperscript{1)}, Muzammil Hussain\textsuperscript{2)}, Xiaoling Zhang\textsuperscript{2)}, Jianqing Tian\textsuperscript{2)}, Xingzhong Liu\textsuperscript{2)}, Yuxi Duan\textsuperscript{1)}, Meichun Xiang\textsuperscript{2)}

\textsuperscript{1)}Nematology Institute of Northern China, College of Plant Protection, Shenyang Agricultural University, China
\textsuperscript{2)}State Key Laboratory of Myology, Institute of Microbiology, China academy of sciences, China

\textbf{Purpose}: Cereal cyst nematode (CCN) is major plant-parasites of agronomic crops worldwide. Hitherto, several fungal species have come to fore diminishing cyst nematode in field soils. However, the information on detailed characterisation of fungal microbiota inhabiting the cyst nematode stages (female and brown cyst) and microbial succession from female to cyst is still limited.

\textbf{Methods}: The CCN white females that were attached on the wheat root and mature brown cysts present in the soil were collected from wheat grown fields located at four different locations in central China and their associated fungal communities were investigated using high-throughput sequencing.

\textbf{Results}: The results showed that there were no significant differences in observed species and Shannon diversity index of fungal communities in the white females and cysts across four locations. However, the fungal microbiota composition was different between white females and cysts. The phylum Mortierellomycota was more abundant in the white female than that in cyst, while Ascomycota had higher relative abundance in the cyst compared to white females. An operational taxonomic unit (OTU) enrichment analysis using combined samples for the white females as well as for the cyst from four locations revealed that only a small subset of OTUs were significantly enriched in white females and cysts. These OTUs were belonging to genera such as Mortierella, Fusarium, Alternaria, Aphroditeola, Cladosporium, Cryptococcus, Olpidium, Penicillium, Periconia and Moleospora. However, the relative abundance of previously well-documented nematode egg-parasites such as Pochonia chlamydosporia, Purpureocillium and other potent taxa were not significantly different between white female and cyst.

\textbf{Conclusions}: Our results indicated that the white females and cysts harbor diverse mycobiota and the majority of fungal taxa remain in steady-state composition from the white females to mature brown cysts.
Temporal dynamics of fungal DNA assemblages evaluated by eDNA metabarcoding in a forest river water in Japan

Shunsuke Matsuoka1), Yoriko Sugiyama2), Yoshito Shimono3), Masayuki Ushio4), Hideyuki Doi1)
1)Graduate School of Simulation Studies, University of Hyogo, Japan
2)Graduate School of Human and Environmental Studies, Kyoto University, Japan
3)Graduate School of Bioresources, Mie University, Japan
4)Hakubi Center, Kyoto University, Japan

Purpose: Recent studies have shown the usefulness of environmental DNA (eDNA) from river water in understanding both terrestrial and aquatic fungal compositions. However, knowledges on temporal variations in fungal DNA assemblages in river water are still limited. Here, we aimed to describe the fungal DNA assemblages in a forest river water and their temporal dynamics by fungal eDNA metabarcoding.

Methods: The study was conducted in a mixed broad-leaved forest (30-years old secondary forest, c.a., 0.6 ha) in southern Kyoto, Japan. Water samples (in total 3 L) were collected monthly from December 2016 to November 2018 (two-years). Fungal DNA assemblages were detected with MiSeq sequencing of fungal rDNA (ITS1) region.

Results and conclusions: In total, 4388 operational taxonomic units (OTUs) were obtained. Of these, 1680 were assigned as Ascomycota, 732 as Basidiomycota, and 68 as Chytridiomycota. We found that the river water contained divergence in functional groups, including saprotrophic, parasitic, and symbiotic fungi associated with plants and animals in both aquatic and terrestrial habitats. The OTU compositions changed continuously and temporally, and showed a periodic change in the compositions resembling each other by the investigation months regardless of the year. The strength of this periodicity varied among functional groups, being strong in saprophytes and weak in symbionts. Our results indicated that the fungal DNA assemblages in river water were composed of fungal DNA from various taxonomic and functional groups including terrestrial fungi, and suggested that temporal changes of the fungal communities in the riverine forest can be evaluated by examining the eDNA in the river water.
Notes, outline and divergence time of Basidiomycota

Rui-Lin Zhao
Institute of Microbiology Chinese Academy of Sciences, China

Basidiomycota constitutes a major phylum of the kingdom Fungi, which is second in species numbers to the Ascomycota. Our work attempts to provide an overview of all valid, currently used Basidiomycota genera published so far in a single document and the divergence times of families and the above. In this study, an outline of basidiomycota was presented which included 1928 valid, currently used genera with 1261 synonym names, which are from 241 families, 68 orders, 18 classes and 4 subphyla. For these 1928 valid genera, we provided a brief note for each genus including information of their classification, accepted species number, type species, life mode, habitat, distribution, and sequence information. Furthermore, totally 771 species from 60 orders and 185 families were included in the phylogenetic and dating analyses. Three datasets of subphyla Agaricomycotina, Pucciniomycotina and Ustilaginomycotina were made respectively, which composed of six-gene (LSU, SSU, 5.8s, rpb1, rpb2, ef1) sequences, and their dating analyses were carried out. Our study indicated divergence times of subphyla in Basidiomycota are 406-430 Ma, classes are 211-383 Ma and orders are 99-323 Ma which were generally similar to the previous study. In this study all phylogenetic supported families were dated, the results showed the families from Agaricomycotina diverged during 49-226 Ma, Pucciniomycotina diverged during 85-222 Ma and Ustilaginomycotina 79-177 Ma. Those divergence times as an additional criterion in ranking would provide a clue to resolve the taxonomic problems in the present taxonomic system, and also provide the knowledge to better understanding the phylogeny and the evolution events in Basidiomycota.
Natural history of blue entolomas, *Entoloma virescens*

Kentaro Hosaka
National Museum of Nature and Science, Japan

Among various colors of mushrooms, blue is arguably the most striking and mysterious color. The genus *Entoloma* is known to contain many colorful species, e.g., purple, yellow and red. Among them, *E. virescens* and a few others produce strikingly blue fruit bodies. *E. virescens* is one of the earliest blue entolomatoid species described in the genus. The holotype collection was made in 1854 from "the Bonin Islands" in Japan. Since then, dozens of Japanese mycologists have explored the islands for studying mycoflora, but mysteriously, no additional collections of *E. virescens* were made until 2000's, which means that no definitive records of the species were made for more than 100 years from the Bonin Islands. I am investigating mycoflora of the Bonin Islands (mainly two big islands inhabited by humans, Chichijima Island and Hahajima Island) every year from 2009, and *E. virescens* have been collected almost every year. Interestingly, however, all collections so far were made only from Chichijima Island although I have spent about the same number of days both in Chichijima and Hahajima Islands. Based on those findings and additional DNA sequence data, we propose the possibilities that (1) the current "*E. virescens*" in the Bonin Islands can be different species or individuals from the ones from 1854; and (2) *E. virescens* has secondarily been introduced to Chichijima Island but not to Hahajima Island relatively recently.
Purpose: A condition resulting in vernal patches of stunted or dying cereal crops was called ‘Omphalina Patch Disease’ (OPD) in England after they were linked to coincidental, co-located basidiomes identified as *Omphalina pyxidata*. Original OPD vouchers from the 1980-90s were located in the Royal Botanic Gardens, Edinburgh, where they had been sent for identification. These were analysed both molecularly and anatomically to confirm or correct the identification. A recently published 2018 analysis on the biological status of the *Hymenochaetales* suggested the genus *Loreleia* be split between two families in two orders. In order to address this question and to resolve the generic placement of the patch fungus, an in-depth investigation was conducted to clarify the taxonomy of agaric genera in the *Rickenellaceae*.

Methods: DNA cloning, followed by screening of the clones using higher resolution gel electrophoresis, and plasmid DNA sequencing were required to resolve the phylogenetic signatures of the old herbarium material and of the type species of *Loreleia*, *L. postii*.

Results and conclusions: Neither patch disease sample is *O. pyxidata* (*Tricholomataceae, Agaricales*). Both were conspecific with each other; a species of agaric in the *Rickenellaceae* (*Hymenochaetales*). We determined that *Loreleia* is not split between two families and orders and that a misdetermined voucher had led to an erroneous conclusion. Additionally, *Contumyces* and *Loreleia* remain phylogenetically distinct but both require new circumscriptions.
Phylogeny and diversity of Boletaceae in China, a review

Zhu-Liang Yang
Kunming Institute of Botany, Chinese Academy of Sciences, China

Purpose: Species in the family Boletaceae are economically and ecologically important. Nearly all of the species in the family are ectomycorrhizal partners forming symbiotic associations with higher plants of more than ten families. Boletaceae are very rich in species, diverse in morphology, and complicated in anatomy and relationships among genera. As a result, Boletaceae have been regarded as one of the most difficult fungal groups for taxonomists.

Methods: We have collected and analyzed over 2800 specimens of Boletaceae from the northern hemisphere, representing about 400 species (ca. 40% of the known species) belonging to more than 60 genera (nearly all the known genera) within the family. We employed integrated and inter-disciplinary approaches, including macro- and micro-morphological characterizations, ultrastructural investigations of basidiospores, and molecular phylogenetic analyses of multigene nucleotide sequences and partially microsatellite genotyping.

Results and Conclusions: Our studies elucidated the higher-level phylogenetic relationships of Boletaceae and proposed a new higher-level classification for the family. Seventeen new genera and 115 new species have been documented and published by Chinese mycologists based on morphological and molecular criteria since 2011. The evolutionary history of *Boletus reticuloceps* in correlation with its adaptation to historical climate fluctuations, ecological differentiations and physical barriers as well as plant hosts was analyzed. The studies provided deep insights into species diversity, phylogeny, and co-evolution of boletes with their host plants.
A transcriptomic atlas of mushroom development reveals conserved genes behind fruiting body development

Laszlo G. Nagy
Biological Research Center, HAS, Hungary

Purpose: Mushroom formation is one of the most spectacular and complex processes in the fungal world, comparable to the development of higher plants and animals in terms of its complexity. Yet, its genetic bases, in particular, conserved developmental genetic events are hardly known. Here we identify conserved developmentally regulated genes in fruiting body transcriptomes of six Agaricomycetes, Coprinopsis cinerea, Rickenella mellea, Phanerochaete chrysosporium, Armillaria ostoyae, Schizophyllum commune and Lentinus tigrinus.

Methods: We obtained developmental transcriptome data for the entire course of fruiting body development of these species and inferred the phylogenetic history of developmentally regulated genes in a dataset of 202 genomes.

Results: We find that 10-40% of the genes are differentially regulated during fruiting body development in the examined species, comprising functions related to cell wall synthesis and modification, mRNA stability, cell growth and regulation of transcription, among others. By studying the conservation of developmentally genes and their evolution through a comparative analysis of 202 fungal genomes, we aim to understand the origins of fruiting body-related genes and to understand the minimal gene set required to initiate and develop agaricomycete fruiting bodies.

Conclusion: This is the first systematic comparative analysis of developmentally regulated genes in mushroom-forming fungi, and illuminates some of the conserved functionalities that fungi deploy during fruiting body development.
Taxonomy and phylogeny of selected Boletales from northern Thailand, challenges and opportunities in drug discovery

Boontiya Chuankid¹, Santhiti Vadthanarat², Benjarong Thongbai³, Marc Stadler³, Kevin David Hyde¹, Olivier Raspe⁴,⁵

¹Center of Excellence in Fungal Research, School of Science, Mae Fah Luang University, Thailand
²Department of Biology, Faculty of Science, Chiang Mai University, Thailand
³Department of Microbial Drugs, Helmholtz Centre for Infection Research (HZI), Germany
⁴Meise Botanic Garden, Belgium
⁵Wallonia-Brussels federation, General service of university teaching and scientific research, Belgium

Purpose: The order Boletales consists of a large number of genera with more than 1000 species. Most Boletales have a stipe and a cap, with tubular hymenophore. However, some have a lamellate hymenophore (e.g., Phylloporus, Gomphidius) or completely different growth forms (gasteroid like in Scleroderma, or even crustose/polyporoid like in Serpula). Most Boletales species form ectomycorrhizae with the roots of forest trees, but others are saprobic or facultatively ectomycorrhizal (Serpula, Phlebopus), or mycoparasitic (e.g., Pseudoboletus, Buchwaldoboletus). To elucidate the species diversity of selected Boletales in Thailand, the investigation of Phylloporus and Retiboletus species were conducted using both morphological and molecular data. Aside from the various taxonomic novelties, new and unique secondary metabolites also interesting to study. Phlebopus is one of the most popular edible mushrooms in northern Thailand and possible to grow in culture media, which are key components for bioprospecting and value-addition.

Methods: Boletales have been collected in northern Thailand since 2014, focusing on taxonomy and multigene phylogeny. Maximum likelihood (ML) and Bayesian inference (BI) analysis were performed with the three-gene dataset (atp6, rpb2, tef1).

Results and conclusions: Morphological and molecular analyses seem to be useful for delimitation of species. Two species of Phylloporus were described as new: P. pusillus and P. subrubeolus. Three other species corresponded with previously described taxa: P. brunneiceps, P. castanopsidis, and P. rubiginosus. For Retiboletus, two taxa are similar to R. fuscus and R. nigrogriseus, with one new species. A preliminary study of extracts from Phlebopus basidiomata and cultured mycelium showed antimicrobial activity.
Diversity and temporal dynamics of zoosporic fungi in aquatic ecosystems

Maiko Kagami1), Kensuke Seto2), Silke van den Wyngaert3)

1) Graduate School of Environment and Information Sciences, Yokohama National University, Japan
2) Ecology and Evolutionary Biology, University of Michigan, USA
3) Leibniz-Institute of Freshwater Ecology and Inland Fisheries, IGB, Germany

Purpose: Aquatic ecosystems remain frequently overlooked as fungal habitats, although fungi potentially hold important roles for shaping microbial communities and food web dynamics. Molecular analyses of environmental DNA samples have revealed an unexpectedly large diversity of undescribed fungi in lakes and oceans, so called “dark matter fungi” (DMF). DMF are likely to be particularly common on the early diverging branches of the fungal tree of life, for example, Chytridiomycota, Rozellomycota (Cryptomycota) and Aphelidiomycota.

Methods: Combining molecular techniques with isolation and microscopic observations, we have examined the species composition and temporal dynamics of DMF in two lakes; shallow and eutrophic Lake Inba, Japan and deep and mesotrophic Lake Stechlin in Germany.

Results and Conclusions: Most of DMF were parasites of phytoplankton belonging not only to Chytridiomycota (chytrids) but also Rozellomycota and Aphelidiomycota. These fungal groups produce zoospores, which can swim in water and utilize various organic matters. Metabarcoding analysis revealed unique temporal dynamics of these fungi in different lakes. In Lake Stechlin, Germany, diverse phytoplankton were infected by different chytrids with high host specificity. Seasonal succession of chytrids corresponded well with the occurrence of their host/substrate including phytoplankton and pollen. While, in Lake Inba, Japan, diverse fungi infected only two dominant diatoms and seasonal succession patterns were less clear with generalists and specialists cooccurring at the same time. We established several dual cultures of parasitic chytrids and their host algae and examined their thallus morphology zoospore ultrastructure, and molecular phylogeny. We revealed that parasitic chytrids belong to novel lineages in known orders, or even order-level novel lineages in the Chytridiomycota. Further studies will surely discover more host parasite combinations.
Chytridiomycete fungi from freshwater samples collected at Korea

Sun Jeong Jeon, Hyo Jin Lim, Hyang Burm Lee
Dept. of Agricultural Biological Chemistry, College of Agriculture & Life Sciences, Chonnam National University, Korea

Purpose: In a survey of indigenous fungal diversity in Korea, five strains of EML-JCW1-1, -HRW1-1, -HRW1-6, -19CPW5-1 and -19CPW6-1 belonging to Chytridiomycetes were isolated from freshwater samples collected at Jeju and Gwangju, South Korea.

Methods: The strains used in this study were isolated from freshwater samples collected at Cheonjeyeon in Jeju, Hwangnyonggang and an artificial pond located in Chonnam National University campus, Gwangju. The Strains were isolated using a bait method and incubated on PmTG medium. The strains were cultured on PmTG. gDNA was extracted and the 28S rDNA was amplified with LR0R-LR5F primers pair. To identify the fungus at the species level, detailed morphological studies and rDNA sequence analyses were performed.

Results: BLASTn search indicated that the identity values of 28S rDNA sequences of EML-JCW1-1, -HRW1-1, -HRW1-6, -19CPW5-1 and -19CPW6-1 isolates represented 100% (740/740 bp), 99.6% (790/793bp), 99.6% (725/728bp), 98.5% (811/823) and 98.7% (857/868 bp) with Globomyces pollinis-pini Barr-003 (GenBank acc. no. DQ485532), Chytriomyces hyalinus ARG097 (GenBank acc. no. JX905513), Protrudomyces lateralis ARG071 (GenBank acc. no. NG_060073), Gorgonomyces sp. ARG036 (GenBank acc. no. EF585614), and Betamyces americameridionalis ARG063 (GenBank acc no. EF585624), respectively.

Conclusions: Our study showed that the EML-JCW1-1, -HRW1-1, -HRW1-6, -19CPW6-1 and -19CPW6-1 strains were identified as novel or undescribed species in Korea. The findings of such chytridiomycete fungi are significantly important in diversity study of rare fungal taxa.
Production of single cell protein and isolation of new hydrophobins from marine fungi growing on complex substrates

Catalina Landeta¹), Maria Elena Lienqueo¹,²)
¹)University of Chile, Chile
²)Centre for Biotechnology and Bioengineering CeBib

Seaweed and waste from the algae industry have a high concentration of complex carbohydrates that restricts the efficient obtaining bioproducts <Balina et al., 201>. The marine fungi are often included in screening for new metabolites, and their ability to assimilate complex polymers <Wang et al., 2016>. Since a couple of decades ago some species of fungi have been used to produce Single Cell Protein (SCP), for human food and animal feed, and others important proteins from fungi are the Hydrophobins (HFBs) <Cicatiello et al., 2016; Kamat et al., 2013>. The objective of this study was to develop and optimize a method for utilizing seaweed and seaweed waste to feed marine fungi and extract from these SCP and HFBs. In this study, the growth of 10 strains of marine filamentous fungi from collection NBCR, in Japan, was preliminarily evaluated. The protein concentration of the pre-selected fungi was evaluated in two different wastes from the algal industry (Waste A and B) and <Macrocystis pyrifera>. In the case of HFBs, 4 marine fungi were evaluated and 4 different methods were set up to extract HFBs of class I and II, from the mycelium and the culture broth in two different minimal mediums. The highest concentrations of protein were obtained with Dendryphiella salina. The productivity found for D. salina was 7.9, 3.3 and 2.6 mg/g day using M. pyrifera, waste A and B respectively. D. salina and Penicillium pinophilum have the ability to produce foam during the growth in shaken cultures, thus indicating the production of biosurfactants. The best medium for improve the production of HFBs was the medium minimal with alginate 0.2%. D. salina and P. pinophilum produced adequate amounts of putative HFBs of Class I in the culture broth: 280 and 258 mg/L, respectively. This work shows that D. salina and P. pinophilum can assimilated seaweed and theirs proteins could be used in different biotechnological applications.
New genus (*Dactylariopsis*), novel species and first records of asexual freshwater fungi in Thailand

Nattawut Boonyuen¹,², Charuwan Chuaseeharonnachai¹,², Watcharee Saotap¹,², Salilaporn Nuankaew¹,², Sayanh Somrithipol², Papichaya Kwantong¹,², Nattapol Pornputtapong³, E.B. Gareth Jone⁴

¹National Biobank of Thailand (NBT), National Science and Technology Development Agency (NSTDA), Thailand
²BIOTEC, National Science and Technology Development Agency (NSTDA), Thailand
³Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand
⁴Department of Botany and Microbiology, College of Science, King Saud University, Kingdom of Saudi Arabia

**Purpose:** During an investigation of freshwater fungi associated with submerged woods in Thai forests, four micro-fungi (i.e. *Dactylariopsis altospora* gen. et sp. nov., *Vanakripa thailandica* sp. nov., *Endophragmiella bitunicata* sp. nov. and *Triadelphia hexaformispora* sp. nov.), are described as new taxa to science. In addition, *E. multiramosa* (collected in Bueng Kan Province) and *E. resinae* (Nakhon Ratchasima Province) are reported for the first time as new records for Thailand. Descriptions, illustrations, and distributions are given for each species.

**Methods:** Based on a combination of morphological and phylogenetic analysis of a concatenated dataset of multi-loci.

**Results and conclusions:** *D. altospora*, *T. hexaformispora* (Nan Province), *V. thailandica* (Chiang Mai Province) confirms their placement of *D. altospora*, *V. thailandica*, and *T. hexaformispora* within Sordariales, Microascales and Conioscyphales, respectively. Morphologically, our comparison data indicated that *E. bitunicata* sp. nov., discovered in Nakhonayok Province is uniquely distinct from accepted species in the genus. This study supports the establishment of the new taxa and increases our knowledge of freshwater fungal asexual morphs in the Kingdom of Thailand.
Morphotype characterization and PacBio amplicon sequencing of ribosomal DNA of fungi associated with shallow corals in the Western Gulf of Thailand

Satinee Suetrong1,2), Sita Preedanon1,2), Sirapong Papan3), Oraphin Pracharoen1,2), Supicha Saengkaewsuk1,2), Anupong Klaysuban1,2), Noppol Kobmoo1), Thippawan Yoocha4), Wirulda Pootakham4), Panida Unagul1,2), Sittiporn Pengsakun5), Thamasak Yeemin5), Jariya Sakayaroj3)

1) National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand
2) National Biobank of Thailand (NBT), National Science and Technology Development Agency (NSTDA), Thailand
3) Walailak University, Thailand
4) National Omics Center (NOC), National Science and Technology Development Agency (NSTDA), Thailand
5) Marine Biodiversity Research Group, Faculty of Science, Ramkhamhaeng University, Thailand

Corals are conduits to numerous microorganisms, including zooxanthellae, protist, prokaryotes (bacteria and archaea) and viruses.

**Purpose:** The goal of this study is to investigate the species diversity and community structure of fungi associated with shallow corals in the western Gulf of Thailand (focusing on a robust coral and susceptible coral species to bleaching), using metagenomics approaches from PacBio SMRT sequencing system together with bioinformatic analysis.

**Methods:** The samples of three shallow coral *Porites lutea*, *Pavona decussata* and *Pocillopora damicornis* in the Western Gulf, southern Thailand (Chumphon, Prachuap Khiri Khan and Surat Thani provinces) were collected by scuba diving. The fungal communities as inferred by PacBio ribosomal amplicon sequencing for unculturable fungi, and the principle components analysis (PCA) for the occurrence of culturable fungi were explored.

**Results and conclusions:** Eighty-seven fungal strains isolated were found to belong to 18 orders in the Phyla Ascomycota and Basidiomycota. The Capnodiales was predominant fungi analysed by ribosomal DNA sequence and PacBio analyses. Principal component analysis showed an effect of the provinces on the fungal communities along the first and second components, but no significant effect of coral species. The community composition of fungi associated with shallow corals in Surat Thani province was different from the other provinces. The community composition of Prachuap Khiri Khan was slightly different to that of Chumphon which overlapped with the other provinces. The result from this study inferred that the diversity of fungi associated with shallow corals in the Western Gulf of Thailand is abundant.
Diversity of Freshwater Fungal Communities in Wicheon Stream of South Korea

Jaeduk Goh, Namil Chung, Hye Jin Hwang, Hye Yoen Mun, Sangkyu Park, Young-Hwan Park
Nakdonggang National Institute of Biological Resources, Korea

Purpose: Freshwater is diverse and complex environment for microorganisms. In freshwater ecosystem, fungi are known as a key player in nutrient cycling, especially in organic matter decomposition. Well-known freshwater fungi are a group of polyphyletic fungi that include a high diversity of phyla. However, there are few studies of metagenomics approaches about seasonal dynamics of fungal communities in freshwater.

Methods: In order to investigate structure of fungal community in freshwater environment, we surveyed four spots in Wicheon stream - a branch stream of Nakdonggang River - every three months in 2018, and collected total 48 samples (three types of environmental samples - water, sediment of stream, and soil of hyporheic zone). We performed amplicon-based pyrosequencing using ITS region and taxonomic assignment.

Results and conclusions: A total of 2,200,325 reads with hits for fungi belonging to 3,557 operational taxonomic units (OTUs) were obtained from the total 48 samples. As a result, we presented structure of fungal community at various levels: phylum, class, order, family, and genus. Mostly fluctuation of diversity index showed reduction in winter and summer compared to spring and fall. The major orders were Pleosporales, Hypocreales, Capnodiales, Sordariales, Helotiales, and Xylariales in Ascomycota; Rhizophydiales in Chytridiomycota; and Cystofilobasidiales and Agaricales in Basidiomycota. Interestingly, there are big differences in construction of fungal communities depending on sample type, sampling site, and collection season. Moreover, molecular analysis showed that there are many unknown fungal species in freshwater more than known fungi. These results indicated that environmental factors could be important factors on freshwater fungal community. Based on this study, we will try to evaluate methods of fungal isolation for acquiring of more diverse fungal isolates and investigate of fungal ecological roles in freshwater.
Cold adapted fungi isolated from sediment in the East Siberian Sea

Masashi Asano¹, Yuta Tuchiya¹, Masato Kida¹, Hirotoshi Sakagami¹, Akihiro Hachikubo¹, Satoshi Yamasita¹, Hirotugu Minami¹, Yung Mi Lee², Young Keun Jin³, Masaaki Konishi¹

¹Kitami Institute of Technology, Japan
²Division of Polar Life Sciences, Korea Polar Research Institute (KOPRI), Korea
³Division of Polar Earth-System Science, Korea Polar Research Institute (KOPRI), Korea

Purpose: Taxonomical distribution, life-cycles and biological function of fungi inhabiting in the Arctic Sea have not been described enough. To investigate the physiological characteristics of fungi from the Arctic Sea, we attempted to cultivable fungi from marine sediments from East Siberian Sea during a research cruise by R/V Araon (Cruise No. ARA09C).

Methods: Surface sediments (approx. 1 g) were spread on YM agar and incubated at 4°C for 3 months. Yeast-like colonies observed as a single colony were picked to obtain pure cultures. Twenty nine strains were isolated, and a part of strains were taxonomically characterized by sequencing of D1/D2 regions in ribosomal RNA large subunit.

Results: All strains grew well below 15°C, and recognized as psychrophiles. Interestingly, AST36-1 and AST31-1, which were identified as Glaciozyma sp. and unknown species in Camptobaciidae, Kriageriales, respectively, showed scaly cells, less pseudohyphae, and bisexual budding. Arctic yeast Glaciozyma sp. (formerly known as Leucosporidium sp.) was reported to have a complex ice binding site which may allow interactions with multiple faces of the ice crystal (Lee et al. 2012). The different species in the genus Glaciozyma had been isolated from Italian glaciers (Turchetti et al. 2011).

Conclusions: The results implied that the Glaciozyma related species would be wide spread in cold region from glaciers to sediment at the Arctic Sea.
Cell fusion and heterokaryon incompatibility: Variety of *Aspergillus oryzae* industrial strains used in Japanese traditional food fermentation

Jun-ichi Maruyama
The University of Tokyo, Japan

**Purpose:** *Aspergillus oryzae* is the industrial filamentous fungus used in Japanese traditional food fermentation such as manufactures of sake, soy sauce and *miso*. As sexual reproduction has not been found in *A. oryzae*, it is quite difficult to breed strains with industrially useful characteristics. We previously identified two mating types of *A. oryzae* strains (MAT1-1 and MAT1-2), indicating the potential for sexual reproduction. Cell fusion is the first process in sexual reproduction, and filamentous fungi also undergo cell fusion during the vegetative growth (asexually) to form heterokaryon. In this study, we examined cell fusion ability among various *A. oryzae* industrial strains.

**Methods:** Methods to sensitively detect the cell fusion were developed by employing auxotrophic complementation and BiFC (Bimolecular Fluorescence Complementation) technique.

**Results and conclusions:** The methods enabled us to demonstrate the ability of cell fusion in *A. oryzae*. There are numerous *A. oryzae* strains industrially used for sake, soy sauce and *miso*. Most of the *A. oryzae* industrial strains are capable of cell fusion, however, cell fusion was not detected with many strain pairs, a phenomenon related to heterokaryon incompatibility. Molecular investigation of the heterokaryon incompatibility by genome editing technique and comparative genomics would help efficiently induce sexual reproduction for crossbreeding in *A. oryzae*.

How has sake yeast acquired high fermentation ability?

Daisuke Watanabe
Kyoto University, Japan

**Purpose:** Sake yeast strains that belong to the budding yeast *Saccharomyces cerevisiae* exhibit higher rates of alcoholic fermentation and ethanol yields in the sake mash than the other types of *S. cerevisiae* strains. Although this has traditionally been regarded to be caused by their higher resistance against ethanol and various environmental stresses, our recent studies revealed that they are rather defective in stress responses.

**Results:** Our genomic and transcriptomic approaches have led to the identification of the sake yeast-specific loss-of-function mutations in the RIM15 gene, which encodes a Greatwall-family protein kinase that plays important roles in the responses to environmental changes. Surprisingly, this mutation markedly contributed to the increase of alcoholic fermentation rate. Furthermore, we successfully revealed the mechanism how the impaired Rim15p functions enhanced alcoholic fermentation. In response to stresses, Rim15p activates downstream transcriptional factors Msn2p and Msn4p (Msn2/4p) and Hsf1p, which upregulate the UGP1 gene that encodes UDP-glucose pyrophosphorylase. Enhanced UDP-glucose synthesis diversifies the intracellular glucose flux from glycolysis to the storage and structural carbohydrates anabolic pathways. Dysfunction of Rim15p leads to maintenance of the high fermentation performance and defective synthesis of carbohydrates to protect themselves even under the stressful environments.

**Conclusions:** Thus, we first reported the causal mutations for the high alcoholic fermentation ability of the industrial yeast strains. These findings have drastically changed how we understand the relationship between ethanol tolerance and ethanol production ability of yeast cells. Moreover, application of our finding is promising in improvement of the fermentation performance of other yeast strains used in various kinds of fermentation industries, such as food, brewing, and biofuel industries.
Potential of *Rhizopus* spp. as an Inoculum in Determining the Quality of Tempe

Anastasia Tatik Hartanti, Meda Canti, Kevin Filbert
Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, Indonesia

*Rhizopus* spp. are the main mold in tempe fermentation. Currently, tempe inoculum in Indonesia is dominated by *Rhizopus microsporus* var. oligosporus as the impact of the commercialization of one the inoculum. The potential of other variety of *R. microsporus* and other species, such as *R. delemar* and *R. stolonifer* as an tempe inoculum were needed to be studied.

**Purpose:**
This study were to determine the quality of tempe produced by several *Rhizopus* strains.

**Methods:**
In this study, we used *R. microsporus* (ATH1, ATH24, ATH26, and ATH40), *R. delemar* (ATH53 and ARPE), and *R. stolonifer* (AR1) as inoculum of tempe. As the control, we used commercial inoculum.

**Results and conclusion:**
The results showed all the tempe produced were white in colour, while ATH24 and ATH26 were yellow colour. Protein content (46-58%) and fat content (more than 8%) of all tempe produced were higher than the Indonesian National Standard (SNI). Antioxidant activities of all tempe produced was not significant difference compared to the control. Overall preference attribute of sensory test showed tempe AR1 was significantly different compared to control. Tempe AR1 and ATH26 were more preferred than other tempe. Antibacterial test results showed all tempe extracts inhibited growth of gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*), except tempe ATH 26 and ATH 40 extracts did not inhibit *S. aureus*. All tempe extracts were not inhibited the growth of gram-negative bacteria (*Escherichia coli* and *Salmonella typhimurium*). All inoculums used in this study have the potency to be developed as tempe inoculum.
**Extracts from *Agaricus blazei* Murril suppress the expression of immune checkpoint molecules in cancer cells**

Hajime Kobori¹), Etsuko Harada²), Naoto Tada¹), Toshihiro Morizono¹), Masaaki Toda²), Corina N. D’Alessandro-Gabazza²), Esteban C. Gabazza²)

¹)Iwade Research Institute of Mycology CO., Ltd, Japan
²)Department of Immunology, Mie University School of Medicine, Japan

**Purpose:** *Agaricus blazei* Murril (Himematsutake strain Iwade-101) is an edible and medicinal mushroom of Agaricus family. It is being cultivated at the Iwade Research Institute since 1975, Dr Inosuke Iwade being the first to develop a system for its artificial cultivation. *Agaricus blazei* Murril extracts have immunomodulatory, anti-cancer and anti-allergic activities. Here, we evaluated whether the alkali and subcritical extracts of hot-water extraction residues of the *Agaricus blazei* Murril fruiting body possess immune checkpoint inhibitory activity.

**Methods:** The *Agaricus blazei* Murril fruiting body was treated with hot-water at 80°C -100°C for 3h to collect its extraction residues. The extraction residues were treated with a 3%-10% sodium hydroxide for 24h to filter the extract solution. The filtrate was successively concentrated under low pressure to collect an alkaline extract. The hot-water extraction residues of the *Agaricus blazei* Murril fruiting body was subjected to subcritical extraction at 2-5 MPa and at 120°C-200°C for 5 to 240min before filtration. A subcritical extract was obtained by successive freeze-drying of the filtrate. The effect of both the alkaline extract and the subcritical extracts on the expression of the receptor tyrosine kinase Axl and the immune checkpoint molecules PD-L1 and PD-L2 were evaluated in vitro using the adenocarcinoma cell line A549.

**Results:** Culture of A549 cells in the presence of alkaline extract or the subcritical extract significantly inhibited the expression of Axl and PD-L1 and PD-L2.

**Conclusion:** Extracts from *Agaricus blazei* Murril may have anti-cancer activity by downregulating Axl, PD-L1 and PD-L2.
Probiotication of Mangosteen Extract-Based RTD by *Lactobacillus plantarum* and Its Anti-obese Validation by Selected Yeast Originated from Fruits

Nilam Fadmaulidha Wulandari  
Research Center for Biology, Indonesian Institute of Sciences (LIPI), Indonesia

**Purpose:** Beverages containing mangosteen peel extract with three different concentration (1-3% w/v), probiotics, honey water and black jelly have been made. The objectives of this research was to know good probiotication, anti-obese property, antioxidant activity, organoleptic and agglutination.

**Methods:** Previously, encapsulation of probiotic *Lactobacillus plantarum* Mar8 at log 8/ml by black grass jelly was prepared. Each concentration of mangosteen extract (1-3% w/v) was mixed with probiotic, honey water and black jelly. Selected yeast originated from indonesian native fruits was used for petite essay and agglutination.

**Results and Conclusion:** The study revealed the highest activities of antioxidant, agglutination and petite test of the drinks. Toxicity and organoleptic properties will be discussed. This potential functional drink has beneficial to reducing obesity.
New edible fungi from Southeast Asia: discovery to production

Samantha Chandranath Karunarathna\textsuperscript{1,2}, Peter Edward Mortimer\textsuperscript{1}, Jianchu Xu\textsuperscript{1,2}, Kevin David Hyde\textsuperscript{1,3}

\textsuperscript{1}Kunming Institute of Botany, Chinese Academy of Sciences, P.R. China
\textsuperscript{2}World Agroforestry Centre, East and Central Asia, P.R. China
\textsuperscript{3}Center of Excellence in Fungal Research, Mae Fah Luang University, Thailand

**Purpose:** The forests of Southeast Asia have the potential to be a rich source of cultivatable edible fungi. Although significant amounts of research on the taxonomy and phylogeny of edible mushrooms have been carried out, far fewer studies have focused on the domestication of wild fungi. Today, the most commonly cultivated strains are temperate species, but tropical and subtropical mushrooms are both abundant and highly diverse, with many species having long histories of human consumption. In addition, many new species have recently been introduced to science, including numerous species of high nutritional and medicinal value. The domestication and cultivation of tropical mushrooms therefore provides an enormous opportunity for Southeast Asian countries. Most tropical and subtropical mushrooms, if provided with appropriate conditions, grow and produce fruiting bodies more quickly than temperate species. Tropical and subtropical mushrooms can be produced using cheap, readily available waste products such as sawdust, corn cobs, rice straw, sugarcane bagasse, and other forest and agricultural residues, making them an ideal crop for smallholder farmers.

**Methods:** We have collected and isolated numerous strains of wild mushroom species from Southeast Asian forests, and have published some initial results documenting our progress in domesticating these species.

**Results and conclusions:** We showed for the first time that it is possible to domesticate the following fungi: *Pleurotus giganteus*; a new Thai-French hybrid strain of *Agaricus subrufescens*; *A. flocculosipes*; *A. subtilipes*; *Auricularia thailandica*; *A. cornea* (white); *Panus roseus*; *Macrolepiota dolichaula*; *Ganoderma australe*; *G. resinaceum*; *G. gibbosum* and *G. leucocontextum*. These discoveries may create new opportunities for the mushroom growing industry and for smallholder farmers in Southeast Asia in particular.
Utilizing mushrooms for conservation and rural development

Peter Edward Mortimer
Kunming Institute of Botany, China

**Purpose:** Mushrooms have been recognized for their potential value as an alternative, sustainable source of income for rural communities. This includes wild harvested and cultivated mushrooms as both systems provide novel aspects for alleviating poverty and improving household nutrition.

**Methods:** Forest surveys were conducted in the Chin State, Myanmar, to assess the diversity of mushrooms present in the region. Furthermore, interviews were conducted in villages to help determine the local knowledge relating to the use and trade of mushrooms. Training was provided to local communities on the identification and harvesting of wild mushrooms, and the cultivation of *Pleurotus ostreatus.*

**Results:** We collected 37 mushrooms species with economic potential. The surveyed areas included coniferous (Pinaceae) and broad leaf (Betulataceae and Fagaceae) forests. A number of poisonous mushrooms were also collected (31 species), this is of importance due to the high occurrence of mushroom related fatalities in the area. The interview data suggests a low level of knowledge relating to the identification, use, and trade of mushrooms in the Chin State, and a high degree of fear regarding the use of wild mushrooms. Mushroom cultivation programs were successful, after 3 training sessions the communities were able to grow *P. ostreatus,* at sufficient volumes to generate enough income to sustain their production lines.

**Conclusions:** The Chin State has the potential for developing a mushroom industry. There is a high number of wild, economically valuable mushrooms, however a strong fear of mushroom poisoning makes the use of wild mushrooms unappealing to the local communities. In this regard, mushroom cultivation been well received by local communities, and has thus far proven profitable. The potential trade associated with wild mushrooms is likely to provide an incentive for rural communities to conserve the forest systems surrounding their villages.
Analysis of bacterial community structure for evaluating maturation of compost for *Agaricus blazei* Murrill cultivation

Naoto Tada¹, Natsumi Sugawara², Mitsuo Kawade¹, Akihiro Saito³, Akikazu Ando²

¹Iwade Research Institute of Mycology CO., Ltd, Japan
²Graduate School of Advanced Integration Science, Chiba University, Japan
³Department of Materials and Life Science, Faculty of Science and Technology, Shizuoka Institute of Science and Technology, Japan

**Purpose:** The maturation of the compost for *Agaricus blazei* Murrill cultivation has been evaluated with sense and experience, conventionally. In this study, we analyzed chemical and microbial properties of the compost during the fermentation process, in order to find an indicator for maturation of the compost.

**Methods:** Compost was prepared three times independently. During the fermentation process, colony numbers of microorganisms (bacteria, fungi and actinomycetes), bacterial community structure, C/N ratio, and fresh yield of the fruit bodies were investigated. The bacteria, fungi, and actinomycetes were isolated from the compost using YG, Rose Bengal or HV media, respectively. Bacterial community structure was investigated by PCR-DGGE analysis. Effects of the microbial isolates on the growth of *A. blazei* Murrill mycelia was examined by counter culture method.

**Results:** In the first preparation of compost, the yield of the fresh fruit body became maximum levels after the C/N ratio and bacterial community structure were stabilized. The results were reproductively observed in the following two compost preparations. The bacterial community structures in the matured composts were similar regardless of the preparation batches. Some DNA-bands were specifically observed in the composts with high yields of fruit bodies, indicating the presence of bacteria specific to matured composts. *Acinetobacter* sp. isolated from the matured composts had a positive effect on growth of *A. blazei* Murrill mycelia.

**Conclusions:** These results suggests that not only C/N ratio but also some bacteria could be used as the indicators for maturation of composts for *A. blazei* Murrill cultivation.
Development of cultivar-specific DNA markers for *Lentinula edodes* (Berk.) Pegler based on SNP data via a MIG-seq approach

Akihiko Kinoshita¹, Mitsuhiko P. Sato², Kazuhiro Miyazaki¹, Rikuo Fukui³, Ayumi Matsuo², Osamu Kurashima⁴, Motomi Ito⁴, Yoshihisa Suyama²

¹Kyushu Research Center, Forestry and Forest Products Research Institute, Japan
²Kawatabi Field Science Center, Graduate School of Agricultural Science, Tohoku University, Japan
³Edible Mushroom Spawn Association, Japan
⁴Department of Systems Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Japan

Purpose: *Lentinula edodes* (shiitake) is a popular cultivated edible mushroom, with more than 200 cultivars in Japan. A rapid and precise cultivar-discrimination system will be useful for the management of existing cultivars and the development of new varieties. Our study objective was to develop cultivar-specific DNA markers based on genome-wide SNP data with MIG-seq (multiplexed ISSR genotyping by sequencing) technique.

Methods: A MIG-seq analysis was conducted for more than 70 shiitake cultivars provided by manufacturers and institutions. We identified SNPs specific to cultivar 5K-16 by comparing all tested cultivars, after which we designed sequence-specific primers. The target DNA was amplified and analyzed by agarose gel electrophoresis to assess the utility of the primers.

Results and conclusions: We detected more than 3,000 SNPs among the analyzed cultivars based on the MIG-seq data. To date, five primer sets that efficiently and specifically identify 5K-16 have been produced. Thus, our novel method for developing markers specific to 5K-16 based on genome-wide SNP data is useful for generating cultivar-specific DNA markers.
Adaptive evolution of sugar metabolism networks in yeast

Feng-Yan Bai, Shou-Fu Duan, Ri-Peng Zhang, Qi Yin, Jun-Yan Shi
State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, China

Purpose: The budding yeast *Saccharomyces cerevisiae* has been used worldwide for food and beverage fermentation for thousands of years. The domesticated populations of the yeast show significantly elevated maltose or galactose utilization abilities compared with its wild lineages. The purpose of this study is to illuminate the molecular mechanisms of the improved traits of domesticated yeast lineages.

Methods: We sequenced *S. cerevisiae* isolates representing different wild and domesticated lineages using the Illumina and the PacBio long-read sequencing strategies and performed SNP, copy number variation, structure variation, introgression and horizontal gene transfer analyses. The phenotypic consequences of the genetic changes were confirmed by gene deletion or swap experiments.

Results and conclusions: We found a remarkable expansion of gene contents, copy numbers, structural complexities and translocation events in the MAL network from the wild to domesticated lineages of *S. cerevisiae*. These polygenic changes in the MAL network are collectively responsible for the significant elevation of maltose metabolism in the domesticated lineages of *S. cerevisiae*. The Milk lineage of *S. cerevisiae* has swapped all its structural GAL genes with early diverged versions through introgression and duplicated the introgressed GAL2 gene. The rewired GAL network has achieved galactose-over-glucose preference switch, abolished glucose repression, and conversed from a strictly inducible to a constitutive system through polygenic changes in the regulatory components of the network. The reverse evolution of the GAL network confers a competitive advantage to the Milk lineage of *S. cerevisiae* in spontaneously fermented milks.
Oral Session 7

2-007-2

Origin of isolates - big but obscure data

Andrey Yurkov, Lorenz Reimer, Sabine Gronow
Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Germany

Purpose: Our understanding of biodiversity heavily relies on specimens preserved in herbaria and culture collections. Collection catalogues provide information about genetic, biochemical and physiological properties of organisms. Furthermore, the deposited material is usually associated with information about country and substrate of isolation. Description of the substrate of isolation does not follow any standard and varies in terms of complexity of the provided information, language, wording, spelling and heterogeneity of recorded environmental data. This makes any analysis of thousands of records extremely difficult, if possible at all.

Methods: Here we report a novel approach to classify and analyse data of the source of isolation in the DSMZ culture collection. We allowed a record to be described with several keywords, analogous to hashtags used in social media. Each keyword was characterised within an own hierarchical ontology-like structure. Additionally, we extracted host data and included Latin names.

Results: Strains were associated with 1-6 (out of 370) keywords that were used to classify sources of isolation into 8 categories and 61 subcategories. Using the advantage of the ontology-like structure, we were able analyse the linking of strains based on their origin on different hierarchical levels, e.g. Environmental > Aquatic > Marine.

Conclusions: We explored the application of this approach on collections of fungi, yeasts and bacteria in a few institutions and collections. With these results, we provide examples on how advanced classification of substrates can improve the use of data from culture collections and other repositories for research in microbial ecology, medicine and biotechnology.
Surveillance of molecular epidemiology of *Cryptococcus gattii* in Taiwan

Kuo-hsi Lin\(^1,2\), Wen-Hsin Chung\(^1\)

\(^1\)National Chung Hsing University, Taiwan
\(^2\)Taichung Tungs’ Metroharbor Hospital

**Purpose:** *Cryptococcus gattii* species complex is a yeast pathogen causing cryptococcosis mainly in non-HIV infected patients. Studies had discovered various species of trees as ecological niches of *C. gattii* and, therefore, sources of infection. We screened trees and the soil around these trees for *C. gattii* isolation and determined the molecular epidemiology of *C. gattii* in Taiwan.

**Method:** We screened various trees and rhizosphere soil of trees in parks and schools in Western Taiwan to isolate *C. gattii* from the environment. The multilocus sequence typing (MLST) of the isolates was performed, and the mating type of the isolates was determined.

**Results:** From November 2017 to May 2018, a total of 82 isolates of *C. gattii* had been isolated from wood debris and soils. The isolation rate was higher in the parks or schools that were established early, especially in Japanese-ruled era. Thirty-three of them were VGI, and the others were VGII. Three domestic sequence types (STs) that had not been reported in other parts of the world were discovered in Taiwan, which were ST328, ST546, and ST548. The diversity of ST is region-dependent. A park in mid-Taiwan had the highest diversity of ST of *C. gattii*. The *Cryptococcus gattii* was isolated in limited species of trees. The top 3 trees that *C. gattii* was most commonly isolated from are *Eucalyptus robusta*, *Pithecellobium dulce*, and *Melaleuca leucadendra*, and the positive rate was 45/116 (38.8%), 22/59 (37.3%), 10/94 (10.6%), respectively.

**Conclusion:** Isolation of *C. gattii* from the environment can enrich our knowledge of local molecular epidemiology of this yeast. This molecular epidemiology data is helpful in studies of the evolution of *C. gattii*, the determination of the source of infection and disease prevention.
Prevalence of *Candida* spp. in Endocervical, Vaginal and Urine Samples of Reproductive Women and In-Vitro Susceptibility Pattern of the Isolates

Llyrha Mae Escaner Maghari-Capio
De la Salle Medical and Health Sciences Institute, Philippines

Vulvo-vaginal candidiasis (VVC) is an inflammatory condition of female genital tract and most encountered problem that affects a large fraction of women in a population caused by different species of the genus *Candida*. This study aimed to assess the prevalence of *Candida* spp. in the endocervical, vaginal and urine sample of patients treated at the university hospital in the Philippines and to evaluate the in-vitro sensitivities of the isolated *Candida* species to four commonly used antifungal agents. Samples were taken from consenting patients of gynecology clinics and isolates were identified using phenotypic and biochemical tests. Susceptibility analysis was performed using Kirby Bauer Method. Of the 86 patients, the prevalence of *Candida* spp was isolated in 37.2% (n=32) corresponding to a prevalence of approximately 59.36% (n=19) for VVC and 40.63% (n=13) colonization. More than 46.87% (n=15) of the isolates were identified as *Candida albicans*; *C. non-albicans* was isolated at a rate of 12.5% (n=4) in symptomatic patients and 9.37% (n=3) *C. albicans* and 31.23% (n=10) non albicans in asymptomatic patients. The prevalence of resistance against itraconazole, ketonazole, fluconazole and nystatin, were 16.66%, 43.75%, 29.16% and 5.20% respectively. The high rate of resistance to triazoles (ketonazole and fluconazole) observed in this study suggests that antifungal prescription should be only given once the proper identification of the *Candida* species has been performed. Furthermore, improperly prescribed antifungal agents may lead to drug resistant.
Diversity of yeasts and yeast-like fungi isolated from household air-conditioners

Shigeki Inaba¹, Atsushi Yamazaki¹, Kazumi Sasaki¹, Kazunobu Kuwabara², Takahiko Horiguchi², Masashi Nakamura²,³, Kayoko Matsunaga²

¹NITE Biological Resource Center, National Institute of Technology and Evaluation, Japan
²Fujita Health University, Japan
³Hoyu, Co., Ltd., Japan

Purpose: The aim of this study is to survey yeasts and yeast-like fungi flora in household air-conditioners (ACs). Fungi are important aeroallergens in private houses and ACs have known to be one of supply sources of such fungal allergens. While some studies have reported filamentous fungal contamination in ACs, research focusing on yeasts and yeast-like fungi is relatively limited.

Methods: Yeasts and yeast-like fungi flora in ACs of private houses was studied in 2018. Using the swabbing method, total 37 dust samples accumulated on internal parts of 18 ACs, mainly filters and heat-exchangers, were collected in Chubu region, Japan. The samples were spread onto PDA plates supplemented with chloramphenicol and incubated at 25 and 40°C. Yeast colonies appeared on the plates were isolated and identified based on the nuclear ribosomal ITS and partial 26S rRNA region sequences and the morphological features.

Results and conclusions: In total, 71 isolates were obtained from the dust samples, and 30 species were recognized. At 40°C, 4 ascomycetous yeast species, Candida metapsilosis, C. parapsilosis, Meyerozyma caribbica and M. guilliermondii, which are known as opportunistic pathogens, were isolated. The occurrences, however, were very low and the species were isolated from only 1, 1, 1 and 3 of 18 ACs, respectively. At 25°C, 23 described (4 ascomycetes and 19 basidiomycetes) and 4 undescribed basidiomycetous species were isolated. The basidiomycetous yeast species are belonging to 3 subphylla, Pucciniomycotina, Ustilaginomycotina and Agaricomycotina. Rhodotorula mucilaginosa was the most dominant species and appeared from 10 of 18 ACs. The results suggest that taxonomically diverse yeasts and yeast-like fungi, including opportunistic pathogens and new species candidates, inhabit in the household ACs.
Root endophytic *Chaetomium cupreum* increased Al tolerance in *Miscanthus sinensis* growing at an old mine site

Toshikatsu Haruma¹,², Keiko Yamaji², Kazuyoshi Ogawa², Hayato Masuya³, Yurina Sekine¹, Naofumi Kozai¹

¹Japan Atomic Energy Agency, Japan
²University of Tsukuba, Japan
³Forest Research and Management Organization, Japan

**Purpose:** Aluminum (Al) causes plant toxicity in acid soil such as mine sites. *Miscanthus sinensis* has Al tolerance via producing chlorogenic, citric, and malic acids to detoxify Al. Some root endophytes produce siderophores, which detoxify Al by chelating. *Chaetomium cupreum* isolated from roots of *M. sinensis* growing at a mine site showed high siderophore production. The purpose of this study was to clarify Al-tolerance mechanisms in *M. sinensis* by considering the interaction with *C. cupreum*.

**Methods:** The siderophore was identified as oosporein by HPLC/ESI-HDMS and XRD. The stability constant of oosporein-Al was calculated to evaluate Al-detoxification efficiency via titration. *Chaetomium cupreum* was used for soil-inoculation test with *M. sinensis* to clarify chemical Al tolerance via measuring concentrations of Al by ICP-OES, chlorogenic acid, and oosporein by HPLC/MS. In water-inoculation test, Al localization was observed by confocal laser microscopy to clarify physical Al tolerance.

**Results:** The stability constant of Al and oosporein was lower than chlorogenic acid, and higher than citric and malic acids. In soil-inoculation test, *C. cupreum* enhanced plant growth, seemed to increase chlorogenic acid production, and produced oosporein in the roots. Al was localized in cell walls of roots. *Chaetomium cupreum* changed Al localization in roots, and accumulated Al in the hyphae to decrease Al toxicity to *M. sinensis*.

**Conclusions:** Oosporein could detoxify Al more efficiently than citric and malic acids. *Chaetomium cupreum* enhanced Al tolerance in *M. sinensis* via increasing detoxified Al concentration by chlorogenic acid and oosporein, changing Al localization, and accumulating Al in the hyphae.
Mechanism of heavy-metal tolerance in shade plant *Aucuba japonica* via the possible function of root-endophytes

Kohei Doyama¹, Keiko Yamaji², Toshikatsu Haruma²,³

¹Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan
²Life and Environmental Sciences, University of Tsukuba, Japan
³Advanced Science Research Center, Sector of Nuclear Science Research, Japan Atomic Energy Agency, Japan

**Purpose:** *Aucuba japonica* is an evergreen shrub and grows normally in the forest floor of our research site, which has been contaminated with heavy metals, such as copper, lead, and zinc. Recently, root-endophytes are reported to enhance heavy-metal tolerance of plants. Our purpose is to clarify the mechanism of heavy-metal tolerance of *A. japonica*, considering the interaction with root-endophytes.

**Methods:** *Aucuba japonica* is growing in forest floor of deciduous mixed forest, where the light condition and temperature are changing through a year, therefore, we collected plants both in July 2017 (summer) and January 2018 (winter). Leaves, branches and roots were analyzed for heavy-metal concentration by ICP-OES and identification of detoxicants, phenolics by HPLC/DAD, and organic acids-TMS by GC/MS. Distribution of zinc in roots was also observed by staining with zinpyr-1, which has Zn-selective fluorescence under confocal laser microscope.

Endophytic fungi were isolated from surface-sterilized root segments, and evaluated microbial ability to chelate zinc by the culture medium containing insoluble zinc.

**Results:** *Aucuba japonica* highly accumulated zinc in roots, and produced citric acid and aucubin as detoxicants both in summer and winter. Zinc was localized in cell walls of epidermis, cortex and stele, and also detected in fungal structures and hyphae in cortical cells.

Root-endophyte, *Pezicula* sp. was most frequently isolated, showing Zn-chelating abilities.

**Conclusions:** *Aucuba japonica* accumulated zinc in roots, suggesting zinc-tolerance by sequenstering zinc in cell walls and producing detoxicants such as aucubin and citric acid through the year. *Pezicula* sp. might enhance the heavy-metal tolerance via producing Zn-detoxicants.
Community and Species-Specific Responses of Soil-Borne Fungi to Copper Oxide and Zinc Oxide Nanoparticles

Jonathan Jaime Gurimbao-Guerrero¹, Teresita U. Dalisay², Ireneo B. Pangga², Nolissa D. Organo³, Pattavipha Songkumarn⁴

¹Bicol University College of Science Department of Biology, Philippines  
²Institute of Weed Science, Entomology and Plant Pathology, University of the Philippines Los Banos, Philippines  
³Soil Science Division, Agricultural Science Institute, University of the Philippines Los Banos, Philippines  
⁴Kasetsart University Faculty of Agriculture, Bangkok, Thailand

Purpose: This research was conducted to evaluate the effects of copper oxide and zinc oxide nanoparticles (NPs) and bulk forms on soil fungal community and to fungal plant pathogens.

Methods: Lipa clay loam soil was treated with 300 ppm CuO or ZnO NPs or bulk forms and incubated in sterile pots for 15 days. Colony-forming units was checked on days 1, 8 and 15. Functional richness, growth, and stress index were determined using Biolog FF plates and expressed as absorbance values. In a separate set up, Rhizoctonia solani, Sclerotium rolfsii, Fusarium oxysporum f.sp. lycopersici and Phytophthora palmivora were grown on amended potato dextrose agar for seven days. Radial growth, protein leakage, organic acid and morphological changes were determined through direct plate observation, UV-vis spectrophotometry, gas chromatography, fluorescent microscopy and scanning electron microscopy, respectively.

Results: The results indicated that CuO and ZnO do not have significant effects to CFU, respiration, growth and stress index to soil fungal community. Among fungal plant pathogens, P. palmivora was most susceptible to CuO in both bulk and nano-forms. R. solani and S. rolfsii were inhibited but recovered from the metal stress thereafter. F.o. f.sp. lycopersici showed cultural changes and decreased production of organic acids compared to control.

Conclusion: While CuO and ZnO showed toxicity, it is the nature of the compound and not particle size that provides the antifungal property in vitro. It is recommended that a range of sizes be used and that other measures of toxicity be employed to comprehensively account for the effects of CuO and ZnO NPs.
Uptake of cesium by ectomycorrhizal fungi and its transfer into plants

Sumika Ogo¹,², Takashi Yamanaka¹, Keiko Akama¹, Junko Nagakura¹, Keiko Yamaji²
¹Forestry and Forest Products Research Institute, Japan
²Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan

Purpose: High concentrations of radionuclides were released into the environment because of the accident at the Fukushima Dai-ichi Nuclear Power Plant in Japan in 2011. Fungi play an important role in the dynamics of radiocesium in the forest.

Methods: We measured the uptake of cesium (Cs), rubidium (Rb), and potassium (K) by ectomycorrhizal (EM) fungi by using inductively coupled plasma mass spectrometry. In addition, we examined the effect of EM formation by Astraeus hygrometricus on the uptake of Cs and K by Pinus densiflora.

Results: The genera Suillus, Pisolithus, and Rhizopogon accumulated Cs, Rb, and K more efficiently than those accumulated in other species when cultured with NH₄ as a nitrogen source, whereas Astraeus and Scleroderma efficiently accumulated Cs, Rb, and K when they were cultured with NO₃. The growth of seedlings was enhanced after EM formation. Cs concentrations in the shoots of EM seedlings significantly increased than in those of non-EM seedlings when CsCl was not added to the medium, suggesting that A. hygrometricus could solubilize Cs fixed in soil particles. Moreover, K concentration in the shoots of EM seedlings significantly increased than in those of non-EM seedlings when CsCl was added.

Conclusions: We observed that the nitrogen source as well as the fungal species affect the uptake of Cs, Rb, and K. In addition, different mechanisms might control the transfer of Cs and K from soil into pine seedlings.
Detection of autophagy-related structures in the fruiting bodies of *Pleurotus ostreatus*

Yuma Ozaki\(^1\), Tadanori Aimi\(^2\), Norihiro Shimomura\(^2\)

\(^1\)The United Graduate School of Agricultural Sciences, Tottori University, Japan  
\(^2\)Faculty of Agriculture, Tottori University, Japan

**Purpose:** Recent studies have revealed that autophagy is involved in the phenomenon of fungal morphogenesis such as sporulation and germination in conidium, and appressorium formation in pathogenic fungi. However, limited information is available regarding the involvement of autophagy in the process of mushroom fruiting. In the present study, we aimed to detect autophagy-related structures in the fruiting bodies of *Pleurotus ostreatus*.

**Methods:** Specimens were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, followed by fixation in osmium tetroxide in 0.1 M phosphate buffer. These samples were embedded in resin, and ultrathin sections were visualized under an electron microscope.

**Result and Conclusion:** Electron microscopic study revealed that the double membraned structures were detected in the cytosol ranging from 500-2,000 nm in diameter. The internal composition of the double membrane structure was morphologically identical to that of the cytosol. The double membraned structures appeared to be in contact with the vacuolar membrane, suggesting that the double membraned structure was an autophagosome, which is typically observed in the process of macroautophagy. In contrast, cytosol or mitochondria surrounded by membrane was observed in the vacuole, indicating that a part of the cytosolic component was sequestered into the vacuole. While the membranes sequestering cytosolic component into vacuoles are indistinguishable from that of the vacuoles, invaginated vacuolar membrane inside the lumen was observed, signifying the involvement of the microautophagy process. These findings suggest that autophagy is involved in the process of fruiting body formation in *P. ostreatus*.
Why study endophytic fungi? The case of Chinese mesona (*Platostoma palustre*)

Roland Kirschner¹, Chung-Wei Hsieh²

¹School of Forestry & Resource Conservation, National Taiwan University, Taiwan
²Department of Life Sciences, National Central University, Taiwan

**Purpose:** The herbaceous plant Chinese mesona (*Platostoma palustre*) is the source for the production of herbal tea and grass jelly as regional specialty in Southeast Asia and Taiwan. One aim was to address fungal diversity associated with this plant, because hitherto no fungal species has been recorded for this crop. Another aim of this presentation is to exemplify general advantages and limitations of such kinds of biodiversity approaches.

**Methods:** Endophytic fungi were isolated from surface-sterilized healthy roots, stems, and leaves and identified by ITS and protein gene sequences and morphology.

**Results:** Over 150 isolates were obtained from 15 healthy plants of *P. palustre*. The most common species were in genera comprising many important plant pathogens. An infection experiment with an endophytic strain confirmed latent pathogenicity. Observation of leaf disease suggested pathogenicity of another fungus which was also isolated as endophyte.

**Conclusions:** In spite of overall species similarity among fungi commonly isolated in the numerous endophyte studies so far, our example indicates that this approach may contribute data to plant-fungus species ratio estimates and allow some risk assessment of the fungus-plant association in agriculture. This kind of study, however, does not contribute to deeper fundamental understanding of plant-endophyte interaction.
Plant associated *Plectosphaerella* species in Japan

Yuuri Hirooka¹, Honami Miyata², Toshiyuki Usami³, Toyozo Sato⁴

¹Department of Clinical Plant Science, Hosei University, Japan
²Saitama Agricultural Technology Research Center, Japan
³Graduate School of Horticulture, Chiba University, Japan
⁴Department of Agro-Food Science, Niigata Agro-Food University, Japan

**Purpose:** Species of the genus *Plectosphaerella* (Plectosphaerellaceae, Sordariomycetes) are well known as plant associated fungi in the world. Although 14 plant diseases caused by the genus have been recorded in Japan, the taxonomic position of each pathogen to species level was not often discussed. The objective of this study was to know the diversity of the plant associated *Plectosphaerella* species in Japan based on detail morphological observation and multi-locus phylogenetic analyses (ITS, LSU, TEF, TUB and CAL).

**Methods:** We examined 46 isolates of Japanese *Plectosphaerella* species in this study. Among them, 28 isolates were found from our field surveys, and the other 18 isolates were received from the Genetic Resources Center, NARO (NARO Genebank), Japan. Its morphological examination was performed on SNA at 25°C after 7 days. Molecular phylogenetic trees were produced based on five loci.

**Results and Conclusions:** Our phylogenetic analyses reveled that 15 isolates made 10 monophyletic clades within the genus *Plectosphaerella*. Based on our detail morphological analysis, each clade did not match any of known species. Therefore, at least 10 unknown species are present in the genus. The host plants of the unknown species are *Sagittaria trifolia* (Alismataceae), *Cucurbita moschata* (Cucurbitaceae), *Solanum tuberosum* (Solanaceae), *Zantedeschia aethiopica* (Araceae), *Lactuca sativa* var. *crispa* (Asteraceae), *Oryza sativa* (Poaceae), *Musa* sp. (Musaceae), and *Ranunculus* spp. (Ranunculaceae). A new identification key for all species of *Plectosphaerella* that included known and newly discovered species in this study is proposed herein.
Rice seed-borne fungi, with special reference to Microdochium, Sarocladium and dematiaceous hyphomycetes

Jie-Hao Ou, Chi-yu Chen
National Chung Hsing University, Taiwan

Purpose: Numerous fungi are carried by rice seed. In addition to the saprophytic species, some species can cause plant diseases, some can produce toxins and some are opportunistic to cause human diseases. Consequently understanding rice seed-borne fungi has been the concern of plant quarantine. The aim of this study is to establish a comprehensive guideline for the identification of rice seed-borne fungi with fully description, illustration, and molecular information. Taxonomic treatments are carried out where applicable.

Methods: A survey of fungi associated with rice seed was conducted during 2012 to 2019. All isolated strains were examined morphologically and subjected to multi-locus phylogenetic analyses.

Results and conclusions: More than four hundred fungal strains were obtained, from which 112 species have been identified. In Sarocladium, four species were successfully identified, including one new species. Particularly, a unique fluorescence-based method by utilizing invisible near-ultraviolet light was developed in the help of species separation. In Microdochium, three species were identified, including one new combination. For dematiaceous hyphomycetes, more than 15 species were identified. The placement of those species in allied genera was evaluated morphologically and molecularly. The abundance of fungi on rice seed is beyond expectation. There are more awaiting to be explored.
Seed fungi are an ecological group of fungi that colonize on decaying seeds or fruits. In this study of seed fungi in Thailand, seeds and fruits were collected from forest floors, incubated in the laboratory, and observed under the microscope for fungi which were morphologically identified and isolated. Most of these seed fungi were encountered as in anamorphic or asexual stages, and the present molecular phylogeny helps to find their accurate position of taxonomic classification. The study reveals that Ascomycota is the major group of seed fungi. The major classes are Sordariomycetes (94 species), Dothideomycetes (31 species), and Incertae sedis or a class of uncertain placement (38 species) while Eurotiomycetes, Leotiomycetes, and Orbiliomycetes have only one species in each class. The major orders of seed fungi in Dothideomycetes are Tubeufiales (14 species) and Pleosporales (12 species) while there are many major orders in Sordariomycetes, such as Chaetosphaeriales (33 species), Hypocreales (14 species) and Microascales (13 species). Genera with species abundance include Dictyochaeta, Helicosporium, and Thozetella while the abundant species on seeds are Menisporopsis theobromae, Cryptophialoidea secunda, and Kionochaeta ramifera. The similarity between seed fungi and leaf litter fungi is discussed. The results from other studies on seed fungi in Thailand are also reviewed and compared.
Taxonomic circumscription and phylogenetics of microfungi associated with Rosaceae

Dhanushka Nadeeshan Wanasinghe¹², Kevin David Hyde¹², Jian Chu Xu¹, Peter Edward Mortimer¹
¹Key Laboratory for Plant Biodiversity and Biogeography of East Asia (KLPB), Kunming Institute of Botany, Chinese Academy of Science, China
²Center of Excellence in Fungal Research, Mae Fah Luang University, Thailand

Purpose: Fungal occurrence on different hosts or different tissue types on the same host is an important consideration in the estimation of global fungal numbers and implications for diversity estimates. Rosaceae is one of the largest families of Angiosperms, including more than 100 genera and about 3100 species with a diversified distribution. There have been several studies where microfungi have been recorded or characterized from members of the Rosaceae. But very few of these taxa are presently known from cultures and most of these published records lack illustrations and descriptions, or DNA sequence data and thus it is very difficult to confirm their names or investigate taxonomic relationships.

Methods: We aimed at providing a compilation of recently collected fungal taxa from Rosaceae in Europe and Asia based on morphological and phylogenetic characterisation. All data presented herein are based on morphological examination of specimens, coupled with phylogenetic sequence data to better integrate taxa into appropriate taxonomic ranks and infer their evolutionary relationships.

Results: This study provides some insights into the diversity of fungi on Rosa species and especially those on Rosa spines that resulted in the characterisation of eight new genera, 45 new species, and nine new host records. We also collected taxa from Rosa stems and there was 31% (20/65) overlap with taxa found on stems with that on spines.

Conclusions: Because of the limited and non-targeted sampling for comparison with collections from spines and stems of the same host and location, it is not possible to say that the fungi on spines of Rosa differ from those on stems. The study however, does illustrate how spines are interesting substrates with high fungal biodiversity. This may be because of their hard structure resulting in slow decay and hence are suitable substrates leading to fungal colonisation.
Current taxonomy of *Penicillium* and *Talaromyces*

Robert A. Samson
Westerdijk Fungal Biodiversity Institute, The Netherlands

The studies on the taxonomy of *Penicillium* have a long history starting at the start of 1900. Since then the interest to classify the species of *Penicillium* have only increased because the genus has a common worldwide occurrence with a significant economic impact. Until the 1980 the studies primarily used phenotypic characters which complicated the species delimitation because the lack of standardization of methods. Although Kenneth Raper and Charles Thom recommended growth media for the study of *Penicillium* and other mycologists used physiological criteria the classification of the genus remained troublesome. This has changed since the introduction of the polyphasic taxonomy approach which combines phenotypical, physiological, biochemical and molecular characteristics. In the last decade this has resulted that the phylogeny of *Penicillium* has been elucidated with a species concept which has been generally accepted. Phylogenetic studies has clearly indicated that taxa formerly belonged to *Penicillium* subgenus *Biverticillium* have to be separated from *Penicillium* and they are now accommodated in *Talaromyces*. In this presentation the current taxonomic status of both genera will be given with an outlook how the taxonomy of these genera will develop.
Updating the taxonomy of the genera *Aspergillus*, *Penicillium* and *Talaromyces* in South Africa

Cobus Meyer Visagie, Neriman Yilmaz
Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa

**Purpose:** South Africa is a biodiversity hotspot with high endemicity. During the morphological era, a trend started of not identifying local *Aspergillus* or *Penicillium* to species level. DNA sequencing combined with a published accepted species list changed this, resulting in ±35 new species described from South Africa in recent years. The goal of this project was to update identifications of strains accessioned in the National Collection of Fungi (PPRI) and the Medical Research Council (MRC) collection.

**Methods:** A thousand PPRI and MRC strains were examined. Of this, 588 strains were sequenced using Beta-tubulin (BenA) for *Penicillium* & *Talaromyces*, and calmodulin (CaM) for *Aspergillus*. Obtained sequences were compared to a locally curated sequence database to make identifications. For strains representing new or rare species, additional internally transcribed spacer rDNA (ITS), BenA, CaM and RPB2 sequences were obtained. New species were described using recommended standardized methods.

**Results:** A total of 588 strains were re-identified to 186 species (*70 Aspergillus*, *90 Penicillium* and *26 Talaromyces*) based on 1057 DNA sequences generated. Amongst these, 27 represent new species (*8 Aspergillus*, *15 Penicillium* and *4 Talaromyces*).

**Conclusions:** South Africa can make significant contributions to a better understanding of these important genera. Not only were rare and new species discovered, but additional infraspecies variation for well-known species were captured. This data is crucial for more robust species delineations, and will thus aid future identifications whether based on culture dependent or - independent techniques.
Characterization and proposal of two new species in *Aspergillus* section *Nigri*

Cai Bian¹, Yikelamu Alimu¹, Yoko Kusuya¹, Tetsuhiro Matsuzawa², Hiroki Takahashi¹,
Takashi Yaguchi¹

¹Chiba University, Japan
²University of Nagasaki

**Purpose:** *Aspergillus* section *Nigri* (section *Nigri*) is an important group of species in food mycology, medical mycology and biotechnology. *Aspergillus niger* (*A. niger*) is a member of section *Nigri*, and second most frequently isolated from clinical specimens in Japan. Here we propose two new species in section *Nigri* supported by the genomic and phenotypic analysis.

**Methods:** We investigated a total of 11 *A. niger* related strains including six clinical and five environmental strains isolated in Japan and Kenya, respectively. We performed the phylogenetic analysis based on both calmodulin gene and the whole genome sequences. We observed and compared their morphological characters such as colony size on CYA, MEA at both 25 and 37 degree Celsius. We also measured their drug resistance to ITCZ and VRCZ.

**Results:** Among 11 strains, five and six strains were classified as *A. costaricaensis* and *A. tubingensis*, respectively, based on the phylogenetic analysis by using calmodulin gene sequences. However, according to the genome-wide phylogenetic analysis, we observed that these strains were located in two new clades.

**Conclusion:** The genomic and phenotypic data indicated two new species in section *Nigri*. 
Taxonomic Re-evaluation of White and Black-Koji Molds

Seung-Beom Hong1), Osamu Yamada2), Robert Samson3)
1) National Institute of Agricultural Science, Korea
2) National Research Institute of Brewing, Japan
3) Westerdijk Fungal Biodiversity Centre, The Netherlands

Makgeolli is Korean traditional rice wine that had been the most consumed alcoholic beverage in 1970s in Korea. The Makgeolli have been made by Nuruk that is a Korean traditional fermentation starter. However a lot of the Nuruk have been replaced by White koji mold since 1930s in Korea. The White koji mold was called as Aspergillus kawachii in Korea. Authors tried to find correct scientific name of the White koji mold. As a result, authors elucidated that White koji mold is albino mutant of Black koji mold that is originated from Okinawa and used for making Awamori Shochu. Therefore, authors re-evaluate scientific names of all species that were used as White and Black koji molds. According to polyphasic taxonomy, White and Black-koji mold species can be divided into three species, A. luchuensis, A. niger and A. tubingensis. A. awamori, A. kawachii, A. inuii, A. nakazawai and A. coreanus were synonymized into A. luchuensis, A. batatae, A. aureus (or A. foetidus), A. miyakoensis and A. usamii (including A. usamii mut. shirousamii) were synonymized into A. niger and A. saitoi and A. saitoi var. kagoshimaensis were synonymized into A. tubingensis in this study. A. luchuensis mut. kawachii, A. niger var. usamii and A. niger mut. shirousamii were also suggested particular names for A. kawachii, A. usamii and A. shirousamii, respectively because of their industrial importance. The history and modern taxonomy of White and Black-Koji molds will be further discussed.
3-O11-3

Genomic landscape of secondary metabolism in *Aspergillus* species

Daisuke Hagiwara\(^1,2,3\)

\(^1\)Faculty of Life and Environmental Sciences, University of Tsukuba, Japan
\(^2\)Microbiology Research Center for Sustainability (MiCS), University of Tsukuba
\(^3\)Medical Mycology Research Center, Chiba University

**Purpose**: Filamentous fungi produce a broad range of secondary metabolites such as penicillin, aflatoxin, and gliotoxin. Increasing numbers of sequenced fungal genomes revealed their potentials to produce more diverse metabolites than previously discovered. However, most of the biosynthetic genes are hardly expressed under laboratory conditions. Although fungal genome sequences are accumulated, transcriptomic view for evolution of SM biosynthesis was sparse. To gain deeper insight into fungal secondary metabolism (SM), here we conducted comparative genomics and transcriptomics analyses using strains of closely related *Aspergillus* species.

**Methods**: Genomes of *Aspergillus* fumigatus, *A. lentulus*, *A. udagawae*, *A. fischeri*, and *A. pseudoviridinutans* were compared with regard to SM genes. Transcriptomic data were obtained from 4 different cultures such as potato dextrose broth, Sabouraud broth, Czapek-Dox, potato dextrose agar.

**Results and conclusions**: The 5 *Aspergillus* species possess 34-75 SM core genes for polyketide synthase and non-ribosomal peptide synthase, and 13 types of the SM gene cluster are evolutionarily conserved in all species tested. Transcriptome analysis revealed that rate of active (expressed) genes for the lineage-conserved SM core genes was higher than that for species-specific SM core genes. This finding is suggestive of evolutionary diversification at transcriptional level besides genomic rearrangement or cluster gain and loss event.
Classification of *Aspergillus fumigatus* related species in Japan and their antifungal susceptibilities

Takashi Yaguchi
Chiba University, Japan

*Aspergillus fumigatus* is usually reported as both the most common member of the section in soil worldwide and the most common cause of aspergillosis. Its related species, *A. lentulus*, *A. udagawae* and *A. viridinutans* have been also reported as causative agents of aspergillosis, and the numbers of cases are increasing. Their susceptibilities against antifungal drugs, especially azoles, are different from that of *A. fumigatus*. Therefore it is essential to identify their species correctly for the appropriate treatment.

Recently, *A. viridinutans* clade (including *A. udagawae*) was re-classified into *A. udagawae*, *A. acensis*, *A. aureoles*, *A. wyomingensis*, *A. siamensis*, *A. felis*, *A. pseudoviridinutans*, *A. arcoverdensis*, *A. frankstonensis* and *A. viridinutans* s.s. (Hubka et al., 2018).

However, there has been no report to date as to the isolation of the related species from the environment in Japan.

The purpose of this study is to make a comparison between clinical isolates and environmental ones of the related species on susceptibilities against antifungal drugs and genetic diversity. First, we tried to isolate the related species from the environment in Japan by the baited method using corn and found some isolates belonging to *A. lentulus*, *A. udagawae*, *A. aureoles*, *A. wyomingensis*, *A. felis* and *A. pseudoviridinutans*. Then we examined their antifungal susceptibilities using the Dry Plate (Eiken Chemicals, Japan) according to the CLSI E38-E3 method. Some isolates of *A. lentulus* and *A. udagawae* have resistance properties against VRCZ, while some of *A. felis* and *A. pseudoviridinutans* showed resistance against both VRCZ and ITCZ. These resistances closely resembled those of clinical isolates in pattern, degree and frequency, therefore they are considered as inherent resistance.
Phyllosphere epiphytic and endophytic fungal community and network structures differ in a tropical mangrove ecosystem

Liang-Dong Guo, Hui Yao, Xiang Sun, Chao He, Pulak Maitra, Xing-Chun Li
State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, China

Purpose: Revealing the relationship between plants and fungi is very important in understanding biodiversity maintenance, community stability and ecosystem functioning. However, differences in the community and network structures of phyllosphere epiphytic and endophytic fungi are currently poorly documented.

Methods: In this study, we examined epiphytic and endophytic fungal communities associated with the leaves of six mangrove species using Illumina MiSeq sequencing of internal transcribed spacer 2 (ITS2) sequences.

Results: A total of 635 operational taxonomic units (OTUs) of endophytic and epiphytic fungi were obtained at a 97% sequence similarity level; they were dominated by Dothideomycetes and Tremellomycetes. Plant identity had a significant effect on the OTU richness of endophytic fungi, but not on epiphytic fungi. The community composition of epiphytic and endophytic fungi was significantly different, and plant identity had a greater effect on endophytic fungi than on epiphytic fungi. Network analysis showed that both epiphytic and endophytic network structures were characterized by significantly highly specialized and modular but lowly connected and anti-nested properties. Furthermore, the endophytic network had higher levels of specialization and modularity but lower connectance and stronger anti-nestedness than the epiphytic network.

Conclusions: This study reveals that the phyllosphere epiphytic and endophytic fungal communities differ, and plant identity has a greater effect on the endophytic fungi than on epiphytic fungi. These findings demonstrate the role of host plant identity in driving phyllosphere epiphytic and endophytic community structure.
Fungal-bacterial mutualistic mechanism; fungal highway and bacterial toll

Norio Takeshita
University of Tsukuba, Japan

Physical spaces and nutrients are prerequisites to the survival of organisms while no interspecies mutual strategy is documented that satisfies them. Here we find that bacterial cells co-cultured with fungus travel along the mycelia at a rate of ~30 µm s⁻¹ and disperse with fungal colony expansion. This bacterial dispersal requires intact flagella and results in expanded bacterial colonization, indicating that mycelia are beneficial “highways” for bacteria to explore spatial niches. Transcriptome analysis indicates that the species interact through thiamine. The wild-type bacterium, but neither the thiamine biosynthesis- nor flagella-deficient strain, is obligatory for growth of the thiamine auxotrophic fungus, indicating that the bacterium travels along mycelia to deliver thiamine to the fungus. These evoke a novel mutualistic strategy that facilitates the communicating species to compete for environmental niche and nutrient respectively.
Ecological aspects of ammonia fungi in serpentine soil

Hiroto Fukayama\textsuperscript{1)}, Tatsuya Fukuda\textsuperscript{1,2)}, Mana Yasui\textsuperscript{3)}, Akira Suzuki\textsuperscript{2)}
\textsuperscript{1)Graduate School of Environment and Information Studies, Tokyo City University, Japan
\textsuperscript{2)Faculty of Knowledge Engineering, Tokyo City University, Japan
\textsuperscript{3)Waseda Research Institute for Science and Engineering, Waseda University, Japan

\textbf{Purpose:} Serpentine soils are usually toxic to many plant taxa which limit their diversity comparing with those on adjacent non-serpentine soils. Ammonia fungi distributed widely from subarctic to tropical forests and colonize on the forest floors immediately after an enrichment disturbance by a large input of ammonium-nitrogen. Ammonia fungi have been recorded from various habitats, but no ecological studies of them have been done in serpentine soil. The aim of our study is to elucidate ammonia fungi in serpentine soil by comparison of mycobiota, and biomass and morphological features of their reproductive structures appeared on the serpentine and non-serpentine soils.

\textbf{Methods:} We applied urea (800 g/m\textsuperscript{2}) in spring on the floors of ever green broad-leaved forests inhabited on both soils in Chiba Prefectures, Japan, and examined fungi occurred on the urea plots.

\textbf{Results and Conclusions:} Dominant saprobic and ectomycorrhizal ammonia fungi collected from both soils were similar. It suggests that serpentine soil does not affect mycobiota of ammonia fungi. Among them, occurrence frequency and biomass of basidiomata of an ectomycorrhizal ammonia fungus \textit{Hebeloma spoliatum} in the serpentine soil was significantly smaller than those in non-serpentine soil. The basidiomata of \textit{H. spoliatum} appeared on the serpentine soil formed significantly shorter stipes with smaller pilei than those in non-serpentine soil. The result was more remarkable in stipes. This is the first report about morphological differentiation of fungi in serpentine soil. The ecological aspects of ammonia fungi in the serpentine soil would be caused by its physico-chemical properties.
Influence of woody plant community composition on the distribution of macrofungal community composition: a case study in Yunnan Province, China

Huili Li¹, Jiayu Guo¹, Lei Ye¹,²,³, Jianchu Xu¹,⁵, Peter Mortimer¹

¹Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences, China
²Institute of Excellence in Fungal Research, Thailand
³Center of Excellence in Fungal Research, Mae Fah Luang University, Thailand
⁴School of Science, Mae Fah Luang University, Thailand
⁵World Agroforestry Centre, East and Central Asia, China

Purpose: We aimed to map the spatial distribution of macrofungal and woody plants community composition in these three sites; to assess whether there has a consistent relationship between macrofungal community composition and woody plant community composition among different functional groups; to further explore what factors affect the macrofungal community composition.

Methods: This study investigated the distribution of macrofungi and woody plant community composition across three sites in Yunnan, as visualized by distribution maps.

Results: The spatial distribution of macrofungi were significantly correlated with the density of woody plants. In two sites, Zhongdian and Baoshan, the community composition of ectomycorrhizal fungi and ectomycorrhizal plants were significantly correlated, but no such relationship was found in our third site, Xishuangbanna. Macrofungal distribution was further affected by geographical and climatic factors at each site, and by the slope of individual plots.

Conclusions: We concluded that dominant woody plant species could be used as an indicator of macrofungal community composition. Thus, forest management of the density of woody plants, coupled with conservation of dominant woody plants species, would be a reasonable strategy for supporting the conservation of macrofungi.
3-O13-1

Cordyceps biodiversity and industrialization of *Cordyceps militaris*

Ting-Chi Wen1), Yuan-Ping Xiao1,2), Feng-Yao Long1), Ji-Chuan Kang1), Kevin D. Hyde2)

1)The Engineering and Research Center for Southwest Bio-Pharmaceutical Resources of National Education Ministry of China, Guizhou University, China
2)Center of Excellence in Fungal Research, Mae Fah Luang University, Thailand

**Purpose:** Cordyceps sensu lato create tremendous economic value due to their medicinal and nutritional importance. The objective of this study is classification of Cordyceps s.l. and optimization of large-scale production conditions for *C. militaris* and cordycepin.

**Methods:** Molecular phylogeny and morphology.

**Results:** More than 120 species of Cordyceps s.l. have been identified from China, Thailand and Russia based on morphology and multi-gene phylogenetic analyses. Among them, 30 are new to the science. In the industrial application works, (1) Solid-state fermentation for fruiting-body and cordycepin production: the optimization strategies in solid medium culture lead to a fruit body yield increased 67.96% (about 1.73 g/bottle) and cordycepin yield in fruiting-body increased 55.36% (0.87%). Larger particle size of rice in the medium offers better fruit body growth, and the cordycepin production prefers smaller particle size. (2) Solid-state fermentation only for cordycepin production: medium components glucose, peptone, adenine and histidine have been examined. The levels of variables for CCD experiments were selected according to the above results of the One-factor-at-a-time method; maximum response of 18.92 mg/g cordycepin at levels of glucose 26.25 g/L, peptone 26.25 g/L, adenine 7.50 g/L, and histidine 4.50 g/L as optimized medium components. This is the first report for improving the cordycepin production by using additives in this method. Isolates from colony sector mutation could be used for screening high-yield strains in cordycepin production and colony colour is one of the markers to detect fruit body and cordycepin production.

**Conclusions:** This study enriches the biodiversity of Cordyceps s.l. This method provides an effective way for increasing the *C. militaris* fruit body and cordycepin production at a large scale in order to improve industrial applications.
Diversity of entomopathogenic fungi infecting Orthopterida in Thailand

Donnaya Thanakitpipattana, Kanoksri Tasanathai, Suchada Mongkolsamrit, Artit Khonsanit, Supaporn Lamlerthton, Janet Jennifer Luangsa-ard
1) National Center for Genetic Engineering and Biotechnology, Thailand
2) Center of Excellence in Fungal Research Faculty of Medical Science, Naresuan University, Thailand

Purpose: The study on diversity of entomopathogenic fungi infecting Orthopterida (Orthoptera, Phasmatodea) aimed to make a species list of these rare specimens in Thailand and clarify the taxonomic and phylogenetic positions using multiple gene regions of the genomic DNA.

Methods: Species diversity of entomopathogenic fungi were surveyed and collected from 5 collecting sites: Khao Yai National Park in Nakhon Ratchasima and Saraburi provinces, Khao Luang National Park in Nakhon Si Thammarat province, Khlong Nakha Wildlife Sanctuary in Ranong province, Ban Hua Thung Community Forest in Chiang Mai province and Ban Phao Thai Community Forest in Phitsanulok province. Fifty-two Orthopterida samples were collected and characterized based on morphological and molecular analysis by using nuclear ribosomal small and large subunits (SSU and LSU), the largest and second largest subunits of RNA polymerase II (RPB1 and RPB2) and elongation factor 1-α (TEF).

Results and conclusions: Six new species and two new genera from 15 specimens were identified as Mayucordyceps sp., Metarhizium sp.1, Metarhizium sp.2, Neotorrubiella sp., Ophiocordyceps sp.1 and Ophiocordyceps sp.2. The most common genera found in this study were Ophiocordyceps and Metarhizium.
Harpellales from Japan by wider host family collection

Hiroki Sato
Forestry and Forest Products Research Institute, Japan

Harpellales is an order in the Kickxellomycotina which are living in the gut of aquatic insects, especially juveniles of Ephemeroptera, Plecoptera and Diptera. Over 250 species have been described to date. **Purpose:** We are studying to clarify the species diversity of Harpellales in Japan comparing with those of Asian countries.

**Methods:** Literature research. Observation of the fungi in the guts of aquatic insects by dissection.

**Results and conclusion:** Forty nine species have been recorded from Asia. Thirty one species in 15 genera, are known from China, 20 species in 12 genera from Japan, 7 species in 5 genera from India, and 5 species in 4 genera from Thailand. Only *Harpella melusinae* is recorded from all the four countries. To compare with the species number, genus number is not so much lower than China. This could reflect the larger number of host families being examined. The families investigated in China: Simuliidae, Chironomidae, Tipulidae and Culicidae in Diptera; Baetidae, Ephemerellidae and Heptageniidae in Ephemeroptera; Plecoptera sp. In Japan, in addition to the families researched in China, harpellalean species have been recorded from Blephariceridae and Thaumaleidae in Diptera, Leptophlebiidae in Ephemeroptera, and Nemouridae in Plecoptera. In this study, we explored to examine not or less investigated host families in Japan. We found about 20 more harpellalean species from Capniidae, Chloroperlidae, Peltoperlidae and Taeniopterygidae in Plecoptera, Caenidae, Ephemerellidae and Isonychiidae in Ephemeroptera. In total about 40 species are recognized suggesting a wide diversity in Japan.
Reclassification of the genus Polycephalomyces; phylogenetic position of the type species P. formosus

Sayaka Ban¹), Kohei Yamamoto²), Takashi Yaguchi¹), Akira Nakagiri³)
¹)Medical Mycology Research Center, Chiba University, Japan
²)Tochigi Prefectural Museum, Japan
³)Fungus/Mushroom Resource and Research Center, Faculty of Agriculture, Tottori University, Japan

Purpose: The genus Polycephalomyces Kobayasi (Hypocreales; Sordariomycetes; Ascomycota) comprises parasitic species on hypogaeal larvae of insects and/or other entomopathogenic Cordyceps sensu lato. The species produce white to cream synnemata, whose tips are swollen and globose with aggregated phialides and conidia. Conidia are non-septate, phialidic, produced numerously at the tip of synnema with mucilage, but several species also produce them along the side of the synnema. The shape of conidiogenous cells and conidia at the tip of synnema (acremonium-like; α-type) differs from those at the sides of synnema (hirsutella-like; β-type). Our recent phylogenetic study revealed that eight teleomorphic species of Ophiocordyceps have close affinity with Polycephalomyces. More recently seven new species were added to the genus, but, some of them seemed conspecific with formerly described and not re-collected species. Thus, we aimed to re-classify such classical species described during 1940s-70s from Japan by re-sampling and multigene phylogenetic analyses.

Results and Discussion: The type species P. formosus Kobayasi was found distinguishable from P. ramosus (Peck) Mains in absence or presence of β-type conidia, though sometime ambiguous. Nine isolates identified as P. formosus from Japan were phylogenetically apart from P. formosus (ARSEF 1424) which has been recognized as the authentic specimen, but close to Cordyceps pleuricapitata Kobayasi & Shimizu. Anamorphic morphology of C. pleuricapitata is similar to Polycephalomyces except β-type conidia formation, and we concluded that ARSEF 1424 was P. ramosus. Phylogenetic trees showed the two separate clades of Polycephalomyces intermingled with Perennicordyceps. Thus, the reclassification of the genus Polycephalomyces is needed.
Phylogeny and systematics revision of *Cordyceps* sensu stricto in Taiwan

Wei-Yu Chuang1), Meng-Ling Wu2), Kuei-Jr Liao1), Hiran Anjana Ariyawansa1)
1) Department of Plant Pathology and Microbiology, College of Bio-Resources and Agriculture, National Taiwan University, Taiwan
2) Taiwan Forestry Research Institute, Council of Agriculture, Executive Yuan, Taiwan

**Purpose:** *Cordyceps* species are entomogenous fungal group and have a long history of use as tonics and folk medicines that can be useful to cancer and diabetes treatments in traditional Chinese medicines. Numerous name changes have taken place in cordyceps-like taxa, after the classification based on morpho-molecular data and the application of one fungus one name after the amendment of ICN. Knowledge of the *Cordyceps* in Taiwan is based on relatively few records that are scattered throughout the literature. Therefore, Surveys of cordyceps-like species were conducted to establish a baseline on the information of *Cordyceps* s. s. in Taiwan through a search of bibliographic records, analysis of existing specimens kept in herbaria and new collections made by the authors.

**Methods:** A total of 39 fresh cordyceps-like isolates were collected and their morphological characteristics were recorded. Phylogenetic reconstruction using single and multi-locus (ITS, LSU, tef1, rpb1 and rpb2) DNA sequences data were used to evaluate the natural classification of new strains.

**Results:** Within this phylogenetic framework and considering the diagnostic morphological characters, four new species are described. In addition, three new reports are also made.

**Conclusions:** This study delivers a robust basis for a more comprehensive exploration of diversity and biogeography of *Cordyceps* s. s. in Taiwan.

**Keywords:** *Cordyceps*, Entomogenous fungi, New record, New species, Phylogenetic analysis
Evolution of Eastern Asian and North American Disjunct Distributions in Powdery Mildews

Susumu Takamatsu
Mie University, Japan

Purpose: The eastern Asian-eastern North American floristic disjunction represents one of the most prominent intercontinental disjunctions of closely related plant species. Similar disjunct distributions are also found in some macrofungi, but never in microfungi. The comparison of powdery mildew species and their host plants among eastern Asia, North America, and Europe reveals that tree-parasitic species are most abundant in eastern Asia, while herbaceous plant-parasitic species are most common in Europe. North America is an intermediate position between eastern Asia and Europe. This study was conducted to investigate the evolution of eastern Asian and North American disjunct distributions in powdery mildews using molecular clock.

Methods: During the molecular phylogenetic studies of powdery mildews, I found that some powdery mildew species closely related in morphology and phylogeny distribute disjunctly in eastern Asia and North America on the same plant genera, viz., Cystotheca lenestris-C. kusanoi (host: Quercus), Neoerysiphe cumminsiana-N. hiratae (host: Asteraceae), Pleochaeta polychaeta-P. shiraiana (host: Celtis), Erysiphe syringae-E. syringae-japonicae (host: Syringa), Erysiphe pulchra-E. cornicola (host: Cornus), E. magnifica-E. maginoliicola (host: Magnolia), and E. abbreviata-E. alphitoides (host: Quercus). Divergence time of these disjunct taxa was estimated using molecular clock of rDNA ITS sequences.

Results: The results suggested that the splitting of six of the seven disjunct combinations occurred during the global climatic cooling period that took place throughout Neogene and Quaternary.

Conclusions: This implies that in most taxa the disjunction may have resulted from vicariance events. However, the possibility of other factors cannot be ruled out.
Purpose: *Golovinomyces* is an important genus of Erysiphaceae. They can cause powdery mildew diseases by infecting a wide range of host families, such as Asteraceae, Solanaceae, Cucurbitaceae, which make them economical value. Few researches were conducted on taxonomy and molecular phylogeny of *Golovinomyces* in China. We collected specimens all over China and tried to clarify their distribution, diversity and phylogeny in China.

Methods: Fresh samples were mounted in sterile water, and dried specimens were scraped from the leaf surface with a clean scalpel, and were mounted in a drop of lactic acid on a microscope slide.

Results: More than 1000 specimens were collected all over the China. And twenty-two species and one variety were identified belonging to *Golovinomyces*. Among them there is one new combination, *G. latisporum* comb. nov., five new records to China, *G. tabaci*, *G. bolay*, *G. monardae*, *G. asperifolii*, *G. macrocarpus*, one new records host family, Boraginaceae, three new record host genera, *Lagopsis*, *Rubia* and *Picris*. The host plants of Golovinomyces scattered among 16 families, 46 genera, 78 species. These fungi distributed in 12 provinces, 2 autonomous regions and 1 municipality. Molecular phylogeny results based on ITS and 28S rDNA sequences showed that all these species of *Golovinomyces* formed twelve groups. But some plurivorous species need more molecular evidence. The circumscriptions of *G. ambrosiae*, *G. spadiceus* and *G. circumfusus* were conducted based on the morphology and multigene (ITS, 28S rDNA, IGS, TUB2, CHS1) phylogeny.

Conclusions: The biodiversity of *Golovinomyces* in China was clarified and it is still need further investigation to try to find more species. The results provided some scientific evidences for evaluating the phylogeny of powdery mildews.
Taxonomic revision of *Blumeria graminis* species complex

Miao Liu¹, Uwe Braun², Susumu Takamatsu³, Sarah Hambleton¹, Parivash shoukouhi¹

¹Ottawa Research & Development Centre, Agriculture and Agri-Food Canada, Canada
²Martin Luther University, Germany
³Faculty of Bioresources, Mie University, Japan

**Purpose:** The causal pathogen of powdery mildew diseases on cereal crops was long known as *Erysiphe graminis*. Speer made the combination *Blumeria graminis* in 1975. In the up-to-date classification, *Blumeria graminis* is the only species in this genus that belongs to the monotypic tribe *Blumerieae*, and was recorded on over one hundred genera in *Poaceae* worldwide. Multi-locus sequences analyses by Inuma and colleagues recovered nine lineages correlated with host specialization. Our study including samples from North America confirmed the separation of the lineages, however challenged the strict host specialization. The purpose of this study is to characterize the genotypic variation with phenotypic characters, and provide formal taxonomic names.

**Methods:** Multi-locus DNA sequences (rDNA, CHS, and two anonymous gene regions) for specimens from North America were developed, others were downloaded from GenBank. Phylogenetic analyses were conducted. Morphological examination was performed for the representative specimens of each lineage from herbaria BPI, DAOM, G, HAL and TNS.

**Results:** Eight species were recognized based on molecular phylogenies combined with morphology and known host and geographic range. The proposed names are: *B. graminis* s.str. on *Elymus*, *Hordeum*, *Secale*, *Triticum* etc.; *B. hordei* sp. nov. on *Hordeum*; *B. avenae* sp. nov. on *Avena*; *B. dactylidis* sp. nov. on *Anthoxanthum odoratum* and *Dactylis glomerata*; *B. bulbigera* comb. nov. on *Bromus* spp.; *B. bromicathartici* sp. nov. on *Bromus catharticus*; *B. graminicola* sp. nov. on *Elymus*, *Lolium*, *Millium* and *Poa*; *B. americana* sp. nov. on *Elymus* and *Hordeum* found in North America.

**Conclusions:** *Blumeria graminis* species complex harbours a high level of genetic variation that merits multiple species recognition.
Australia: A continent without native powdery mildews?

Levente Kiss1), Niloofar Vaghefi1), Kaylene Bransgrove2), John D. W. Dearnaley1), Yu Pei Tan3), Craig Marston4), Roger G. Shivas3), Susumu Takamatsu1,5)

1) University of Southern Queensland, Australia
2) Department of Agriculture and Fisheries, Plant Pathology Herbarium, Australia
3) Department of Agriculture and Fisheries, Biosecurity Queensland, Ecosciences Precinct, Australia
4) Department of Agriculture and Water Resources, Australia
5) Mie University, Faculty of Bioresources, Japan

Purpose: In contrast to Eurasia and North America, the powdery mildews (Ascomycota, Erysiphales) are an understudied group in Australia, with over 900 species known globally, and less than 50 species recorded from Australia. Some of these records are doubtful as the identifications were presumptive, being based only on host plant-pathogen lists from overseas. Our goal was to provide the first comprehensive database of all the powdery mildew species present in Australia.

Methods: We compiled an up-to-date list of all the taxa known to occur in Australia based on published DNA barcode sequences and identified 117 freshly collected specimens, and 30 herbarium specimens based on morphology and DNA barcodes.

Results: Altogether, 39 species representing 10 genera were confirmed in Australia, including two genera and ten species newly recorded during the project. In Eurasia and North America the number of powdery mildew species is more than 10x higher. Interestingly, powdery mildew infections have been recorded on only eight native Australian plant species, and were caused by polyphagous taxa known to infect many other host plants.

Conclusions: Our data indicates that (i) the native Australian vegetation may have evolved without being exposed to native powdery mildews; and (ii) all the species of the Erysiphales that are present in Australia may have been introduced since the European colonisation of the continent.
Bacterial quorum sensing (QS) is a well-characterized communication system that governs a large variety of collective behaviors. By comparison, QS regulation in eukaryotic microbes remains poorly understood, especially its functional role in eukaryote-specific behaviors, such as sexual reproduction. Cryptococcus neoformans is a prevalent fungal pathogen that has two defined sexual cycles (bisexual and unisexual) and is a model organism for studying sexual reproduction. Here, we show that the QS peptide Qsp1 serves as an important signaling molecule for both forms of sexual reproduction. Qsp1 orchestrates various differentiation and molecular processes, including meiosis, the hallmark of sexual reproduction. It activates bisexual mating, at least in part through the control of pheromone, a signal necessary for bisexual activation. Notably, Qsp1 also plays a major role in the intercellular regulation of unisexual initiation and coordination, in which pheromone is not strictly required. We identified the atypical zinc finger regulator Cqs2 as an important component of the Qsp1 signaling during both bisexual and unisexual reproduction. The absence of Cqs2 eliminates the Qsp1-stimulated mating response. Unveiling the regulon of Cqs2 through ChIP-seq identified the regulatory network responsible for QS-coordinated sexual development. We found that Cqs2 can directly orchestrate the expression of the regulators dominating the various stages during sexual reproduction, including meiosis and sexual structure (basidium) maturation that represent two concomitant pre-sporulation events. Cqs2 governs the regulatory coordination of meiosis and basidium maturation through the direct control of Pumilio-family regulator Pum1. We further demonstrated that the coordination of these two events is required for the formation of infectious spores likely as a key commitment mechanism. Together, these findings extend the range of behaviors governed by QS to sexual development and meiosis.
How a fungus integrates glucose sensing with carbon catabolite repression and development to adapt to living plants versus decaying litter

Monika Schmoll¹, Guofen Li¹, Wolfgang Hinterdobler¹, David Turra², Stefanie Kindel¹, Ursula Sauer¹, Aroa Rodriguez-Iglesias¹, Stephane Compant¹, Antonio diPietro², Monika Schmoll¹

¹AIT Austrian Institute of Technology GmbH, Austria
²University of Cordoba, Spain

Purpose: The natural habitat of fungi is a complex environment, where appropriate interaction with other microbes, but also with plants is crucial for successful colonization and propagation. While considerable research was done on bilateral interactions, only little is known on sensing, recognition and priorities for the output, which we investigated here.

Methods: We applied chemotropic analyses, microscopy, plant interaction assays, secondary metabolite analysis and analysis of development.

Results: We show chemotropic growth towards glucose in a concentration dependent manner in *Trichoderma reesei*. This reaction is dependent on the glucose sensing G-protein coupled receptors CSG1 and CSG2 as are morphological changes during growth on a natural substrate. Additionally, the whole downstream G-protein pathway as well as adenylate cyclase and protein kinase A are required for glucose induced chemotropic growth. Constitutive activation of G-protein alpha subunits abolishes concentration sensitivity.

In the absence of carbon catabolite repression, chemotropic sensing is shifted to higher concentrations, indicating that the concentration dependence reflects an adaptation to typical amounts of glucose released from decaying plant material. Thereby, *T. reesei* prioritizes some signals over others. Deletion of CSG1 and CSG2 abolishes chemotropic sensing of a plant and the ability to efficiently colonize living plant roots. As in *Fusarium oxysporum*, *T. reesei* senses plants using its pheromone receptors. Accordingly, sexual development is slightly enhanced in the presence of a plant and fruiting bodies are formed on top and around plant roots. The fruiting bodies clearly react to the plant roots and interact with plant tissue.

Conclusions: We conclude that sensing of a specific glucose concentration is rated for relevance and applied for regulation of enzyme production relevant for plant interaction or litter degradation.
Regulation of fruiting body development in Winter Mushroom

Shaojie Li1,2, Taju Wu1,2, Xianyun Sun1,2, Zhenying Zhang1,2, Chengcheng Hu1,2

1) State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, China
2) University of Chinese Academy of Sciences, China

Most of the edible mushrooms cannot be cultivated or have low yield under industrial conditions, partially due to the lack of knowledge on how fruiting body development is regulated. From Winter Mushroom (Flammulina velutipes), one of the most popular industrially cultivated mushrooms, three novel regulators of fruiting body development were identified.

FvCPC-2 is a protein containing a seven-WD40 repeats domain. The fruiting body development could be completely impaired by Fvcpc2 knockdown. Overexpression of Fvcpc2 could shorten the cultivation time by 3 days. FvCPC-2 regulates the expression of some genes important for fruiting body development. The ortholog of FvCPC-2 in Neurospora crassa, CPC-2, was reported as a positive regulator of protoperithecium formation. Fvcpc2 could restore the fertility phenotype to the ∆cpc-2 mutant, indicating that FvCPC-2 and its ortholog CPC-2 have the same function.

PDD1 is a transcription factor with a HMG-box domain. It increased transcription during fruiting body development. The pdd1 knockdown strain with 89.9% reduction in the pdd1 transcription failed to produce primordia, while overexpression of pdd1 promoted fruiting body development. PDD1 positively regulated several genes related to fruiting. PDD1 homologs are widely present in other basidiomycetes. Transcription factor LFC1 has a ZnCys domain, and its encoding gene decreases transcription during fruiting body development in F. velutipes. The lfc1 overexpression strains delayed primordia formation and produced abnormal fruiting body with stubby stipe and irregular cap. Conversely, knockdown of lfc1 promoted fruiting body development. Thus, LFC1 is a negative regulator of fruiting body development in F. velutipes.
Function of pcc1 in dikaryotization in *Pleurotus ostreatus*

Tatpong Boontawon, Takehito Nakazawa, Masato Horii, Masahiro Sakamoto, Yoichi Honda
Graduate School of Agriculture, Kyoto University, Japan

**Purpose:** Understanding the molecular mechanisms controlling dikaryon formation in Agaricomycetes, which is basically controlled by the A and B mating type loci, would contribute to improving mushroom cultivation and breeding. Various mutations in pcc1, which encodes an SRY-type HMG protein, activates A-regulated pathway(s) to form pseudoclamps in monokaryon of *Coprinopsis cinerea* (Murata et al., 1998 Genetics; 2009 Mycoscience). This study investigated the role(s) of pcc1 gene in dikaryon formation in *P. ostreatus* with the aim of understanding its conserved and diverse role(s) between *P. ostreatus* and *C. cinerea*.

**Methods:** Total RNA was extracted from seven-day-old monokaryon, PC9 (A2B1) and #64 (A64B64), and dikaryon, PC9xPC15 (A2B1 A1B2) and #64xPC15, on YMG agar plates, followed by qRT-PCR.

**Results and conclusions:** Unlike in the case of *C. cinerea*, pseudoclamp cells were not observed in a *P. ostreatus* pcc1 disruptant from 20b/1 (A2B1 ku80::Cbx<sup>e</sup> pyrG-1), pcc1d#1. When strain pcc1dF<sub>1</sub>#1 (A64B64 ∆pcc1), a F<sub>1</sub> progeny from a cross between pcc1d#1 and #64 (A64B64), as a donor was mated with 14 F<sub>1</sub> progeny (pcc1<sup>+</sup>) from dikaryon pcc1d#1xPC15, as a recipient, clamp cells were observed within 5 days after mating. However, clamp cells were not observed after mating between pcc1dF<sub>1</sub>,#1 and 14 F<sub>1</sub> strains (∆pcc1). It was shown that pcc1 transcript accumulates much more abundantly in dikaryon strains than in monokaryons in *P. ostreatus*, which is consistent with the results in *C. cinerea*. In conclusion, it was suggested that the role(s) of pcc1 in dikaryon formation is different between the two Agaricomycetes.
Genetic coordination of protein O-mannosyltransferase C and HOG pathway controls sterigmatocystin production and development in *Aspergillus nidulans*

Tram Le¹,², Kiminori Shimizu¹,³)

¹)Department of Biological Science and Technology, Tokyo University of Science, Japan
²)Biotechnology Center of Ho Chi Minh City, Vietnam
³)Medical Mycology Research Center, Chiba University, Japan

**Purpose:** Sterigmatocystin (ST), a precursor of the carcinogenic compound aflatoxin, is produced by the filamentous fungus *A. nidulans* during its developmental process. In this study, we investigate involvement of protein O-mannosyltransferase C (PmtC) in ST production and fungal development in *A. nidulans* through a genetic coordination with HOG pathway.

**Method:** To examine influences of PmtC in ST production and fungal development, pmtC deletion strain (ΔpmtC) was generated by replacing the entire coding sequence of pmtC gene with a marker gene.

**Results and conclusion:** Deletion of pmtC gene caused no conidiation and an apparent decline of vegetative growth. A tremendous decline of ST level was recognized in the ΔpmtC mutant at the fourteenth day after inoculation. Expression levels of aflR gene, an essential transcription factor for the ST biosynthetic genes, were also down-regulated in the ΔpmtC. Overexpression of aflR gene could not improve ST production by the ΔpmtC mutant compared to the wild-type. The loss of pmtC led to a reduction of the AflR protein, but not to its nuclear localization. Remarkably, impaired vegetative development, conidiation and low ST yield caused by the loss of pmtC were partially restored under osmotic pressure. Conidiation in ΔpmtC was not restored under osmotic conditions when the hogA gene, encoding the protein required for cellular response to hyper-osmolarity, was deleted. However, ST yield and vegetative growth in the ΔhogA ΔpmtC double deletant still restored under high osmolality and were comparable to the ΔpmtC single deletant, suggesting a partial genetic connection between hogA and pmtC.
The *Neurospora crassa* COT1 kinase - a regulator of polar growth: Interactions with MOB2A, type 2A phosphatase and the RNA-binding protein GUL1

Oded Yarden¹, Inbal Herold¹, Liran Aharoni-Kats¹, Hila Shomin-Levi¹, Diego L. Delgado-Alvarez², Marisela Garduno-Rosales², Rosa R. Rosa R. Mourino-Perez²

¹The Hebrew University of Jerusalem, Israel  
²CICESE, Mexico

**Purpose:** Determine the interactions between the NDR kinase COT1 that plays a role in polar growth and development in *Neurospora crassa* and other fungi, and other cellular components.

**Methods:** Classical/molecular genetics, biochemistry and microscopy were used to identify the interactions and their phenotypic significance.

**Results and conclusions:** Osmotic, oxidative as well as other stress conditions result in the partial suppression of the defects observed when COT1 function is impaired, demonstrating a functional link between COT1 and stress response. COT1 interacts with co-activators (MOB2A/B), phosphatases (e.g., PP2A) and downstream effectors (e.g., GUL1). Production of MOB2A phosphomimetic mutants demonstrated the potential significance of MOB2A phosphorylation on the physical interaction with COT1 and its unique functions in conidiation/germination. Inactivation of the PP2A regulatory subunits rgb-1 and b-56 conferred severe growth defects. Partial suppression of defects was observed in the rgb-1RIP strain in cot-1 phosphomimetic mutants, demonstrating that altering COT1 phosphorylation state can bypass the requirement of RGB1. Another component of the COT1 complex that undergoes phosphorylation is GUL1 (the homologue of yeast Ssd1p). Deletion of gul-1 results in partial phenotypic suppression of the cot-1 (ts) mutant and affects transcript abundance of multiple genes in the COT1 pathway. GUL1 can form aggregates that exhibit high mobility, which is dependent on a functional cytoskeleton. Under stress conditions, a significant increase in GUL1 aggregate association with nuclei was observed. Using RNA Antisense Purification and Immunoprecipitation, we have determined that GUL1 is a bona fide RNA-binding protein and can physically associate with several RNA species.
De novo assembly and annotation of the red and white Antrodia cinnamomea complete genome sequences

Ting-Fang Wang  
Academia Sinica, Taiwan

Antrodia cinnamomea, an endemic fungus of Taiwan, has received much attention in pharmacological and biotechnology fields, because it has long been used for the prevention and treatment of several illnesses including hangover, liver diseases, cancer and hypertension. The medicinal effects of A. cinnamomea are thought to come from its rich contents of triterpenoids and polysaccharides. Although most wild dikaryotic strains produce lustrous and orange-red mushrooms, few isolates generate milky white fruiting bodies. To reveal the phylogenetic relationship between orange-red and white A. cinnamomea strains, we isolated four sexually compatible monokaryons (W1, W2, V5 and V7). W1 and W2 are derived from the arthospores of a white dikaryotic strain (SN1), whereas V5 and V7 are from the arthospores of an orange-red dikaryotic strain (HC1). Next, we applied PacBio RSII and Illumina sequencing platforms to these four monokaryons for high-quality genome assembly. Gene prediction and annotation were performed with a funannotate pipeline. Comparative analyses of these four haploid genomes show great potential, mainly, due to the annotation of putative sequences that could be employed in basic researches and biotechnological approaches.
Genomic basis of fungal bioluminescence in *Mycena* species

Huei-Mien Ke1), Chan-Yi Ivy Lin1), Hsin-Han Lee1), Yu-Ching Liu1), Chiung-Chih Chang1), Jo-Wei Hsieh2), Pao-Yang Chen2), Hsiao-Wei Kao3), Isheng Jason Tsai1)

1) Biodiversity Research Center, Academia Sinica, Taiwan
2) Institute of Plant and Microbial Biology, Academia Sinica, Taiwan
3) Department of Life Sciences, National Chung Hsing University, Taiwan

Bioluminescence is present in many species ranging from marine bacteria to terrestrial fungi, earthworm, and firefly. Currently more than 78 fungal species are known to display bioluminescence. Although the underlying chemical reaction in all bioluminescent species are believed to involve a luciferin and a luciferase enzyme were discovered last year, its regulation and ecological role remains elusive.

To further understand the global view of expression profile of bioluminescence and its evolution, comparative transcriptomics and comparative genomics were conducted. Five species belonging to *Mycena* genus including four bioluminescent species and one nonbioluminescent species were selected for genome sequencing. In these four bioluminescent fungi, differential expression genes were either identified from comparison between higher and lower bioluminescent mycelium in the same species or identified from the correlation between bioluminescent intensity of tissues and gene expression levels.

Our results reveal that all bioluminescent fungi shared common ancestry. Three gene families including luciferase (luz), hispidin-3-hydroxylase (H3H), and fatty acid desaturase were up-regulated according to the intersect of up-regulated genes among four bioluminescent species. The gene luz is absent in the nonbioluminescent species. There are 6-30 paralogues of h3h in the four bioluminescent fungi but with different gene expression levels. These systematically understanding will further unravel the roles of fungi in ecological niches and evolution in the bioluminescence of *Mycena* fungi in general.
SrpkF participates in the early phase cellobiose- and cellulose-responsive induction of the cellulase genes in *Aspergillus aculeatus*

Natsumi Kobayashi, Ryohei Katayama, Jun-ichi Sumitani, Shuji Tani, Takashi Kawaguchi
Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Japan

**Purpose:** The goal is to identify new regulator(s) controlling the expression of cellulase genes in *Aspergillus aculeatus* that produces a number of cellulose- and hemicellulose-degrading enzymes.

**Methods:** Factors controlling the expression of cellulase genes were screened from the T-DNA inserted mutant library of *A. aculeatus*. We identified serine-arginine protein kinase-like gene (*srpkF*) as a new regulator. To figure out the function of *SrpkF* on the gene expression in response to cellulose, the effect of deletion and overexpression of *srpkF* were assessed by quantifying the expression of the cellulase genes.

**Results and conclusions:** The expression of *srpkF* elevated under the carbon starvation and in the presence of Avicel 98 and 44 times higher, respectively, than that in the presence of glucose. The expression levels of FIII-avicelase (*cbhI*) and Flb-xylanase (*xynIb*) genes were reduced to 37 and 19% by the deletion of *srpkF* in the presence of Avicel, respectively. However, the deletion of *srpkF* did not affected for the expression of *xynIb* in response to xylose. The expression of *sprkF* was 9 times higher in OE-*srpkF* than that in MR12 in the presence of Avicel. Overexpression of *srpkF* significantly increased the *cbhI* and *cmc2* expression at the early phase of induction in response to cellobiose but not *xynIb* in response to xylose. These results suggested that *SrpkF* was produced under the carbon starvation condition and contribute to the early phase of induction in response to cellulosic carbon sources.
Chemical genetic analysis of zoospore development by using beta-rubromycin in *Phytophthora infestans*

Shuji Tani¹, Naotaka Nishio¹, Kenji Kai¹, Howard Judelson², Takashi Kawaguchi¹

¹Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Japan
²Department of Microbiology and Plant Pathology, University of California, Riverside, United States

**Purpose:** The goal is to understand the relevant molecular mechanisms of cyst germination in *Phytophthora infestans* by chemical genetics.

**Methods:** We first screened for microbial culture inhibiting a specific stage of the sporangium development. Actinomycetes strains were mainly isolated from soil in Japan and applied for the screening. Testing samples were prepared by mixing equal volume of acetone and microbial culture. Among more than 700 tested samples, one sample constantly inhibited cyst germination in *P. infestans*.

**Results and conclusions:** The bioactive sample was extracted from 76.4 liter culture broth of the isolated strain with equal volume of ethyl acetate, and which were applied for series of column chromatography. We finally obtained 2.9 mg of the bioactive sample, namely sample A. The ESI-MS, ¹H-NMR, and ¹³C-NMR analyses of the sample A resulted in the identification of beta-rubromycin as a cyst germination inhibitor. Beta-Rubromycin inhibited cyst germination (IC₅₀=19.8 micro g/L) but not zoospore release, encystment, and appressorium formation among sporangium development under cool temperatures. Beta-Rubromycin inhibited cyst germination more effectively than beta-rubromycin (IC₅₀=58.5 micro g/L); demonstrating that naphthazarin moiety is critical to inhibit cyst germination. The expression of rio kinase genes was increased in the presence of beta-rubromycin. Silencing and overexpression of rio kinase gene resulted in abnormal morphological development under cool temperatures.
Advances in the taxonomy of phytopathogenic fungi

Yasmina Marin Felix¹,²), Pedro W. Crous²)
¹Department Microbial Drugs, Helmholtz Centre for Infection Research, Germany
²Westerdijk Fungal Biodiversity Institute, the Netherlands

Purpose: Phytopathogenic fungi are important agents of plant disease, resulting in major annual losses to agricultural and forestry industries. Since the advent of molecular DNA techniques, many species of plant pathogenic fungi have been shown to represent species complexes or to be members of genera that are para- or polyphyletic. Moreover, for many genera and species type material has not been designated or/and the vast majority of these taxa were described before the DNA phylogenetic era and thus lack DNA barcodes. To address these issues, the “Genera of Phytopathogenic Fungi” initiative was launched, whose main objective is to provide a stable platform for the taxonomy of phytopathogenic fungi.

Methods: For each genus, a morphological description and information about its pathology, distribution, hosts and disease symptoms are provided. In addition, these data are linked to primary and secondary DNA barcodes of the presently accepted species.

Results: Hitherto, 62 genera of phytopathogenic fungi have been studied. For some of these genera new barcodes were generated allowing us to redefine them, e.g. Dichotomophthora and Metulocladosporiella. Moreover, new species and new genera such as Verkleyomyces and Wingfieldomyces were introduced to accommodate new taxa. Moreover, numerous new combinations were proposed to correct the classification of known species of phytopathogenic fungi. Finally, some asexual-sexual links were resolved, as in the case of Pyrenophora and Drechslera.

Conclusions: Since the start of the “Genera of Phytopathogenic Fungi” project, 62 genera have been treated, resulting in the introduction of five new genera, 88 new species, 38 new combinations, four new names and 13 typifications. This project has therefore revealed huge potential for advancing the taxonomy of phytopathogenic fungi.
The dynamics of *Raffaelea quercivora*, a causal agent of Japanese Oak Wilt

Yukiko Shirouzu Takahashi1), Norihisa Matsushita2), Taizo Hogetsu2)

1) Forestry and Forest Products Research Institute, Japan
2) The University of Tokyo, Japan

**Purpose:** Japanese Oak wilt (JOW) caused by *Raffaelea quercivora* is one of the most serious forest pests in Japan. To comprehend the dynamics of *R. quercivora*, detail distribution of hyphae and construction of genets were investigated in inoculated or naturally infested oaks.

**Method:** Hyphal distribution, dysfunction of water conduction and host protective reactions were investigated in xylem of inoculated oak saplings with a fluorescent-conjugated lectin staining technique. Fungal genets were investigated on galleries and mycangia of the vector beetle with microsatellite markers.

**Results and conclusion:** Time-cause observation of inoculated saplings revealed that hyphae were confined within a relatively small area near the inoculation site. Affected area causing water occlusion and accumulation of defensive substances were limited around them. It suggests that JOW may not be induced by dysfunction of a small number of vessels but by that of many vessels, and it requires *R. quercivora* hyphae spread from many galleries bored by beetles during mass attack. While diverse genotypes of *R. quercivora* are patchy distributed in each galleries and were carried by a female beetle, which means that the beetle unloads and loads *R. quercivora* repeatedly. Because hyphae are able to extend among galleries, the mosaic pattern could be the result of invasions from other galleries. Although the teleomorph of *R. quercivora* is not found, it might maintain genetic diversity diverse without sexual reproduction. The beetle might be playing a role to maintain the genetic diversity of their symbiotic fungus.
One stop shop-taxonomic update with molecular phylogeny for important phytopathogenic genera

Ruvishika Shehali Jayawardena, Kevin David Hyde
Center of Excellence in Fungal Research, Mae Fah Luang University, Thailand

Many fungi are pathogenic on plants and are responsible for causing significant damage to agriculture and forestry, affecting the economy. They are a part of the natural ecosystem and play a major role in regulating plant numbers. However, morphological identification of plant pathogenic fungi are often hampered by the scarcity of discriminatory taxonomic characters and the endophytic or inconspicuous nature of these fungi. One stop shop (OSS) is a series of papers focused on providing a stable platform for the taxonomy of plant pathogenic fungi. Genera included in these paper series are associated with plant diseases. However, some may not be well-known plant pathogens and Kochs’ postulates may have not conducted in order to establish their pathogenicity. The aims of this series of publications is to facilitate the present and future studies of plant pathogenic fungi by providing phylogenetic backbone trees, recommendation of correct names, disease symptoms and latest taxonomic notes. The website, www.onestopshopfungi.org, hosts a database for plant pathogenic fungi. This fungal database allows mycologists and plant pathologists to understand disease symptoms, host distribution, classification, morphology and provides an updated phylogeny which will enhance current understanding of plant pathogens and gain better insights into the current fungal classification system.
Phylogeny and taxonomy of *Cronartium* and allied genera

Peng Zhao, Xiao-Hua Qi, Lei Cai
State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, P. R. China

**Purpose:** The pine blister rust diseases, caused by the *Cronartium* species (*Basidiomycota, Pucciniales*), are one of the three most important forest diseases worldwide. Species in this genus usually infect two-needle or five-needle pines, and several species have been listed as quarantine pests in many countries because of their severe ecological damage and economic losses to forestry. However, relationship of genus *Cronartium* with other genera in rust fungi is still unresolved, and species delineation within genus is also in a state of disorder, which further hampered the pathogen recognition and disease control. This study was conducted to clarify the intergeneric relationships of the genus *Cronartium* with related genera and the interspecific relationship within genus.

**Methods:** 705 specimens were loaned from worldwide herbaria and they were used for molecular analyses and morphological examination.

**Results and Conclusions:** At the generic level, the monophyly of genus *Cronartium* and its relationship with related genera in families *Coleosporiaceae, Melampsoraceae* and *Pucciniastaceae* were confirmed. A novel genus, *Quasipucciniastrum*, typified by *Q. agrimoniae*, sp. nov., is proposed based on distinct morphological characters and phylogenetic placement. Within genus *Cronartium*, 18 species including three potential novel species were confirmed based on the association of morphological characteristics, geographic origins and host range with phylogenetic relationships. Host alternation of 9 *Cronartium* species were confirmed. Our morphological and molecular studies further emphasized the importance of several morphological characters in aecial stage for species recognition.
Re-evaluation of *Phoma erratica* var. *mikan* from *Phoma* rot disease

Haruna Matsuyama¹, Yukako Hattori², Keiichi Motohashi¹)

¹Department of International Agriculture Development, Tokyo University of Agriculture, Japan
²Graduate School of Bioresources, Mie University, Japan

**Purpose:** *Phoma erratica* var. *mikan* Hara is the causal agent of Phoma rot disease, affecting fruits and leaves of several citrus hosts in Japan. This specie was synonymized *Phyllosticta citricarpa*, which causes Citrus Black Spot disease. CBS has been suspected to emerge in Japan, therefore, *Pho. erratica var. mikan* should be re-evaluated.

**Methods:** In this study, *Pho. erratica var. mikan* was re-evaluated with morphological characteristics and molecular analysis.

**Results and Conclusions:** The morphological identification of *Pho. erratica var. mikan* in this study cannot differentiate from genus *Phyllosticta* spp.. The phylogenetic analysis shows that *Pho. erratica var. mikan* are formed distinct clades from *Phy. citricarpa*. Additionally, *Pho. erratica var. mikan* formed a monophyletic clade with *Phy. citrichinaensis*. Hence, *Pho. erratica var. mikan* is taxonomically distinct from *Phy. citricarpa* and it should be transfer to genus *Phyllosticta*. 
Diversity of *Fusarium* Species Infecting Banana in Malaysia

Anysia Hedy Ujat¹, Yukako Hattori², Chiharu Nakashima², Clement Kiing Fook Wong¹, Dzarifah Zulperi¹

¹Faculty of Agriculture, Universiti Putra Malaysia, Malaysia
²Graduate School of Bioresources, Mie University, Japan

**Purpose:** The Fusarium wilt is caused by mainly four different races of *Fusarium oxysporum f. sp. cubense* (*Foc*). *Foc* tropical race 4 (*Foc-TR4*) or *F. odoratissimum* had raised the concern of plant pathologist all around the world as this strain of pathogenic fungi can infect most of banana cultivar, making it the most destructive strain in comparison to others. On the other hand, it is known that each physiological race of *Foc* has a different host range of the banana cultivar but there are no morphological differences among them. There is a need to assess the diversity of *F. oxysporum f. sp. cubense* infecting the various banana cultivars in Malaysia to estimate the severity of this disease and the damage caused in order to effectively control this disease.

**Method:** Using Fusarium selective medium, 38 fungus samples suspected to be *F. oxysporum* was isolated from symptomatic tissue of corm, root and stem of different local banana cultivar infected by Fusarium wilt in Malaysia. The banana samples consist of dessert, plantain and cooking banana cultivars. These isolates were subjected to identification and classification by using histone H3 and TEF 1-alpha gene. Analysis of race identification was conducted using TR4 specific primer and also LAMP-FLP analysis.

**Result:** Based on the phylogenetic analysis of histone H3 and TEF 1-alpha, the isolates could be classified into *F. fujikuroi*, *F. odoratissimum*, *F. grosmichelii* and unknown *Fusarium oxysporum* species complex (*FOCS*). Result shows that the 27 out of 38 of the sample are positive for Tropical Race 4 (*TR4*).

**Conclusion:** Previous studies of TR4 focuses on the symptoms on the Cavendish. However, to prevent the prevalence of Fusarium wilt caused by TR4, further study regarding the diversity of this fungus from different banana cultivar, including the local cultivar of various genome, is urgently needed.
Robust bioprocess for the sustainable production of the fungal anti-cancer lead compound illudin M

Lillibeth Chaverra-Munoz, Theresa Briem, Tian Cheng, Clara Chepkirui, Stephan Huettel, Marc Stadler
Helmholtz Centre for Infection Research, Germany

**Background and purpose:** Fungi and especially Ascomycota and Basidiomycota are excellent examples of organisms with an incredible versatile secondary metabolism. This feature has been used to establish biotechnological processes to obtain interesting natural products with unique biological activities that are potential starting points in drug discovery programs like the illudins produced by the basidiomycetes Lampteromyces and Omphalotus. These sesquiterpenoids are being used as base molecules for the development of new antitumor agents. Because the strong and unspecific cytotoxicity of the natural compounds have prevented their direct use in cancer therapy, semisynthetic conjugates based on illudin M have been designed with a strongly improved therapeutic index. Current research on that molecule class is ongoing to further improve their selectivity towards malignant cells and this purpose requires a reliable supply of larger amounts of illudin M. The aim of this project is to develop a stable up-stream and down-stream process for the biotechnological production of illudin M in multi-gram scale using *Omphalotus nidiformis* as a model organism for its production.

**Methods:** Several parameters such as morphology, pH, agitation, oxygen transfer, influence of seed culture density and media components were studied using shake flasks and subsequent stirred-tank experiments with regard to improve culture conditions enhancing a scalable production of the compound. Strategies for product recovery and purification were also developed.

**Results and conclusions:** Preliminary results suggest that through optimization of culture conditions and following an improved strategy for down stream processing it is possible to achieve 500 mg/L of pure illudin M.
**A study of the biodiversity and secondary metabolites of fungal endophytes from medicinal plants in Guizhou**

Ji-Chuan Kang, Ting-Chi Wen, Yi-Xin Qian, Si-Xuan Zhou, Xiao-Ya Ma

Engineering Research Center of Southwest Bio-Pharmaceutical Resources, Ministry of Education, Guizhou University, China

**Purpose:** For solving the problems of plant shortage, environmental disruption and obtaining novel structural as well as bio-active natural products, we have been studying the fungal endophytes and their secondary metabolites from medicinal plants in Guizhou province for a number of years.

**Methods:** We isolated and combined bio-activity analysis in vitro, chemical composition identification and gene detection in fungal endophyte to screen and evaluate fungal strains.

**Results:** We isolated over five thousand fungal strains from medicinal plants including Artemisia carvifolia, Artemisia japonica, Blumea balsamifera, Camptotheca acuminata, Dendrobium orchids, Ginkgo biloba, Nothapodytes pittosporoides, Reineckia carnea, Taxus brevifolia. Dozens even hundreds of different fungal isolates were obtained from each plant species. Most of the fungal endophytes belong to Ascomycota which mainly distribute in Pezizomycete, Dothidiomycete and Sordariomycete. Fungal endophyte taxa were subjected to vary kinds of factors, for example, the age of host, organ, humidity of sample site, altitude, season, surrounding plant, extent of environmental contamination and so on. We combined bio-activity analysis in vitro, chemical composition identification and gene detection in fungal endophyte to screen and evaluate 1003 fungal strains. Fifty-six of them showed anti-inflammatory, antitumor, antioxidant, anti-pathogen and P-gp inhibitory bio-activity to different extent. At present, we accomplished the study for secondary metabolites of ten fungal strains that possess high bio-activity. More than 200 bio-active compounds were isolated. Ten of them have new structures. However, there is a long way to produce bio-active compounds in large scale because of the low production.

**Conclusions:** Therefore, it is necessary to improve cultivation methods or alter inner gene in fungal endophyte by genetic engineering for achieving novel chemical structure as potential new drug.
Spider parasitic fungi as alternative sources of novel secondary metabolites

Wilawan Kuephadungphan¹,²,³, Soleiman Helaly²,⁴, Allan Patrick Macabeo⁵, Charuwan Daengrot³, Patima Phainuphong³, Vatcharin Rukachaisirikul³, Souwalak Phongpaichit³, Janet Jennifer Luangsa-ard¹, Marc Stadler²

¹The National Center for Genetic Engineering and Biotechnology, Thailand
²Department of Microbial Drugs, Helmholtz Centre for Infection Research, Germany
³Faculty of Science, Prince of Songkla University, Thailand
⁴Department of Chemistry, Faculty of Science, Aswan University, Egypt
⁵Laboratory for Organic Reactivity, Discovery and Synthesis (LORDS), Research Center for the Natural and Applied Sciences University of Santo Tomas, Philippines

Purpose: Since hypocrealean arthropod-parasitic fungi are revealed to be capable of producing a diverse array of secondary metabolites with various biological properties, a number of species have been intensively studied. Remarkably, some certain species, in particular the parasites of spiders, Gibellula and Hevansia appear neglected. Although there has been an increasing interest toward them in the past years, it is nevertheless limited to specific research groups. According to the fact that their production of secondary metabolites remains largely unexplored, the attempt to investigate and explore them was herein made.

Methods: As Thailand is one of the global biodiversity hotspots, many species of spider-parasitic fungi from various parts of the country were studied for production of secondary metabolites where they were examined using analytical HPLC coupled with diode array and mass spectrometric detection (HPLC-DAD/MS) and compared within and across species according to the multigene phylogenetic tree.

Results and conclusions: So far this has led to the discovery of more than ten unprecedented molecules from three different species and the recognition of their unique patterns of secondary metabolite production. Our findings demonstrate that spider-parasitic fungi constitute a rich, hitherto untapped source for novel metabolites that might eventually turn out to be useful in medicine, agriculture or other applications. We hope that these can also help to raise the general scientific interest in this group of fungi and in particular in the taxonomy and secondary metabolism of the spider pathogens.
Inhibition of Methicillin-resistant Staphylococcus aureus by pteridophyte Nephrolepis cordifolia (Linn.) Presl. and its associated fungal endophytes

Judee N. Nogodula1,2, Ma. Eva C. San Juan2, Reynaldo M. Nogodula3, Roberth Riggs L. Rondilla4, Kin Israel R. Notarte4, Thomas Edison E. Dela Cruz4,5

1) College of Arts and Sciences, University of Southeastern Philippines, Philippines
2) Pharmacy Department, University of the Immaculate Conception, Philippines
3) College of Education, University of Southeastern Philippines, Philippines
4) Fungal Biodiversity, Ecogenomics and Systematics Group, University of Santo Tomas, Philippines
5) Research Center for Natural and Applied Sciences, University of Santo Tomas, Philippines

Purpose: The misuse of antibiotics has led to the rapid development of multi drug-resistant bacteria. Asia, for instance, has one of the highest prevalence rates of healthcare-and community-associated methicillin-resistant Staphylococcus aureus (MRSA). The need to find new antibiotics has brought to the investigation of the tropical fern Nephrolepis cordifolia and its associated fungal endophytes (FE) as sources of metabolites against MRSA.

Methods: The crude extract of N. cordifolia underwent fractionation with butanol and dichloromethane. The fungal endophytes associated with the host pteridophyte were also isolated using a variety of culture media. DNA barcoding with ITS was further employed for molecular identification of selected endophytes. In testing the metabolite bioactivity extracted from the host plant and fungal endophytes, TLC-bioautography was performed for the detection of chemical constituents antagonistic to MRSA. The bioactivity had further validated by minimum inhibitory concentration (MIC) of the test sample against MRSA.

Results and Conclusion: The butanol extract inhibited the MRSA at the Rf values of 0.54 and 0.74, whereas the dichloromethane extract elicited inhibition at Rf value of 0.58. The MIC of the leaf fractions was at 100ug/mL. A total of 49 fungal endophytes were also isolated from the host pteridophyte. Five of these FE inhibited MRSA with zones of inhibition (ZOI) of > 14mm. Genetic analysis identified these fungal endophytes with bioactive metabolites as Fusarium equiseti, Colletotrichum sp., Lassiodiplodia theobromae, Harknessiia sp., and Aspergillus sp. Among these, F. equiseti showed the ZOI of 23mm against MRSA with an MIC value of 12.5 ug/mL. Fractionated extracts from F. equiseti showed a putative terpenoid at Rf value of 0.63.

Keywords: antimicrobials, bioprospecting, Philippine fern, tropical fungi
Biosynthesis of cyathane diterpene to discovery and characterization of a new family of diterpene cyclases

Hongwei Liu
Institute of Microbiology, Chinese Academy of Sciences, China

Purpose: To fulfill the large scale preparation of cyathin diterpenes by synthetic biology, the key biosynthetic mechanism of erinacine A in Hericium erinaceum was studied.

Methods: We presented the identification and verification of the EriG, a member of UbiA superfamily, as the gene responsible for the cyclization of cyathane skeleton in mushroom H. erinaceum. Genome mining using the EriG protein sequence leads to the identification of a new family of ubiquitous UbiA related diterpene cyclase genes in the bacteria and fungi. Using an engineered Escherichia coli, we successfully characterized five new diterpene cyclases and their corresponding products in Chloroflexus sp., Saprospira grandis, Cystobacter violaceus, and Streptomyces lydicus, respectively.

Results: The gene cluster responsible for the biosynthesis of cyathin diterpenes were identified for the first time in H. erinaceum and Cyathus africatus. EriG, a member of the UbiA superfamily, was identified as the enzyme responsible for the cyclization of the cyathane skeleton in the mushroom H. erinaceum.

Conclusions: Furthermore, genome mining using the EriG protein sequence as a probe led to the discovery of a new family of ubiquitous UbiA-related diterpene cyclases in bacteria and fungi.
Elucidation of the life cycle of the endophyte genus *Muscador* based on a polyphasic taxonomic approach

Benjarong Thongbai¹, Milan C Samarakoon² ³, Kevin D Hyde³, Marc Stadler¹, Mark Bronstrup¹, Ulrike Beutling¹, Christopher Lambert¹, Dinushani A Daranagama⁴, Jian Kui Liu⁵, Itthayakorn Promputtha²

¹Helmholtz Centre for Infection Research, Germany
²Faculty of Science, Chiang Mai University, Thailand
³Center of Excellence in Fungal Research, Mae Fah Luang University, Thailand
⁴Dept. of Botany, University of Kelaniya, Sri Lanka
⁵Guizhou Academy of Agricultural Sciences, P.R. China

**Purpose:** The genus *Muscador*, which exclusively consists of endophytes with sterile mycelia that produce volatile antibiotics, had been originally erected based on a highly questionable concept. Even though it was originally accommodated in the Xylariales (or the Xylariaceae, respectively), its affinities so far remained obscure for lack of morphological characters, since the cultures so far isolated did not produce a conidial state. We have recently encountered the sexual states of two xylarialean fungi that produced apiospores from Northern Thailand. Cultures derived from those apiospores were found to produce volatile antibiotics and could also be assigned with certainty to a clade that otherwise only contained species of *Muscador* in a multi locus genealogy of representative species of the Xylariales. With this data at hand, we have tried to elucidate the life cycle of the genus.

**Methods:** Aside from a molecular phylogeny based on ITS, LSU, rpb2 and TUB2 DNA sequence data, the cultures were also subjected to a study of their volatile organic compounds (VOCs), using both, dual cultures for assessment of antimicrobial effects and extensive GC-MS analyses.

**Results and conclusions:** The morphological features and the molecular phylogeny of the new fungi, as well as their VOC profiles and their antibiotic effects in dual culture, will be presented and discussed. Our phylogeny shows that *Muscador* species have affinities to the genera, *Emarcea* and *Induratia*. Phylogenetic and morphological data on both genera would allow for integration of *Emarcea* and *Muscador* in >Induratia, i.e. the genus that was historically described first.
Root disease control by using artificial suppressive soil inoculated microbial consortium

Jamjan Meeboon¹, Makoto Shinohara¹, Kazuki Fujiwara¹, Kenji Miyamoto², Yasuo Kato³, Akinori Ando⁴,⁵, Jun Ogawa⁴,⁵

¹National Agriculture and Food Research Organization, Japan
²Dept. Biosci. Inform., Keio University, Japan
³Biotech. Res. Cent., Toyama Pref. University, Japan
⁴Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto University, Japan
⁵Res. Unit Physiol. Chem. Kyoto University, Japan

Purpose: The presence of disease suppressive soils has been identified worldwide. The mechanism of disease suppression by suppressive soil is still unknown, although its analysis has been advanced from the various viewpoints. A novel cultivating method was developed that can utilize organic fertilizer to inorganic nutrients such as nitrate in the hydroponic solution that can inhibit root diseases such as bacterial wilt disease and Fusarium wilt.

Methods: An attempt was made to create artificial suppressive soil, through the addition of microorganisms to the artificial media rock wool in organic hydroponics culture solution. The addition of the fish-based soluble fertilizer in the inoculated rockwool resulted in the generation of inorganic nutrients. *Fusarium oxysporum* and *Ralstonia solanacearum* were inoculated onto the rockwool.

Results and conclusions: it was found that the growth of the pathogen was inhibited. This result indicates the successful artificial creation of disease suppressive soil from inorganic media and microbial inoculum.
Isolation of different rhizosphere-associated fungi with biological control activities from Korea

A Yeong Heo, Young Mo Koo, Ho Seob Lee, Mi Jung Jo, Dasan Lee, Jeong Hyun Lee, Hyong Woo Choi
Andong National University, Korea

**Purpose:** We aimed to isolate rhizosphere-associated fungi with biological control activities against different plant pathogenic fungi.

**Methods:** In 2019, soil-born fungi were randomly isolated from Andong area, and screened for their antifungal activities against different plant pathogens, including Colletotrichum spp., Alternaria spp., Botrytis spp. and Phytophthora spp., using in vitro dual culture assay.

**Results and conclusions:** From the screening of 300 unknown rhizosphere-associated fungi grown on PDA, some of them showed significant antifungal activity in vitro. To identify the selected fungi, the internal transcribed spacer (ITS) regions were amplified by PCR and sequenced. ITS sequence of each fungi was compared with reference ITS sequences of GenBank at NCBI using the basic local alignment search tool, and identified Trichoderma spp., Mortierella spp., Cupriavidus spp., Aspergillus spp., Byssochlamys spp. and etc. Our rhizosphere-associated fungi with in vitro antifungal activity awaits further studies for the in vivo plant protection activity and the development of novel biological control agents which can be useful for sustainable agriculture.
Metal and Salinity Tolerance in *Trichoderma asperellum* and the Impact on Biocontrol Efficacy against *Fusarium* Pathogens

Jia May Chin, Adeline Su Yien Ting, Yau Yan Lim
Monash University Malaysia, Malaysia

**Purpose:** *Trichoderma asperellum* (isolate T2), a mycoparasite with biocontrol activities, is studied for its tolerance to metal and salinity stress, and the gradual impact of the stress on biocontrol activities. This helps to establish the role of *Trichoderma asperellum* as a biocontrol agent and its ability to retain biocontrol activities when used in soils with high metal and salinity stress.

**Methods:** *Trichoderma asperellum* was first screened for tolerance against varying concentrations (100ppm, 200ppm, 300ppm, 400ppm, 500ppm and 1000ppm) of copper (Cu), lead (Pb), zinc (Zn), aluminium (Al), chromium (Cr), cadmium (Cd) and of salt (50mM, 100mM, 150mM, 200mM and 250mM). This was conducted using plate assays (potato dextrose agar supplemented with metals and salt). The isolate was also tested for antagonistic effect against *Fusarium solani, F. proliferatum* and *F. verticillioides* by dual culture assay.

**Results:** Results showed that *Trichoderma asperellum* was able to tolerate high concentrations of toxic metals (up to 500ppm except for cadmium) and also salinity (up to 250mM). At high metal concentrations, morphological changes (pigmentation) of the mycelium was observed. Tolerance assay revealed that *Trichoderma asperellum* grew under presence of cadmium with less than 100ppm, and other metals with less than 500ppm. *Trichoderma asperellum* exhibited antagonistic activity against Fusarium pathogens and suppressed the growth of *F. solani* (60.75±0.54%), *F. proliferatum* (64.14±0.51%) and *F. verticillioides* (70.57±0.35%) on dual culture assay.

**Conclusions:** It is concluded that *Trichoderma asperellum* is able to tolerate metals and grow in high salinity environment. With these characteristics, *Trichoderma asperellum* can potentially have applications as biofertilizer, and biofungicide in soils high with metal and salinity stresses.
Control of Mango's Post-Harvest fungal diseases in Miyako Island, Okinawa Prefecture, Japan by Hot Water Treatments

Victor Alonso De la Cruz Padilla¹, Hidehiko Kikuno², Chiharu Nakashima³, Mark Joseoh Balanay Cano⁴, Kojiro Omijya⁵, Keiichi Motohashi¹
¹Department of International Agriculture Development, Tokyo University of Agriculture, Japan
²Miyako Subtropical Experimental Farm, Tokyo University of Agriculture, Japan
³Graduate School of Bioresources, Mie University, Japan
⁴College of Science, Polytechnic University of the Philippines, The Philippines
⁵Saion Co. Ltd., Japan

Objective: Mango (Mangifera indica L.) is known as a quality fruit in Japan. As post-harvest diseases, like anthracnose by Colletotrichum spp. and stem end rot by Lasiodiplodia spp., cause serious damage, cost-effective and environment-conscious treatment is required. In this study, the effect of Hot Water Treatments on the control of post-harvest fungal diseases was evaluated.

Methods: Matured fruits of Mango cv. "Irwin" harvested in Miyako Island were examined. Four factorial and one control treatments were applied: dip in hot spring water at 60 °C for 1 minute and then cooling for 10 minutes under running tap water (T1); dip in sterilize distilled water at 60 °C for 1 minute and then cooling for 10 minutes under running tap water (T2); dip in sterilized distilled water at room temperature for 10 minutes (T3); and dip in hot spring water at room temperature for 10 minutes (T4). The fruits were storage at room temperature with AC (24-27 °C) after treatments. The effects were evaluated by preventive value at 0, 6 and 12 days after treatment.

Results and Conclusion: Hot water treatments with hot spring water (T1) and sterilize distilled water (T2) have a significant reduction of the incidence of diseases during storage. Hot Water Treatments at 60°C for 1 minute are effective for control of post-harvest diseases in Mango cv "Irwin".
Use of response surface methodology (RSM) for the optimization of Lasiodiplodia sp. LAMP assay in Philippine ‘Carabao’ Mango (Mangifera indica Linn.)

Chester C. Deocaris1), Monzour Dave L. Manrique1), Ruth Royelle L. Izon1), Dexter M. Foronda1), Jan Bernel P. Padolina2), Chiharu Nakashima3), Lourdes V. Alvarez3,4)

1) Department of Physical Sciences, College of Science, Polytechnic University of the Philippines, Philippines
2) Research Management Office, Polytechnic University of the Philippines, Philippines
3) Graduate School of Bioresources, Mie University, Japan
4) Department of Biology, College of Science, Polytechnic University of the Philippines, Philippines

Purpose: Lasiodiplodia sp. is a plant pathogen associated with the Stem-end-rot (SER) of mangoes. The disease is one of the primary concerns among mango production, including the Philippine Carabao mango cultivar. In this study, we demonstrate the utilization the response surface methodology (RSM) to determine the optimal conditions employed in the rapid detection of Lasiodiplodia sp. using the loop-mediated isothermal amplification PCR (LAMP) for the early detection of the SER pathogen.

Methods: The primers used in the LAMP assay were designed to target the RNA-directed DNA polymerase II (rbp2) gene of Lasiodiplodia sp isolated from SER stricken mango fruits. Optimization started with fractional factorial design using the Fedorov’s (1972) algorithm. The factors considered in the LAMP assay were the following: 1.) Mg 2+ concentration; 2.) temperature; and 3.) time. The presence of LAMP amplicons was measured from the GelRed™ fluorescence of the LAMP reaction tubes using the photographs of reaction and blank tubes. The response variable assigned in the RSM optimization was the ratio of pixel intensities of the test reaction tube and blank tubes.

Results: The LAMP reaction described best fits the second order with interaction response model (R-squared=0.918). In this model, the pure-quadratic (PQ) form of the factors respond significantly (p=0.039) towards synthesis of LAMP amplicons. Canonical analysis of the of the surface model suggests the optimum LAMP reaction conditions: [Mg2+] = 7.59mM; Temperature = 63.9°C; and Time = 42mins.

Conclusions: Response surface methodology (RSM) could be applied to the optimization of the LAMP assay. The estimated values for each factor from the RSM canonical analysis could be used as a benchmark for the further development of the Lasiodiplodia sp-LAMP assay in field applications.
Facilitative and synergistic interactions between fungal and plant viruses in mixed infections

Liying Sun, Ruiling Bian, Tianxing Pang
State Key Laboratory of Crop Stress Biology for Arid Areas and College of Plant Protection, Northwest A&F University, China

**Purpose:** To investigated the infectivity of cryphonectria hypovirus 1 (CHV1, genus Hypovirus), a capsidless, positive ssRNA mycovirus in a model plant, Nicotiana tabacum.

**Methods:** Mechanical inoculation of viral RNAs and fungal inoculation

**Results:** By mechanical inoculation of viral RNAs, CHV1 replicates in inoculated leaves but does not spread to the upper leaves. Co-inoculation with a plant virus such as tobacco mosaic virus (TMV), potato virus Y and cucumber mosaic virus enables CHV1 to systemically infect the whole plants. Likewise, CHV1 systemically infects the transgenic plants expressing TMV movement protein and co-infection with TMV further enhances CHV1 accumulation in this transgenic plants. In the fungal inoculation experiment using a plant pathogenic fungus, Fusarium graminearum, we demonstrated that TMV infection in the plant enables the horizontal transfer of CHV1 from the fungus to plant. Moreover, the presence of CHV1 promotes TMV accumulations in the fungal host.

**Conclusion:** Our results demonstrate that the plant virus infection could facilitate the cross-kingdom infection of a mycovirus from the fungus to plant.
2A-like protease activity is essential for replication and viability of yado-kari virus 1 hosted by yado-nushi virus 1

Subha Das, Mahfuz Alam, Rui Zhang, Sakae Hisano, Yukiyo Sato, Hideki Kondo, Nobuhiro Suzuki
Institute of Plant Science and Resources (IPSR), Okayama University, Japan

Purpose: A unique mutualistic virus-virus interplay has recently been demonstrated in *Rosellinia necatrix*, where a positive sense, single-stranded RNA mycovirus, yado-kari virus 1 (YkV1) snatches the capsid protein (CP) from a co-infecting double-stranded RNA virus, yado-nushi virus 1 (YnV1) to encase its genome and RNA-directed RNA polymerase (RdRp). In return, YkV1 trans-enhances YnV1's accumulation. We hypothesize that YkV1 utilizes YnV1's CP as the replication site using its own RdRp. Interestingly, YkV1's polyprotein contains a 2A-like sequence at its C-terminus. The picornavirus 2A oligopeptide has a conserved motif, DxExNPGP (where 'x' is any amino acid), and cleaves co-translationally between the G and P. Here, we examined whether 2A-mediated cleavage is essential for YkV1's viability.

Methods: YkV1 mutants with amino acid substitutions in the 2A-like region were constructed and their replication competence and self-cleaving capabilities were examined in *R. necatrix* (harboring YnV1) and sf9 insect cells, respectively.

Results and conclusions: Mutants with alanine substitutions of the highly conserved sixth P and seventh G residues of 2A-like motif (DVEKNPGP) failed to replicate. Nevertheless, mutants retained replication competence when amino acids were altered at fifth and eighth residues. Insect cell-based protein expression showed a congruence between virus replication competence and 2A-like protease activity. These results further confirmed that complete cleavage at 2A-like motif is prerequisite for efficient replication of YkV1. Taken together with our previous results, this study indicates that YkV1, while depending on YnV1 CP for trans-encapsidation, utilizes its own RdRp that is likely functional only after being cleaved by the 2A-like protease.
A necrotropic fungal pathogen serves as beneficial endophyte on plants

Daohong Jiang, Hongxiang Zhang, Jiatao Xie, Yanping Fu, Jiasen Cheng, Shufen Cheng, Zhenzhen Zhao
State Key Laboratory of Agricultural Microbiology, Plant Pathology, College of Plant Science and Technology, Huazhong Agricultural University, PR. China

*Sclerotinia sclerotiorum*, a widespread ascomycetous fungus, is a notorious necrotrophic fungal plant pathogen that destroys many important economic crops and vegetable crops leads to huge economic losses, it causes stem rot of rapeseed, a major edible oil crops in China. *S. sclerotiorum* has developed sophisticated strategies to attack plants: it produces oxalic acid and plant cell-wall degrading enzymes to kill host cells and tissues, and it secretes effector-like small proteins to suppress host resistance systems and weaken host metabolism. *S. sclerotiorum* has a wide host range, and is known to attack over 400 species and subspecies of plants distributed in 75 families. Mycoviruses are widespread in nature, and hypovirulence-associated mycoviruses are believed to play a role in counterbalancing plant diseases in nature, and were looked as ideal potential resources for biological control of plant fungal diseases. Previously, various mycoviruses have been identified from *S. sclerotiorum*, including a DNA mycovirus, *Sclerotinia sclerotiorum* hypovirulence-associated DNA virus 1 (SsHADV-1). The virus particles of SsHADV-1 could infect hypha of *S. sclerotiorum* directly, this virus also has been found to drive mycophagous insect as transmission vector. Recently, we found that SsHADV-1-infected strain of *S. sclerotiorum* could not only improve rapeseed yield in field when applied on aerial parts of rapeseed, but also could grow on plants. We further found that SsHADV-1 down-regulates genes involving virulence of *S. sclerotiorum*, and SsHADV-1-infected strain could activate genes in the resistance pathways of rapeseed, hence treated plants showed strong resistance against *S. sclerotiorum* and other fungal pathogen.
The discovery, evolution, transmission of mycoviruses associated with hypovirulence on *Sclerotinia sclerotiorum*

Jiatao Xie¹, Du Hai¹, Minghong Wang², Daohong Jiang¹, Yanping Fu¹, Jiasen Cheng¹

¹State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, PR China
²College of Forestry and Horticulture, Hubei University for Nationalities, PR. China

**Purpose:** *Sclerotinia sclerotiorum* is one of the most damaging pathogens to infect widely hosts. Mycoviruses associated hypovirulence have potential to develop virocontrol agents to management this disease. Vegetative incompatibility system in filamentous fungi is key restricted factor for mycovirus transmission. However, the understanding on mycovirus transmission among vegetative incompatibility groups under natural conditions (infection process) is still limitation.

**Methods and Results:** Here we use a metatranscriptomic approach to identify six novel mycoviruses infecting a single hypovirulent strain SCH733 of *S. sclerotiorum*. These identified mycoviruses with +ssRNA virus genome belong to five viral evolution lineages, and temporarily named as *Sclerotinia sclerotiorum* narnavirus 1 (SsNaV1), *Sclerotinia sclerotiorum* mitovirus B (SsMVB), *Sclerotinia sclerotiorum* deltaflexivirus 3 (SsDFV3), *Sclerotinia sclerotiorum* ourmiavirus 2 (SsOV2), SsOV3, and *Sclerotinia sclerotiorum* diaporvirus 1 (SsDPV1). The genomes analysis revealed that these six mycoviruses not only include close relatives of known mycoviruses, but also distant relatives of known mycoviruses. Strain SCH733 was dual-cultured with a hygromycin resistance gene labeled strain 1980 of *S. sclerotiorum*, and new 1980 isolates were sub-cultured for mycoviruses detection. The results revealed that SsOV2 could be transmissible to strain 1980, but other five mycoviruses failed. The similar horizontal transmission experiments were conducted on the soybean leaves or radish. Interestingly, SsNaV1, SsDFV3, SsOV2 and SsOV3 can stably transmit to strain 1980 and later strain converts into hypovirulent strain, and SsMVB or SsDPV1 is occasionally transmissible to strain 1980.

**Conclusions:** Those results revealed that mycoviruses could break the obstacle of vegetative incompatibility system of phytopathogenic fungi during their infecting plants with some unknown mechanism.
Mycovirus-mediated biological control of chestnut blight in Europe

Daniel Rigling
Swiss Federal Research Institute WSL, Switzerland

Purpose: The chestnut blight fungus, *Cryphonectria parasitica* is native to East Asia and has been introduced into North America and Europe. In both continents, the pathogen has caused a severe disease epidemic on the susceptible American (*Castanea dentata*) and European (*C. sativa*) chestnut species. In Europe, however, many chestnut stands recovered from the disease due to the spontaneous occurrence of hypovirulence. Hypovirulence is caused by *Cryphonectria hypovirus 1* (CHV-1) that infects *C. parasitica* and reduces its virulence and sporulation capacity. The purpose of our research was to identify factors important for successful biological control of chestnut blight using hypovirulence.

Results: Natural dissemination and active biological control applications have led to a high prevalence of the CHV-1 in many regions of Europe. At the population level, several factors contribute to the success of hypovirulence including (1) efficient dissemination of hypovirus-infected propagules; (2) low vegetative incompatibility barriers for virus transmission between fungal individuals; (3) presence of dead chestnut wood that supports the production of hypovirulent inoculum; and (4) ecological fitness of the main biological control agent, CHV-1 subtype I.

Conclusions: Therapeutic treatment of individual chestnut blight cankers with hypovirus-infected *C. parasitica* strains has been highly successful and provides an efficient control method for these perennial infections. High prevalence of CHV-1 in many regions in Europe indicate a self-sustainable biocontrol, which has significantly contributed to the recovery of European chestnut from chestnut blight.
Effects of ectomycorrhizosphere bacterial strains on sporocarp production by the ectomycorrhizal fungus *Laccaria parva*

Keisuke Obase
Forestry and Forest Products Research Institute, Japan

**Purpose:** The effects of ectomycorrhizosphere bacteria on sporocarp production of the ectomycorrhizal fungus *Laccaria parva* were examined in vitro.

**Methods:** Three bacterial strains closely related to *Bradyrhizobium* were selected based on their affinity for *L. parva* strain LL02 in confrontation tests: strain 5_8_1_1 increased hyphal extension areas of strain LL02, strain 6_9_1_1 did not significantly affect hyphal extension areas, and strain 2_2_2_2 decreased hyphal extension areas. *L. parva* LL02 mycelia and a suspension of each bacterial strain were inoculated onto a surface-sterilized pine seedling in a glass bottle and then incubated for 3 months in an illuminated incubator.

**Results:** Plant biomass and the number of root tips did not differ significantly among the treatments, but the percentage of ectomycorrhizal roots was low in the treatment inoculated with strain 2_2_2_2. The frequency with which mature sporocarps occurred was lower in the control and 2_2_2_2 treatments compared with the 6_9_1_1 and 5_8_1_1 treatments. The total biomass of sporocarps was lower in the 2_2_2_2 treatment but higher in the 6_9_1_1 and 5_8_1_1 treatments. The ratio of biomass accounted for by mature sporocarps was low in the control, moderate in the 2_2_2_2 treatment, and high in the 6_9_1_1 and 5_8_1_1 treatments.

**Conclusions:** These results indicate that ectomycorrhizosphere bacteria affect the production and maturation of *L. parva* sporocarps and that the affinity between the fungi and bacteria is likely to underlie this interaction.
Frequency of spore attachment on the body surfaces of Collembola feeding on mushrooms

Taizo Nakamori, Masaya Yonekawa, Masakazu Nakano
Yokohama National University, Japan

Purpose: Among fungi that produce umbrella-shaped sporocarps aboveground, spores are generally believed to be wind-dispersed. However, recent studies have raised the possibility of animal dispersal of these fungal spores. Collembola are micro-arthropods found abundantly in soil. Certain Ceratophysella species (family Collembola) have occasionally been reported in large numbers (thousands) on aboveground fungal sporocarps; therefore, these species are expected to carry fungal spores. The possibility of epizoochory of fungal spores by Collembola species has not been investigated. Therefore, in this study, we examined the frequency of spore attachment on the body surface of Ceratophysella species feeding on aboveground sporocarps.

Methods: Collembola were collected from sporocarps of 16 fungus species, and spore occurrence on the body surface was examined for a maximum of 50 randomly selected Ceratophysella individuals per sporocarp.

Results and conclusions: The frequency of individuals with spores on the body surface varied among fungus species, ranging from 0 to 88%. Although no individual had spores on the body surface in Amanita citrina and A. virosa, 88 and 78% of individuals had spores in Psathyrella cineraria and Cortinarius salor, respectively. Spores were frequently observed on the ventral side of the body, including the ventral tube, claws, antennae, and mouth parts. The results of this study suggest that Collembola contribute to spore dispersal of certain fungus species with aboveground sporocarps, including P. cineraria and C. salor, but not Amanita species.
Predator-prey interactions between ectomycorrhizal fungi and soil nematodes in a coastal black pine forest

Yudai Kitagami\textsuperscript{1,2)}, Yosuke Matsuda\textsuperscript{1)}
\textsuperscript{1)Graduate School of Bioresources, Mie University, Japan}
\textsuperscript{2)Research Fellow of the Japan Society for the Promotion of Science}

**Purpose:** Ectomycorrhizal (ECM) fungi colonize on most fine roots of Japanese black pine \textit{(Pinus thunbergii)} grown in coastal forests in Japan. The fungi can be one of main food resources for belowground fungivorous nematodes. However, the effect of ECM roots and associated fungal species on the abundance of soil nematodes is unknown. The aim of this study was to elucidate the effect of ECM fungi on soil nematodes abundance. To achieve our goals, we examined predator-prey interactions between ECM fungi and nematodes by two approaches; pot and cultural experiments.

**Methods:** For the pot experiment, we constructed a (pot) microcosm with sandy soils collected from a coastal pine forest. The soil-filled pots were incubated for 8 months with or without pine seedlings, and compared the abundance of soil nematodes. For the cultural experiment, the growth rate of fungivorous nematodes in the family Aphelenchoididae was measured grown on two ECM fungal species \textit{(Cenococcum geophilum} and \textit{Rhizopogon roseolus}) once a month for 3 months.

**Results and conclusions:** In the pot experiment, the presence of pine seedlings significantly and positively contributed to the abundance of soil nematodes. In the cultural experiment, the number of nematodes was significantly greater at \textit{C. geophilum} than \textit{R. roseolus} throughout the experimental period. These data indicated that nematodes were increased by foraging ECM fungi with some feeding preferences.
Richness of foliar endophyte communities in tropical forests reflects phylogenetic diversity of host communities

Shuzo Oita¹, Francois Lutzoni², Jolanta Miadlikowska², Alicia Ibanez¹, Anne Elizabeth Arnold¹

¹University of Arizona, USA
²Duke University, USA

Purpose: Endophytic fungi that live within healthy leaves are important in plant health and physiology. They often are transferred horizontally as airborne spores and hyphae, raising the question: what factors shape their richness and community composition? We hypothesize that plants select compatible endophytes from the species pool in the surrounding environment. Under that scenario we expect that (1) endophyte richness in local hosts scales positively with diversity of local plant communities, and (2) leaf chemical or structural defenses plays a role in defining endophyte community assembly.

Methods: We collected fresh leaves from 120 plants (angiosperms representing 79 species in 48 families) across six sites in western Panama, including lowland, lower montane, and upper montane tropical forests. We quantified host diversity as phylogenetic distance; measured physical and chemical defense as leaf mass per area and the concentration of tannins, phenolics, and flavonoids; and examined endophyte communities by sequencing the fungal ITS1 region via Illumina MiSeq.

Results and conclusions: After accounting for climate and forest structure, we found that (1) endophyte richness associated positively with phylogenetic diversity of local plant communities, and (2) hosts differed in their endophyte richness in a manner partially independent of leaf defenses. We conclude that in these forests, hosts filter compatible endophytes from the local species pool via traits that complement leaf defenses, with overall richness influenced by such host traits and their evolutionary relationships. Together these results inform the factors that shape endophyte richness at leaf-to-landscape scales, providing a basis for testing hypotheses regarding the evolutionary processes underlying their community assembly.
Purpose: Bacterial endosymbionts in fungi are known as endofungal bacteria, and they mostly occur in the phylum Mucoromycota. Although the number of researches focused on the biodiversity of these bacteria are increasing, how the endofungal bacteria affect the hosts is still elusive. Recently, we reported that Burkholdericeae-related endobacteria (BRE) associated with 53 isolates consisting of 22 species of Mortierella obtained from Japan. Among these BRE-harboring isolates, a homothallic isolate YTM39, which is described as Mortierella sugadairana, did not produce its homothallic zygospores in zygospore-inducing conditions that are suitable for other non-BRE-harboring isolates of this species. Considering this infertile isolate, we hypothesized that the BRE can affect the zygospore production in this isolate.

Methods: In order to test this hypothesis, we tried to eliminate the BRE from this isolate by single-sporangiospore isolation and antibiotic treatment. Then, we compared the zygospore production of BRE-harboring and BRE-free clonal lines originated from the isolate.

Results and conclusions: As a result, we successfully obtained BRE-free clonal lines by occasional spontaneous loss of the BRE through single-sporangiospore isolation and antibiotic treatment. We also found that the BRE-free clonal lines restored the zygospore production. This finding is the first case showing that a BRE can inhibit sexual reproduction of its fungal host.
The coevolution of mycoparasitic *Tremella* sensu lato species and their hosts

Xin-Zhan Liu, Feng-Yan Bai
Institute of Microbiology, Chinese Academy of Sciences, China

The genus *Tremella* sensu lato is featured by mycoparasitic life style. They can parasitize a variety of species belonging to Basidiomycetes and Ascomycetes. *Tremella* species are delimited mainly by the phylogenetic relationship because of the morphological characters are scarce. Whether hosts and their parasites speciate by cospeciation, or through host switching, is a key issue in host-parasite evolution. Understanding the evolutionary dynamics of parasitism of *Tremella* spp. and their hosts could provide evidence partly for the taxonomy of *Tremella* species. The phylogenies of *Tremella* spp. and hosts were generated based on the ITS and D1D2 sequences of 45 and 100 species, respectively. We investigated the congruence between parasites and hosts phylogenies using distance-based and event-cost based methods. Distance-based test supported an overall congruence between the phylogenies of *Tremella* spp. and their hosts. Reconciliation reconstructions determined host-switching (27-30) other than cospeciation (10-13) is a major impetus driving *Tremella* species diversity. The number of failure-to-diverge case (62) is also high and stable, independently of the cost regimes, which means that some *Tremella* species are able to parasitize closely related species to be generalists.
**Stranger Things: The Simple Holocarpic Oomycetes**

Anthony Buaya\(^1,2\), Sebastian Ploch\(^2\), Alexandra Kraberg\(^3\), Marco Thines\(^1,2\)

\(^1\)Goethe-Universitaet Frankfurt am Main, Department of Biological Sciences, Institute of Ecology, Evolution and Diversity, Germany  
\(^2\)Senckenberg Biodiversity and Climate Research Center, Germany  
\(^3\)Biologische Anstalt Helgoland, Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Germany

**Purpose:** The Oomycetes are fungus-like heterotrophic organisms ubiquitous in aquatic and terrestrial environments. The phylum is best known for its plant pathogens and freshwater saprobes in managed and natural ecosystems. However, little is known about the biology and evolution of the simple holocarpic forms. Occurrence of these bizarre oomycetes was mostly reported decades ago and only a handful have been re-isolated and investigated for their molecular phylogeny. Here, we showcase the taxonomy, phylogeny and diversity of aquatic holocarpic oomycetes encountered in our on-going study.

**Methods:** Samples were isolated from marine and freshwater habitats in Germany (Helgoland (North Sea), Rebstocksee, Aartalobersee, Trais-Horloffsee, Main River, Nidda River) and Norway (Oslo Fjord), and were studied by classical microscopy and molecular phylogeny.

**Results and conclusions:** A number of species that are obligate endobiotic parasites in diatoms (*Coscinodiscus, Pseudo-nitzschia, Rhizosolenia, Pleurosigma, Gyrosigma, Licmophora, Synedra, Achnanthes, Nitzschia*), aquatic oomycetes (*Achlya, Saprolegnia*), algae (*Ceramium, Spirogyra, Ancylistes, Oedogonium*), and freshwater invertebrates (*Ostrocods, Rotifera*) were found. Four species, two new genera, two new families, and one class were newly described, two species rediscovered, and several species combined into new genera based on morphology and molecular phylogeny.

**Keywords:** Straminipila, Oomycota, taxonomy, phylogeny

**Corresponding author:** anthony.buaya@senckenberg.de
Enhancement of quality of Oomycetes in the NARO Genebank (MAFF Collection)

Shihomi Uzuhashi, Takayuki Aoki, Daisuke Tanaka, Hiromi Nakajima, Miyuki Yamazaki, Mamoru Satou
National Agriculture and Food Research Organization (NARO), Japan

Purpose: In the NARO Genebank (MAFF), approximately 1,200 Oomycetous strains are preserved. Confirming the validity of the scientific name of each strain, as well as preserving the strains for long-term with high viability, is crucial for culture collection. The purpose of our study is to increase reliability and utilization by enhancing the quality of Oomycetes in our collection.

Methods: 1) Evaluation of the scientific names. The COX1 (barcode region) and ITS sequences were used for the phylogeny. Morphological observation was also conducted as needed. The name of each strain was evaluated by the results, and also based on the latest taxonomy. 2) Development of a new technique for preservation. Some Oomycetous strains which were known for its low viability during storage were co-cultured with rapeseeds or sesame seeds on PDA or CMFA plates. The infested seeds were used as substrates for freeze-preservation or vitrification.

Results and conclusions: 1) More than 260 strains were re-identified, and about 130 strains were updated by the latest taxonomy. All of sequences determined were stored on the Microorganisms Database for anyone to use. 2) Viability of many strains were much improved compared with the general method using agar discs as substrates, although conditions representing the highest viability were varied depending on the strains. To minimize spending effort, more versatile condition should be selected and applied for culture collection. Anyway, we believe that our Oomycetous collection has become taxonomically more reliable. Continuous updating of the database and effort to preserve the strains more stable is still challenging.
Multiplex LAMP detection of *Phytophthora ramorum*, *P. kernoviae* and *P. lateralis* with plant universal primer set as an internal control

Ayaka Hieno¹, Kayoko Otsubo¹, Haruhisa Suga², Koji Kageyama¹
¹River Basin Research Center, Gifu University, Japan
²Life Science Research Center, Gifu University, Japan

**Purpose:** Recently, several *Phytophthora* species cause destructive disease in forest trees and nursery plants. *P. ramorum* is a causal agent of sudden oak death, most commonly observed on Camellia, Magnolia, Pieris and Quercus spp. *P. kernoviae* causes similar symptoms to *P. ramorum*, bleeding stem cankers, foliar blight, and shoot dieback on Fagaceae and other host plants. Unlike others, *P. lateralis* is especially aggressive to *Chamaecyparis lawsoniana*. Previously, we have designed species-specific LAMP primer sets for rapid detection of these three species in import quarantine inspection. In this study, we try to establish multiplex LAMP with plant universal primer set to determine consequences of false-negative and evaluate the reaction.

**Methods and Results:** We performed fluorescence LAMP assay to distinguish two amplified products from mycelial and plant DNA by peak temperature of anneal derivative curve. By using mycelial and plant DNA, we identified appropriate reaction conditions for these three combinations as follows; reaction temperature is 65°C, concentrations of the species-specific primer is same as before and plant universal primer was 0.08 times lower than before. By the reaction conditions, we were able to detect mycelial DNA when it existed in DNA sample, and if it was absent, plant DNA was detected. We confirmed the multiplex LAMP detection was applicable to inoculated plant samples of Rhododendron, Pieris and Camellia.

**Conclusions:** This method can be used for rapid and accurate detection of these three *Phytophthora* species.
Characterization of *Pleurotus florida* Mycelia as a Functional Food

Janice C. Laforteza\(^1\), Renato G. Reyes\(^2\), Trinidad P. Trinidad\(^1\)

\(^1\)University of Santo Tomas, Philippines
\(^2\)Central Luzon State University, Philippines

**Purpose:** Functional foods are food products that can provide beneficial physiological effects beyond basic nutrition. As functional foods, mushrooms are promising sources of several nutritional and health-beneficial compounds, including proteins, minerals, dietary fibers, and myconutrients. *Pleurotus florida* as the most widely cultivated mushroom, has a large-scale market for human consumption particularly its fruiting bodies, however, studies on its mycelia are scarce. The study aimed to characterize the mycelia of this species as a functional food.

**Methods:** The nutrient composition; dietary fiber and its fermentability in vitro, myconutrients, and antioxidant activity of the mycelia were analyzed using standard methods.

**Results:** Findings showed that its mycelial powder (MP) had a moisture content of 18.13 ± 0.03 g/100g; ash of 12.2 ± 0.06 g/100g; protein of 5.03 ± 0.09 g/100g; fat of 0.1 ± 0.00 g/100g, and carbohydrates of 61.4 ± 0.12 g/100g. Results also revealed that MP is an excellent source of dietary fiber (54.54 ± 3.03 g/100g) and contained high amounts of insoluble (21.72 ± 0.93 g/100g) and soluble (33.14 ± 1.78 g/100g) fiber. MP also produced significant amounts of short chain fatty acids after fermentation in vitro simulating conditions in the colon; acetate (1.92 ± 0.05 mg/g), propionate (0.89 ± 0.03 mg/g) and butyrate (0.31 ± 0.03 mg/g). Mycelial powder contained myconutrients such as phenolics (1.58 ± 0.06 mg GA/g) and flavonoids (0.74 ± 0.00 mg RHE/g) that exhibited antioxidant activity (DPPH - 33.85%, FRAP - 1.30 ± 0.06 mg Trolox/g, ABTS - 0.75 ± 0.00 mg Trolox/g sample).

**Conclusions:** In conclusion, mycelia may be considered as a potential functional food/ingredient and may be utilized by the food industry, thus contribute in the prevention for risk of chronic diseases and in the maintenance of human health.
Nutritional composition of wild edible mushrooms from traditional market in Northern Papua, Indonesia

Supeni Sufaati, Rosye H.R. Tanjung, Septriyanto Dirgantara, Verena Agustini
Cenderawasih University, Indonesia

Purpose: Wild edible mushrooms play an important role as food and income source for people in a rural area. However, there is still a lack of data on the nutritive value of the wild edible mushrooms traded in Papua. This study was done to collect the information on nutrient composition of the most marketable wild edible mushrooms in the traditional market in the lowland of north Papua.

Methods: Wild edible mushrooms were bought, dried and extracted to analyze their macro and micro nutrient. Proximate composition analysis was based on the Official Analytical Chemists (AOC) method. Protein content was determined by Micro Kjeldahl methods, P by spectrophotometer, K by flame photometer, Fe, Zn, Ca, Mg, and Cu, by Atomic Absorption Spectrophotometer (AAS). Amino acids composition were analyzed by Reversed-Phase High Performance Liquid Chromatography (RP-HPLC).

Results: There were 4 wild edible mushrooms sold in several traditional market in Papua province: Volvariella volvacea, Volvariella sp, Pleurotus sp, and Lentinus sajor-caju. Ash, fat, protein, carbohydrates and mineral composition were varied among those mushrooms. Volvariella sp (sago mushroom) has the highest protein (52.7 %) and minerals content. From the eighteen amino acids which were determined, each species showed different amino acid profile. Total free amino acid contents range from 131,356.77 mg/kg in Pleurotus sp to 217,139.39 mg/kg in Volvariella volvacea. The most abundant amino acids present in all samples were glutamic acid, aspartic acid, and leusin.

Conclusion: This result indicated that wild edible mushrooms are good nutrients source to overcome nutrient deficiency in the rural area. Further investigation on antimicrobial and antioxidant activity will be conducted to search potential bioactive compounds of wild edible mushrooms from Papua, Indonesia.
Study on hypoglycemic effect of the crude polysaccharide extract from *Inonotus obliquus*

Suping Guo, Lin Bi, Chengrui Li
Shanxi Institute of Medicine and Life Sciences, China

**Purpose:** *Inonotus obliquus* is a very rare and valuable medicinal fungus which has a therapeutic effect on malignant tumors, heart disease, diabetes and so on. This study was conducted to observe the effect of fasting blood glucose and glucose tolerance in normal and alloxan-induced diabetic mice after given different concentrations of the crude polysaccharide extract from *Inonotus obliquus* (CPIO) by gavage.

**Methods:** Diabetic mice were induced by alloxan (160 mg/kg, i.p.). 24 normal mice were divided into the control group and the high dose CPIO group (500 mg/kg). 60 diabetic mice were divided into the model group, the metformin group (166 mg/kg), the high dose CPIO group (500 mg/kg), the middle dose CPIO group (333 mg/kg) and the low dose CPIO group (166 mg/kg). Each group were given corresponding distilled water, metformin or CPIO by gavage for 30 days. The fasting blood glucose and glucose tolerance were determined in the last day. The results showed that middle and low dosage CPIO (333 and 166 mg/kg body weight) could significantly increase the rate of fasting hypoglycemic (p<0.05); high and middle dosage CPIO (500 and 333 mg/kg body weight) could significantly reduce the 2h glucose (p<0.01, 0.05); high, middle and low dosage CPIO (500, 333 and 166 mg/kg body weight) could significantly increase the rate of 2h blood sugar decline (p<0.05, 0.01, 0.05); middle dosage CPIO (333 mg/kg body weight) could significantly reduce area under curve of glucose (p<0.05).

**Results and Conclusion:** The crude polysaccharides from *Inonotus obliquus* (CPIO) has a certain hypoglycemic effect on alloxan diabetic mice.
Oral Session 23

[Future Perspective for the Use of Medicinal Mushroom in Health and Disease States]

4-O23-4

Antioxidant activities and total phenolic and flavonoid contents in four mushroom of *Phellinus* from Thailand

Khwanyuruan Nakwunakul
Mahasarakham University, Thailand

*Phellinus* is medicinal mushroom with variety of bioactivity compounds and used with traditional medicine for a decade especially in Asia.

**Purpose:** In this study, was investigated antioxidant activities with DPPH, FRAP and ABTS assay.

**Methods:** Four mushroom of *Phellinus* was used such as *P. nigricans, P. nigrolimitatus, P. rimosus* and *P. wahlbergii*, the fruiting body was extracted using boiling water 95°C, ethanol and ethyl acetate.

**Results and conclusions:** Total phenolic content, estimated by Folin-Ciocalteu assay, were found highest content from ethanol extract of *P. nigricans* and *P. rimosus* to be 606.4±1.65 and 603.54±4.2 mg GAE/g, respectively. The aluminum chloride colorimetric method was used for the determination of the total flavonoid content were found highest content from ethanol extract of *P. rimosus* 433.12±5.3 mg EC/g extract. The extract showed good free radical scavenging with DPPH assay in ethanol extract of *P. rimosus* (IC50 49.13±2.1 mg/mL) and ABTS assays in ethyl acetate extract of *P. nigricans* (IC50 5.96±0.13 mg/mL) and reducing capacities by FRAP assay in ethyl acetate extract of *P. rimosus* (0.45±0.04 mg ferrus sulfate/ g extract). The result showed good antioxidant activities are *P. rimosus* and *P. nigricans* using ethanol and ethyl acetate extract.
Optimization and anticancer effect of L-asparaginase production in *Penicillium citrinum* isolated from Malaysian medicinal plant *Pereskia bleo*

Ling Sze Yap, Wai Leng Lee, Adeline Su Yien Ting
School of Science, Monash University Malaysia, Malaysia

**Purpose:** L-asparaginase is commonly used as a chemotherapeutic agent in the treatment of acute lymphoblastic leukemia (ALL) to remove L-asparagine, which is required for the growth of the leukemia cancer cells. With the removal of L-asparagine, the rapid growth of tumor cells can be controlled. However, the commercialized bacterial-derived L-asparaginase has been reported to induce several toxic side effects and cause immunogenic reactions. Therefore, endophytes which live within the plants are proposed as an alternative source of L-asparaginase.

**Methods:** In this study, L-asparaginase producing endophytes were isolated from medicinal plant *Pereskia bleo* (Seven star needle). All endophytes were subjected to plate assay and quantitative assay to detect for the presence of L-asparaginase. The selected endophytes were identified via 18S rRNA gene sequencing. Six variables of growth condition, carbon and nitrogen sources, their concentrations, incubation period, pH, temperature and agitation rate were optimized. Cytotoxicity bioassay was performed on leukemic Jurkat E6 cell using crude extracts derived from endophytes cultured under optimum conditions.

**Results and conclusion:** Results revealed that 10 of the 13 endophytic isolates showed positive results on plate assay and isolate PL4 (*Penicillium citrinum*) showed highest L-asparaginase activity. The optimum conditions for L-asparaginase production were 0.2% glucose, 1.0% L-asparagine, 5 days, pH 5, 30 degree celsius and 140 rpm. The crude extract derived from PL4 showed strong dose dependent cytotoxicity effect against leukemic Jurkat cell. The results demonstrated that isolate PL4 is a potential alternative source for L-asparaginase.
Reexamination of the genus *Albugo* in Azerbaijan

Aynur G. Bakhshiyeva<sup>1,2</sup>, Daisuke Mabuchi<sup>2</sup>, Yukako Hattori<sup>2</sup>, Chiharu Nakashima<sup>2</sup>, Dilzara N. Aghayeva<sup>1</sup>

<sup>1</sup>Institute of Botany, Azerbaijan National Academy of Sciences, Japan
<sup>2</sup>Graduate School of Bioresources, Mie University, Japan

Species of the genus *Albugo* (Pers.) Roussel of the family Albuginaceae, are obligate biotrophic 'pseudofungi' responsible for white blister rust (WBR) on Amaranthaceae, Brassicaceae and other plants. The genus comprises about 56 species worldwide. The genus *Albugo* has been studied in Azerbaijan since mid of 20th century. In total, 28 taxa were recorded in the “Mycoflora of Azerbaijan”.

**Purpose:** The aim of our study is to revise the species of the genus *Albugo* based on the latest taxonomical rearrangements and reveal the diversity of that in Azerbaijan considering morphology and phylogenetic relationship.

**Methods:** Specimens of *Albugo* were derived from the Mycological Herbarium (BAK) of the Institute of Botany, ANAS. About 15 specimens were examined. For the subsequent microscopic examination in Zeiss Stemi 305 (Jena, Germany) microscope and Zeiss Axio Imager A1 (Oberkochen, Germany) for DIC light microscopy were used. The COX2 gene region obtained from 1 newly collected specimens and 4 herbarium specimens of the genus *Albugo* s.l., which have been kept in BAK more than 40 years, were sequenced by using COX2-F and COX2-R primers.

**Results and conclusions:** Based on morphology six species, *A. candida*, *A. candida var. macrospora*, *A. resedae*, *Pustula tragopogonis*, *Wilsoniana bliti*, *Wilsoniana portulacae*, were identified and redescribed as fungi from Azerbaijan. From the results of phylogenetic analyses, *Albugo candida* s.str. was newly recorded from *Tragopogon graminifolius* and *Albugo candida* s.l. on *Arabis hirsuta*, *Albugo* sp. (not *A. resedae*) on *Reseda globulosa*, and two new species of *Wilsoniana* differenciated from the hitherto known species on *Amaranthus*. The phylogenetic analyses using the herbarium specimens indicated the richness of species diversity of the genus *Albugo* and its related genera.
A new *Pythium* species causing lettuce wilt

Akihiro Hayano¹, Kensuke Yamada¹, Ayaka Hieno¹, Haruhisa Suga², Koji Kageyama¹

¹River Basin Research Center, Gifu University, Japan
²Life Science Research Center, Gifu University, Japan

**Purpose:** In 2016, the lettuce showing in the above-ground and root rot was observed in a lettuce field in Kagawa prefecture. An isolate with fast-growing and non-septate hyphae like *Pythium* species was obtained from the root.

**Methods and Results:** The isolate was cultured in a 9 cm petri dish with CMA medium, and after the isolate growth spread throughout, sterilized soil was covered over the colony, and a pre-germinated lettuce seed was sown in the soil. After 7-days incubation in a growth chamber at 25°C in 12-hours photoperiod, the seedlings showed root rot and the isolate was recovered from the root. When the sequence of the rDNA-ITS region was analyzed, it belonged to the molecular phylogenetic clade J of *Pythium* species classified by Levesque and de Cock (2004). Additionally, the nucleotide sequences of coxI, coxII and β-tubulin were examined, and multigene phylogenetic tree was constructed with the other members of clade J, resulting in a single lineage. The morphological characteristics of the isolate were confirmed: the sporangia (av. 27.3 um) was spherical, the oogonium (av. 32.5 um) was smooth and spherical, and the oospore (av. 27.6 um) was aplerotic. The antheridia were diclinous, and the antheridial cells were clavate and crook-necked. One to three the antheridia were attached per oogonium. Mycelial growth was observed between 5°C and 25°C. These characteristics differed from the other members of clade J.

**Conclusions:** The results indicate that the isolate will be a new *Pythium* species as well as a new pathogen in lettuce.
**P1-03**

**Mucoralean fungi in Korea**

Hyang Burm Lee, Thuong T. T. Nguyen  
Dept. of Agricultural Biological Chemistry, College of Agriculture & Life Sciences, Chonnam National University, Korea

**Purpose:** Mucoromycotina and Mortierellomycotina consist of the largest number of described species within Mucoromycota. Many members into this subphylum are important in biotechnological areas. Especially, some species are known as causal fungi of human mucormycosis. However, the knowledge about the taxonomy of mucoralean fungi in Korea is limited. The present study aims to characterize 13 new species and 27 unrecorded mucoralean species in Korea by morphological and molecular study.

**Methods:** Mucoralean fungi were isolated from dung, insect, fruits, freshwater, and soil by using the dilution plating and baiting technique. To identify fungal strains, the ITS, 18S, 28S, EF-1α, and act-1 gene were amplified with the primer pairs ITS1/ITS4, NS1/NS4, LROR/LR5F, MEF11/MEF41, and Act-1/Act-4R, respectively. Phylogenetic analyses were performed using MEGA 7 software. In addition, the micro-morphological features of strains were investigated by LM with an Olympus BX51 microscope and SEM.

**Result:** In this study, 92 isolates representing 43 species belonging to 14 genera were isolated from different sources, including dung, insect, fruits, freshwater, and soil. Among these genera, *Mucor* presented with the highest number of species, followed by *Mortierella*. The phylogenetic analyses of sequence data of the loci, ITS, 18S, 28S, EF-1α, and act-1 showed that 13 species of the genus *Backusella, Absidia, Gongronella, Mucor, Rhizopus, Mortierella*, and *Umbelopsis* were represented as new species and 27 species were identified as unrecorded species in Korea.

**Conclusions:** In this study, 92 isolates representing 43 species belonging to 14 genera were isolated from different sources in Korea. Herein, 13 new species and 27 unrecorded mucoralean species were discovered and characterized through this study. Especially, *Backusella, Blakeslea, Gilbertella*, and *Pilobolus* species known as rare species were found in Korea.
Toward a natural classification of Venturiales

Ying Zhang1), Min. Shen1), Pedro W. Crous2)
1) Beijing Forestry University, China
2) Westerdijk Fungal Biodiversity Institute, The Netherlands

Purpose: 1) to delineate the phylogenetic lineages, families and generic boundaries of Venturiales; 2) and to designate appropriate types to stabilise the application of names of Venturiales.

Methods: Five loci, ITS, LSU, tef1, tub2 nuDNA and rpb2, are used for analysing 110 venturialean taxa representing 27 genera and four families in the current classification of Venturiales.

Results and conclusions: Two new families, viz. Cylindrosympodioidaceae and Cylindrosympodiaceae, as well as eight new genera are introduced, namely Bellamyces, Faguscola, Fraxinucola, Neofusicladium, Parafusicladium, Phaeohlia, Pinicola and Sterilla. In addition, 12 species are described as new to science, and 40 new combinations were proposed. The formation of conidia, i.e. solitary or in chains, showed significance at generic classification. The tendency of sporulation also showed phylogenetic significance in some degree. The current clade of Venturia seems representing a genus complex. The ancestral state of Venturiales is most likely to be saprobic, and plant pathogens seems a new evolutionary state. The type specimens of 50 Venturia species are described and illustrated with 27 species accepted within Venturia s. str.
Members of the genus *Cytospora* are often reported as endophytes, saprobes or phytopathogens, primarily causing canker diseases. They occur on a wide range of hosts and have a worldwide distribution. Although several species have in the past been reported from China, the vast majority are not known from culture or DNA phylogeny. The primary aim of the present study was thus to clarify the taxonomy and phylogeny of a large collection of *Cytospora* species associated with diverse hosts in China. *Cytospora* spp. were collected in northeast, northwest, north and southwest China, indicating that the cold and dry environments favour these fungi. In this study, we provide an assessment of 51 *Cytospora* spp. in China, focusing on 38 species represented by 88 isolates from 28 host genera. Based on a combination of morphology and a six-locus phylogeny (ITS, LSU, act, rpb2, tef1-α and tub2), 13 new species and one new combination are introduced. The majority of the species investigated here appear to be host-specific, although further collections and pathogenicity studies will be required to confirm this conclusion.
Taxonomy and phylogeny of *Diaporthe* associated with dieback diseases in China

Qin Yang\(^{1,2}\), Xinlei Fan\(^1\), Yingmei Liang\(^1\), Chengming Tian\(^1\)

\(^1\)College of Forestry, Beijing Forestry University, China
\(^2\)College of Life Science and Technology, Central South University of Forestry and Technology, China

**Purpose:** *Diaporthe* species have often been reported as important plant pathogens, saprobes, and endophytes on a wide range of plant hosts. Although several *Diaporthe* species have been recorded in China, little is known about species able to infect forest trees. The primary aim of the present study was thus to clarify the taxonomy and phylogeny of a large collection of *Diaporthe* species associated with dieback diseases in China.

**Methods:** The current results emphasized on 38 species from 104 representative isolates involving 22 host genera using comparisons of DNA sequence data for the nuclear ribosomal internal transcribed spacer (ITS), calmodulin (cal), histone H3 (his3), partial translation elongation factor-1\(\alpha\) (tef1) and \(\beta\)-tubulin (tub2) gene regions as well as their morphological features.

**Results and conclusions:** Thirty-one novel taxa are introduced and *Diaporthe eres* was found as the most common species associated with diversity hosts. The current study improves the understanding of species causing diebacks on ecological and economic forest trees and provides useful information for the effective disease management of these hosts in China.
Using divergence times in fungal classification

Jian-Kui (Jack) Liu\textsuperscript{1)}, Milan C. Samarakoon\textsuperscript{2)}, Alan Phillips\textsuperscript{3)}, Sheng-Nan Zhang\textsuperscript{2)}, Kevin D. Hyde\textsuperscript{2)}

\textsuperscript{1)School of Life Science and Technology, University of Electronic Science and Technology of China, P.R. China
\textsuperscript{2)Center of Excellence in Fungal Research, Mae Fah Luang University, Thailand
\textsuperscript{3)Biosystems and Integrative Sciences Institute, Universidade de Lisboa, Portugal

Purpose: The current classification system for the recognition of taxonomic ranks among fungi, especially at high ranking level, is subjective. With the development of molecular approaches and the availability of fossil calibration data, the use of divergence times as a universally standardized criterion for ranking taxa has now become possible. We can therefore date the origin of Ascomycota lineages by using molecular clock methods and establish the divergence times for the orders and families of Dothideomycetes.

Methods: We chose Dothideomycetes, the largest class of the phylum Ascomycota, which contains 32 orders, to establish ages at which points orders have split; and Pleosporales, the largest order of Dothideomycetes contains 55 families, to establish family divergence times. We have assembled a multi-gene data set (LSU, SSU, TEF1 and RPB2) from 391 taxa representing most family groups of Dothideomycetes and utilized fossil calibration points solely from within the ascomycetes and a Bayesian approach to establish divergence times of Dothideomycetes lineages.

Results and Conclusions: Our results indicate that divergence times (crown age) for most orders (20 out of 32, or 63\%) are between 100 and 220 Mya, while divergence times for most families (39 out of 55, or 71\%) are between 20 and 100 Mya. We believe that divergence times can provide additional evidence to support establishment of higher level taxa, such as families, orders and classes. Taking advantage of this added approach, we can strive towards establishing a standardized taxonomic system both within and outside Fungi. To get more reliable calibrations, we also carry out a modern review of estimations regarding ancient lineages of Ascomycota, and we summarize a historical fossil outline with a reliable minimum age for 16 calibrating points. A scheme of Ascomycota ancient lineages is also provided in order to improve divergence time estimations.
Diversity of endolichenic fungi isolated from the lichen Usnea from Malaysia and the Philippines

Krystle Angelique Santiago1,2, Thomas Edison dela Cruz3, Adeline Su Yien Ting1,2

1School of Science, Monash University Malaysia, Malaysia
2Tropical Medicine & Biology Multidisciplinary Platform, Monash University Malaysia, Malaysia
3College of Science, University of Santo Tomas, Philippines

Purpose: Endolichenic fungi (ELF) are organisms residing inside healthy tissues of lichens. However, not much is known of their diversity and distribution in various lichen hosts. This study, therefore, serves as a preliminary survey of ELF present in the lichen Usnea collected from Malaysia and the Philippines.

Methods: Usnea baileyi, U. bismolliuscula and U. pectinata were collected from various altitudes in Bukit Larut, Malaysia and Sagada, Philippines and were surface-sterilized prior to ELF isolation. The isolated ELF were first categorized based on morphology. Molecular-typing was then performed via RAPD-PCR where similarity matrix was generated using UPGMA algorithm. Ecological indices were calculated to assess their diversity and evenness using Simpson’s Index of Biodiversity (D’) and Shannon’s Equitability (EH).

Results: A total of 166 ELF was isolated from the three Usnea species. Of these, 70 were from Malaysia and 96 were from the Philippines. Morphological characterization indicated 24 presumptive genera. This was validated by molecular-typing revealing nine genera. The most common genera were Nemania sp. and Xylaria sp.. Astrocystis bambusae and Pseudopestalotiopsis theae were reported as ELF for the first time. Among the lichen hosts, U. pectinata had the highest ELF diversity (D’=0.700) and an even ELF distribution (EH=0.143).

Conclusion: Usnea harbor diverse species of ELF. Altitude affects ELF diversity more strongly than lichen host and geographic location. Although results are solely based on culturable isolates and may have excluded some fastidious species, this study has provided the first evaluation of ELF in Usnea from Malaysia and the Philippines.
Purpose: The International Code of Nomenclature for Algae, Fungi and Plants (ICN) contains the rules and recommendations dictating the naming of fungi. However, strict application of some articles result in species being invalid even though the species appears to be validly published to a non-specialist. Examples include articles 40.7 (only a single type specimen may be indicated) and 40.8 (must include a statement that the culture is preserved in a metabolically inactive state); especially the combination of these two where the same culture is listed in multiple culture collections as type is problematic. Another example is article 32.1 (conditions for validly publishing a taxon name), which excludes the use of a name in a new combination if the name itself was invalidly published. Some of these articles effectively invalidate names that are commonly accepted by users.

Methods: Examples were obtained from different ranks that are indicated as invalid in MycoBank or Index Fungorum. The original publication was checked to confirm why the name is invalid, as well as any subsequent publications aimed at validating the name in question.

Results and conclusions: Quite a number of species currently indicated as invalid can easily be corrected by simply redescribing them with e.g. only citing a single specimen/culture number. However, there are no rules determining who the author of this species should then be or whose responsibility it should be. Some good practices and suggestions to resolve nom. inval. cases are discussed.
The species diversity of the genus *Lasiodiplodia* in Japan

Yukako Hattori\(^1\), Lynn Nakano\(^2\), Carmelita P Mapanao\(^3\), Arcibel B Bautista\(^3\), Chiharu Nakashima\(^1\)

\(^1\)Graduate school of Bioresources, Mie University, Japan
\(^2\)Department of Bioresources, Mie University, Japan
\(^3\)Polytechnic University of the Philippines

**Purpose:** *Lasiodiplodia* species are well known as plant pathogenic fungi that cause stem end rot, fruit rot, and die-back. In Japan, 29 plant genera of 21 plant families are affected by *Lasiodiplodia*. However, Japanese species of *Lasiodiplodia* has not been elucidated completely. In this study, we aimed to clarify the Japanese species diversity of these fungi based on the phylogenetic relationship and morphological characteristics.

**Methods:** Six isolates were established by the single conidial isolation from the fresh mango fruits, branches, and leaves, collected in Miyako Islands. 5 isolates were also obtained from Mango fruits imported from the Philippines to Japan. Other 39 cultures of *Lasiodiplodia spp.* from various plants were subdivided from culture collections. To observe morphological and cultural characters, the isolates were incubated. Genomic DNA from isolates were extracted and sequenced for rDNA ITS, TEF1, and TUB2 regions. Those concatenated matrix was analyzed with MP and ML by PAUP* and RAxML for phylogenetic studies.

**Results and Conclusion:** As a result of the phylogenetic analysis, Japanese *Lasiodiplodia* species were divided into 3 clades. The first clade is *Lasiodiplodia theobromae*-complex, which consists of isolates from tropical fruit trees, Mango, Cacao, Sugar apple, and Papaya. The isolates on imported Mango were also located in this clade. The second one is sister to *L. jatrophicola*. This clade includes strains, isolated from a broad host from herbaceous plants to woody plants. The third clade is separated from the other clades and each small clade should be treated as a new species.
Fungal Diversity of Limestone Caves in Sabah, Malaysia

Ibrahim Wasti¹,², Jaya Seelan¹

¹Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Malaysia
²Faculty of Natural Science and Sustainability, University College Sabah Foundation, Malaysia

Purpose: Despite Borneo being a biodiversity hotspot, there have been no studies conducted on the mycobiota of its limestone caves. The aims of this study were to isolate and characterize the microfungi in different substrates in caves and compare the diversity between caves. Four limestone caves in Sabah had been selected as ideal study sites.

Methods: Gomantong and Madai caves represented anthropogenically active caves, and Balambangan and Keruak caves represented anthropogenically non-active caves. Opportunistic sampling was undertaken to collect 20 speleothem samples, 14 water samples, 11 bat guano samples, and 9 dead arthropod samples. Only Gomantong caves yielded arthropod cadavers. Morphological and molecular methods were utilized to identify all isolates to at least the genus level.

Results and conclusions: A total of 76 distinct taxa from 35 genera were identified from 180 pure isolates. Identification of 58 taxa (54 species) received molecular confirmation after DNA extraction, amplification, and sequencing. An average of 4.10 species per speleothem site, 3.71 species per water sample, 3.18 species per guano site, and 1.11 species per dead arthropod were recorded. The highest average CFU count for speleothem was 254.0 CFUcm⁻² per isolate, cavern water was 335.0 CFUml⁻¹, guano had 6266.7 CFUg⁻¹, and dead arthropods had 1.11 isolates per cadaver. In order of decreasing frequency, some the genera of fungi identified include Penicillium, Aspergillus, Trichoderma, Fusarium, Purpureocillium, Nodulisporium, Annulohypoxylon, Clonostachys, Talarmyces, Cladosporium, Curvularia, and Paecilomyces. We suggest that fungal abundance in higher in caves with more anthropogenic activity, and distance from the cave entrance may play a role in fungal abundance.
Clavicipitaceous fungi on Commelinaceae, which have been recognized as smut fungi

Eiji Tanaka
Ishikawa Prefectural University, Japan

**Purpose:** A smut-like fungus was found on the flowers of *Murdannia keisak* (Commelinaceae, Monocots) in Japan. Infected flowers were filled with yellow to orange thick-walled conidia, and then the sori changed to olivaceous green. The fungus was identified to be *Ustilago aneilematis*, which have been recognized as a smut fungus (Ustilaginales, Basidiomycota). This study aimed to show that this species is included in the family Clavicipitaceae (Hypocreales, Ascomycota).

**Methods:** Isolates derived from a single spore of the smut-like fungi on *M. keisak* was used for DNA extraction. Molecular phylogenetic analysis was done based on DNA sequences from LSU, SSU, TEF, RPB1, and RPB2.

**Results:** Multi-locus phylogenetic tree showed that the fungus is grouped with the species of the tribe Ustilaginoideae (Clavicipitaceae, Ascomycota). The thick-walled conidia of the fungus developed on the apex of dichotomous or trichotomous conidiophores in contrast to the pleurogenously developing conidia of known Ustilaginoideae spp.

**Conclusions:** Based on these findings and taxonomical considerations, it was concluded that the fungus must belong to a new genus of the tribe Ustilaginoideae in the family Clavicipitaceae. This species is the first clavicipitaceous fungus found to infect a host plant species in the Commelinaceae.
Diaporthalean fungi associated with canker and dieback of tree hosts in China, emphasising on *Cytospora* and *Diaporthe* (Diaporthales)

Chengming Tian, Xinlei Fan
Beijing Forestry University, China

Diaporthales represents an important order in *Sordariomycetes* containing taxa that are mainly isolated as endophytes, saprobes or plant pathogens on various hosts. Taxonomy of these pathogens is difficult due to their uninformative descriptions and similar asexual morphology. The primary aim of the present study was to redefine the taxonomy and phylogeny of a large collection of Diaporthales species associated with diverse hosts in China. In the current study, 12 families are involved here based on morphology and phylogeny. *Cytosporaceae* and *Diaporthaceae* are most common phytopathogenic taxa causing canker. Other nine different families associated with canker and dieback of tree hosts are morphologically treated and phylogenetically compared. These include *Cryphonectriaceae*, *Gnomoniaceae*, *Melanconidaceae*, *Melanocniellaceae* and *Juglanconidaceae*. Four new families (Diaporthosporellaceae, Diaporthostomataceae, Pseudomelanconidaceae, Synnemasporellaceae), and two new genus, *Dendrostoma* (Erythrogloeaceae) and *Sheathospora* (Melanconiellaceae) were introduced. Combined analyses of ITS, LSU, RPB2 and TEF1-α sequence data were used to construct the molecular phylogeny.
Taxonomy and phylogeny of Gymnosporangium in China

Yingmei Liang, Bin Cao
Beijing Forestry University, China

Gymnosporangium species are mainly distributed in the northern hemisphere and approximately 61 species of Gymnosporangium have been reported worldwide. Among them, 16 species have been recorded from China. Most Gymnosporangium species are heteroecious and demicyclic. They usually produce telia on needles, stems, and branches of Cupressaceae and aecia on the leaves and fruits of plants belonging to Maloideae, which resulted in great economic losses. However, species and their interspecific relationships identified by morphological observation are still very confusing and the life cycle of most Gymnosporangium species is not clear, which leads to a lot of problems to taxonomy on the species level. Therefore, it is necessary to use phylogeny to determine the life cycle of Gymnosporangium and to correctly define the species of Gymnoporangium combining morphology and phylogeny. In the current study, a total 27 species of Gymnosporangium were identified in China based on morphology and phylogeny, and the main distribution areas were also listed through our studies. Six new species were found in this research: G. huanglongense, G. lianhuashanense, G. niitakayamense, G. przewalskii, G. xianmiense, G. zhuoniense. One new record species was found in this research: G. unicorne. One new combination species was found in this research: G. echinulatum. Three species are resurrected in this research: G. distortum, G. nanwutaianum, G. sikangense.
Molecular taxonomy and morphology of some fungi from Russia

Napalai Chaiwan, Kevin Hyde
Mea Fha Luang University, Thailand

Fungi are one of the most important components of any ecosystem, they are responsible for plant growth, nutrient cycling, and help maintain plant diversity. Microfungi are a large group of microorganisms which have small (microscopic) fruiting bodies and cell wall structures composed of the chitin, mostly classify to the phylum Chytridiomycota, Zygomycota, and Ascomycota, but also include other, molds, mildews, rusts and smuts. Furthermore, microfungi can act as pathogens, infecting economic crops and resulting in yield losses. Furthermore, microfungi species can be used in antibiotics to treat bacterial infections, as well as used in biological control agents in agriculture. In this study, I will provide some fungi from Russia specimen for study the diversity on their fungal communities. To date, I has found 6 species of fungi including Calophoma sp. from Humulus lupulus, Cytospora sp. from Rosa sp., Keissleriella sp. from Wlnus purmila, Nothophoma sp. from Prunus mahaleb, Preussia sp. from Prunus cerasus and Uzbekistanica sp. from Prunus armeniaca. In this presentation, I illustrate the morphology of some of the fungi. The purpose of this study is to show the diversity of fungi from Russia specimen.
Status of genus *Helvella* (Pezizomycetes, Ascomycota) in Pakistan

Abdul Rehman Niazi, Simab Asghar, Abdul Nasir Khalid, Najam ul Sehar Afshan

University of the Punjab, Pakistan

**Purpose:** The order Pezizales is the most diverse group of ascomycetes represented in Pakistan. The Pezizales, the only order of the Pezizomycetes, is characterized by asci that generally open by rupturing to form a terminal lid called operculum. Most of these Ascomycetes are found in Himalayan moist temperate forests which occupy Kashmir, Murree & Hazara hills, Gilgit and Baltistan, lower Dir, upper part of Khurram Agency and some humid areas of upper Swat, because of moisture and dense vegetation cover. *Helvella* belongs to family Helvellaceae which has worldwide distribution comprising of 59 species specifically recognized by the pileus shape which is saddle like. From Pakistan, 11 species of *Helvella* are previously reported from different northern areas of Pakistan. Fruiting bodies of *Helvella* grow above ground generally; have stems and cap which is irregularly shaped.

**Methods:** During present study, by molecular and morphological data, we have described five new species of *Helvella* with their specific location, as the targeted rDNA of the specimen was amplified by using ITS1F and ITS4.

**Results and Conclusions:** The described species are new to science which extended the status of genus *Helvella* in Pakistan. This study suggests that forests of Pakistan are rich in fungal flora and there is need to explore different areas to document and enlist fungi of Pakistan.
Poster Presentation 1

P1-17

Three New Ascomycete Fungi Isolated from Freshwater and Plant Leaf Samples in Korea

Hyo Jin Lim, Hyang Burm Lee
Environmental Microbiology Lab, Dept. of Agricultural Biological Chemistry, College of Agriculture & Life Sciences, Chonnam National University, Korea

**Purpose:** During an investigation of fungi of the ascomycete in Gwangju, Korea, the isolates CNUFC-MSW24-4, CNUFC-HRS5-12 and CNUFC-NJ1-12 were isolated from freshwater and *Torreya nucifera* leaf samples collected in Wonhyo valley, Hwangnyonggang and arboretum of Chonnam national university.

**Methods:** The three Strains, CNUFC-MSW24-4, -HRS5-12, -NJ1-12 were isolated from freshwater samples and *Torreya nucifera* leaf samples in Korea by using different isolation method. Genomic DNA were extracted. For these strains, ITS, LSU, SSU, ACT, tef1-α and GPDH sequences were amplified with the primer pairs ITS1/ITS4, LROR/ LR5F, NS1/NS4, EF1-728F/ EF1-986R and Gpd1/Gpd2. The purified PCR products were sequenced with an ABI 3700 automated DNA sequencer. Phylogenetic analyses based on ITS, LSU, SSU, ACT, tef1-α and GPDH sequences were conducted, using BioEdit ver. 7.2.6, Clustal X2 ver. 2.0. Their phylogenies were assessed by employing programs available in the MEGA7. Morphological characteristics were observed under Olympus BX51 microscope.

**Result:** Sequences of β-tubulin gene analysis by BLASTn search indicated that the isolate CNUFC-MSW24-4 was closest to *Paraconiothyrium fungicola* (GenBank accession no. JX496359) with identity values of 94.56% (400/423 bp). Sequences of rDNA ITS regions analysis by BLASTn search indicated that the isolates CNUFC-HRS5-12 and CNUFC-NJ1-12 were closest to *Ochroconis* sp. isolate WX-ITS4_H12 (GenBank accession no. MH969430) and *Phyllosticta harai* (GenBank accession no. KU363980) with identity values of 99.57% (687/690 bp) and 97.05% (625/644 bp), respectively.

**Conclusions:** On the basis of their morphological characteristics and phylogenetic analysis, the CNUFC-MSW24-4, -HRS5-12 and -NJ1-12 isolates were identified as a new species of *Paraconiothyrium, Ochroconis* and *Phyllosticta*, respectively.
Multi-locus phylogeny reveals *Phaeodothis mori* sp. nov. from dead leaves of *Morus australis*

Danushka Sandaruwan Tennakoon, Chang-Hsin Kuo, Kevin David Hyde

1) Department of Plant Medicine, National Chiayi University, Taiwan
2) School of Science, Mae Fah Luang University, Thailand
3) Center of Excellence in Fungal Research, Mae Fah Luang University, Thailand
4) Key Laboratory for Plant Biodiversity and Biogeography of East Asia (KLPB), Kunming Institute of Botany, Chinese Academy of Science, China
5) Department of Biology, Faculty of Science, Chiang Mai University, Thailand

In order to establish phylogenetic relationships and resolve a natural classification for species of Dothideomycetes, it is necessary to use multi-gene phylogeny as well as morphology. Phaeodothis mori is a new species collected from dead leaves of *Morus australis* in Fanlu Township area, Dahu forest, Chiayi, Taiwan. Maximum parsimony, Maximum likelihood and Bayesian analyses of combined ITS, LSU, SSU and tef1-α sequence data were performed to clarify the phylogenetic affinities of the species. Phaeodothis mori is distinguished from the other Phaeodothis species based on distinct size differences in ascomata, asci, ascospores and DNA sequence data. The new species is compared with other Phaeodothis species and a comprehensive description and micrographs are provided.

Key words: 1 new species, Dothideomycetes, Pleosporales, phylogeny, taxonomy
Purpose: Ochroconis and Verruconis can be classified to species level by using 18S small subunit (SSU) ribosomal RNA (rRNA) gene that forms secondary structure inside 40S ribosome. Therefore, the hypothetical and secondary structure of 18S SSU involving in speciation of those fungi should be investigated.

Methods: Twenty six 18S rRNA gene sequences of type and reference species of Ochroconis (23) and Verruconis (3) were collected from GenBank and aligned for the secondary structure by using SSUALIGN version 0.1. The aligned dataset was then edited, adjusted and analyzed by BioEdit version 7.2.5, MEGA7 and Microsoft Excel.

Results: The aligned 18S rRNA contained 1,883 positions (p.) and 9 variable (V) regions. Speciation of all investigated species could be detected in variable regions of the gene, 21 species of Ochroconis and 3 of Verruconis were in V2 (p. 125-300), 13 of Ochroconis and 3 of Verruconis in V4 (p. 650-995), 12 of Ochroconis in V8 (p. 1,520-1,675), 5 of Ochroconis in V5 (p. 1,020-1,215), 4 of Ochroconis and 1 of Verruconis in V7 (p. 1,410-1,500), 4 species of Ochroconis in V9 (p. 1680-1830), 3 species of Ochroconis could be found in p. 300-415, and V3 (p. 460-555) and 1 of Verruconis was in V6 (p. 1,290-1,340).

Conclusions: The variable regions V2, V4 and V8 of 18S rRNA were responsible for most speciation of Ochroconis members whereas V2 and V4 were for 3 members of Verruconis.
Additions to pestalotioid taxa in Taiwan

Ichen Tsai, Hiran Anjana Ariyawansa
Department of Plant Pathology and Microbiology, National Taiwan University, Taiwan

Purpose: We aimed to investigate the diversity of pestalotioid taxa in Taiwan.

Methods: Fungal samples were collected from Nantou and Taipei counties. We carried out polyphasic studies using single- and multi-gene (ITS, tef, tub2) phylogenies based on Maximum likelihood and Bayesian inference together with phenotypic data to assess the natural classification of freshly collected isolates.

Results: Based on these data, two novel species are proposed in Pestalotiopsis while three in Pseudopestalotiopsis and are introduced herein as Pestalotiopsis formosana, P. neolitseae, Pseudopestalotiopsis hydeae, Ps. ixorae and Ps. taiwanensis. P. formosana and P. neolitseae, which were isolated from dead grass and living leaves of Neolitsea villosa respectively, are morphologically similar to Pestalotiopsis with concolourous median cells, but vary from the phylogenetically related taxa in the size of conidiomata and conidia, the number of apical appendages and length of basal appendages. Ps. hydeae was obtained from dead leaves of Diospyros sp., while Ps. ixorae and Ps. taiwanensis were isolated from living leaves of Ixora sp. These three new species fit well with Pseudopestalotiopsis in having dark concolourous median cells with knobbed apical appendages, but differ from known taxa by the size of conidiomata and conidia, the number of apical appendages and length of basal appendages. The outcomes of pathogenicity tests discovered that P. neolitseae, Ps. ixorae and Ps. taiwanensis are capable of causing leaf spots on N. villosa and Ixora sp. respectively.

Conclusions: Our study increases the base of evidence concerning the diversity of pestalotioid species in Taiwan.

Keywords: New species, Pestalotiopsis, Phylogeny, Phytopathogenic fungi, Pseudopestalotiopsis
**Lecanicillium aphanocladii** isolated from “Tengu-no-Mugimeshi” in Mt. Kurohime, Nagano Prefecture, central Japan

Mayuka Higo¹, Rei Hokari¹, Masato Iwatsuki¹, Tomotaka Tanabe², Taiga Kasuya³, Kenichi Nonaka¹

¹Kitasato Institute of Life Sciences, Japan
²Togakushi Museum of Natural History, Japan
³Keio University, Japan

**Purpose:** “Tengu-no-Mugimeshi” is a microbial mass, and it has been recognized from mountainous areas in the volcanic zone of Chubu region, central Japan. It consists of several to 10 types of bacteria and fungi, and is composed of different microbes depending on the production area. “Tengu-no-Mugimeshi” used in this study was collected from Mt. Kurohime, northern part of Nagano Prefecture, in 1939 was sealed in glass bottles and preserved at Terao Elementary School, Nagano City. We report here a fungus isolated from this “Tengu-no-Mugimeshi” specimen.

**Methods:** Small portions of the sample were suspended with the Winogradsky solution and diluted to 10 and 100 times. Of each diluted suspension were spread on three isolation media (PDA, MEA, CYA) and incubated at 25°C for 7 days. Its colony characteristics and micro-morphology were observed under the same conditions in three media (PDA, MEA, PCA).

**Results and conclusions:** One verticillium-like fungus was isolated. Colonies on three media were 26-36 mm diam, very raised, white, reverse gray red. It produced diffusing reddish purple pigment only on PDA. Conidiogenous cells produced singly in pairs verticillate or in dense irregular clusters on prostrate hyphae, at first flask-shaped, tapering into thread-like neck, 3.1-9.2*0.7-2.3 um. Conidia solitary, oval to subglobose, 2.4-3.9*1.5-2.3 um.

As a result of multi-gene (SSU, LSU and TEF) phylogenetic analyses, the isolate formed the same cluster as *Lecanicillium aphanocladii* and was supported with a high bootstrap value.

From these micro-morphological characteristics and the multigene phylogenies, it was identified as *Lecanicillium aphanocladii*. 
**Ascomycetes in Yunnan, China**

Rungtiwa Phookamsak\textsuperscript{1,2,3,4}, Kevin D. Hyde\textsuperscript{1,2)}, Jianchu Xu\textsuperscript{1,3,4)}

\textsuperscript{1}Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, China

\textsuperscript{2}Center of Excellence in Fungal Research, Mae Fah Luang University, Thailand

\textsuperscript{3}East and Central Asia Regional Office, World Agroforestry Centre (ICRAF), China

\textsuperscript{4}Centre for Mountain Futures (CMF), Kunming Institute of Botany, China

**Purpose:** Yunnan is one of the world’s richest biodiversity hotspots, maintaining an extremely high diversity of microfungi. However, the studies of microfungi in this province are still poorly documented. In this study, we attempt to improve an accurate species number of microfungi in Yunnan Province, China based on morphological characteristics and multigene phylogenetic analyses.

**Methods:** Saprobic and pathogenic ascomycetes were collected from various plants (e.g., Acer, Artemisia, Caragana, Cirsium, Cycas, Liriope, Lonicera, Mangifera, Pinus, Thysanolaena, Zea as well as many fern species) in both aquatic and terrestrial habitats. The samples were collected from Baoshan, Honghe, Kunming, Lijiang and Xishuangbanna during April to December 2015-2017 and returned to the laboratory for examination. Multigene phylogenetic analyses of a concatenated LSU, SSU, TEF1-α, RPB2 and ITS sequence dataset were carried out based on maximum likelihood and Bayesian inference criteria.

**Results and conclusions:** Based on multigene phylogenetic analyses, 38 taxa distributed in ten orders and 18 families were described and illustrated in “Fungal diversity notes 929-1035: taxonomic and phylogenetic contributions on genera and species of fungi”. Six new genera and 25 new species are introduced in this study. A reference specimen for *Tamsiniella labiosa* is designated and the sexual-asexual morph connection of *Plenodomus sinensis* is reported. In addition, new host records and distributions in Yunnan are reported for *Amarenomyces dactyloids, Muyocopron lithocarpi, Periconia cortaderiae, Phragmocamarosporium hederae and Sphaerelopsis paraphysata*. 
Purpose: The family Lachnaceae (Helotiales, Ascomycota) is characterized by minute, stipitate apothecia covered by partially to totally granulate hairs. Lachnaceae contains 17 genera and its generic taxonomy has been mainly based on the morphology of hairs and ectal excipulum. In Lachnaceae, taxonomic confusion of three genera (Albotricha, Capitotricha and Dasyscyphella) has long been left. In this study, we aimed to clarify the generic concepts of these three genera.

Methods: Species of above three genera were collected mainly from Japan. Generic delimitation was studied considering detailed morphological observation and molecular phylogenetic analysis on three nuclear DNA regions (ITS-5.8S, LSU and RPB2) using ML and Bayesian methods.

Results: Two strongly supported clade appeared in molecular phylogenetic analysis: Clade A composed of Albotricha including type species and Clade B composed of Albotricha and Capitotricha including type species. Species in Clade A shared hairs lacking granules at three or more apical cells and ectal excipulums composed of textra angularis, while species in Clade B shared hairs lacking granules at one or two apical cells and ectal excipulums composed of textra prismatica. Species of Dasyscyphella were separated from the both clade in molecular phylogenetic analysis and no clades supporting more than two species appeared.

Conclusions: Clade A and Clade B was redefined as Albotricha and Capitotricha respectively and no taxonomic treatment of Dasyscyphella were conducted in this study.
Endophytic fungal flora in the living leaves of *Fraxinus excelsior* healthily growing in Hokkaido, Japan

Rieko Ichimura¹, Izumi Okane², Yutaka Tamai³, Yasuhiro Ishiga², Yuichi Yamaoka²

¹Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan
²Faculty of Life and Environmental Sciences, University of Tsukuba, Japan
³Research Faculty of Agriculture, Hokkaido University, Japan

**Purpose:** Ash dieback caused by *Hymenoscyphus fraxineus* (Helotiales) is threatening *Fraxinus* species in Europe. Kato (2018) showed that *H. fraxineus* endophytically inhabits several ashes growing in Hokkaido Pref., Japan by detection of fungal DNA. Among them, *F. excelsior* (European ash) that is known as a susceptible species, but healthily growing in the Sapporo campus of Hokkaido Univ. was included. Other fungi coexisting in the same tissues may affect to *H. fraxineus* and function as one of the biological factors to control the disease. In this study, assemblages of endophytes in healthy leaves of the *F. excelsior* were investigated toward exploration of biological agents for ash dieback control.

**Methods:** Assemblages of endophytes in leaflets and rachises of the healthily growing *F. excelsior* were explored by a culture-based method and a metagenome analysis. *Fraxinus pennsylvanica* (Green ash) and *F. mandshurica* (Manchurian ash) in the site were examined by a culture-based method as well.

**Results and Conclusion:** 22 species of 15 genera in 14 families were isolated from the surface-sterilized leaves of *F. excelsior*. *Aureobasidium pullulans*, *Diaporthe* spp. and *Colletotrichum* spp. were isolated in high frequency. *Talaromyces marneffei* was isolated only from *F. excelsior*. In the metagenome analysis, 186 species were recognized in the leaves that were processed by ultrasonic-washing with dioctyl sodium sulfosuccinate (Aerosol OT). The most frequently detected species was *A. pullulans*, followed by *Papiliotrema flavescens*. Such fungi detected in high frequency may dominate the endophytic flora. Further studies are required to explore the antagonisms of those fungi against *H. fraxineus*. 
Behavior of *Hymenoscyphus fraxineus* in the leaves of four foreign ash species (*Fraxinus* spp.) in Hokkaido, Japan

Saori Kato¹, Izumi Okane², Yutaka Tama³, Takehiro Yamaguchi⁴, Yuichi Yamaoka², Yasuhiro Ishiga²

¹Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan
²Faculty of Life and Environmental Sciences, University of Tsukuba, Japan
³Research Faculty of Agriculture, Hokkaido University, Japan
⁴Hokkaido Research Center, Forestry and Forest Products Research Institute, Japan

**Purpose:** *Hymenoscyphus fraxineus* causes a lethal disease known as “ash dieback” in European ash *Fraxinus excelsior*, in Europe. However, this fungus endophytically inhabits the living leaves of Manchurian ash, *F. mandshurica*, in Japan. *Hymenoscyphus fraxineus* can infect ashes including East Asian and Euramerican species. While *F. excelsior* is susceptible in general, this species are healthily growing in the Sapporo campus of Hokkaido University. *Fraxinus pennsylvanica* and *F. americana* are economically important in North America, concerned attacks by *H. fraxineus* possibly introduced. In this study, we compared the behavior of *H. fraxineus* in the leaves of foreign ash species.

**Methods:** Four ash trees, *F. excelsior*, *F. pennsylvanica*, *F. americana* (Euramerican species) and *F. chinensis* subsp. *rhynchophylla* (East Asian species), growing in Sapporo were investigated. *Fraxinus pennsylvanica* and *F. americana* are weakening in the sites. Symptomless leaves of the trees were continuously collected. Whole DNA were extracted from the leaf samples, and then, the relative amount of fungal DNA of *H. fraxineus* to plant DNA was calculated by using real-time quantitative polymerase chain reaction.

**Results and conclusions:** The fungal DNA was apparently lower in *F. excelsior* healthily growing in the site. In *F. pennsylvanica*, the fungal DNA began to increase at the onset of the defoliation period. That in the leaflets of *F. americana* reached the peak even in late August. In *F. chinensis* subsp. *rhynchophylla*, the fungal DNA was slightly detected and retained low. These results suggested that change of relative amount of fungal DNA in the leaves was different between four ashes.
Population structure and genetic recombination of *Racodium therryanum* revealed by new microsatellite markers

Ayuka Iwakiri, Norihisa Matsushita, Kenji Fukuda
Graduate School of Agricultural and Life Sciences, University of Tokyo, Japan

**Purpose:** *Racodium therryanum* is a fungus that causes snow blight to some coniferous species in nursery and plantation in northern area of Japan such as Hokkaido. Since its sexual stage has not been found in Japan, little is known its life cycle. The purpose of this study is to elucidate the population structure of *R. therryanum* in nursery and plantation area and to examine whether genetic recombination events take place in its life cycle.

**Methods:** From the nursery and two plantation areas 10~20 km apart from each other, a total of 133 isolates of *R. therryanum* were collected after the snow melted.

**Results:** They were classified into 70 multilocus genotypes by using seven newly developed microsatellite markers. Two genotypes dominated in the nursery, while many unique genotypes were observed in plantations, indicating that genotypic diversity was lower in nursery. Pairwise $F_{ST}$ showed the significant genetic differentiation between the nursery and two plantations (0.0405, 0.0629, respectively, $P<0.05$) though it was not observed between the plantations. This result indicates that the nursery is isolated from plantations, while gene flow occurs among plantation areas. Also, multilocus analysis revealed three lineages which were distributed sympatrically across the nursery and plantations. Linkage disequilibrium analysis indicated the random mating of *R. therryanum* within each lineage.

**Conclusions:** These results suggest the complex population structure and hidden recombination events of *R. therryanum*, even though no sexual stage of this fungus has been observed.
Diversity of Microfungi on Para rubber (*Hevea brasiliensis*)

Chanokned Senwanna¹, Ratchadawan Cheewangkoon¹, Kevin Devid Hyde²,³,⁴

¹Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Thailand
²Centre of Excellence in Fungal Research, Mae Fah Luang University, Thailand
³World Agroforestry Centre, East and Central Asia, Heilongtan, People's Republic of China
⁴Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, People's Republic of China

**Purpose:** Para rubber substrates have been reported to be a rich source and favorable host for fungi including saprobes, pathogens and/or endophytes. Fungi on Para rubber have been described and classified based on morphological characteristics; however, the understanding of fungal diversity and specificity in Thailand is poorly known, with limited information about taxonomic placement of fungi. Therefore, it is important to examine many taxa to resolve and clarify their systematic positions. The aim of this investigation is to study the micro fungal diversity on Para rubber collected from different areas of Thailand.

**Methods:** Samples of Para rubber were collected during October 2016 - September 2018. Which includes branches, twigs, petioles, and leaves. Additional molecular data were acquired for more accurate identification of fungi. Phylogenetic analyses of multigene sequence data coupled with morphology were further used to clarify their systematic positions.

**Results and conclusions:** From 174 isolates of fungi, one new genera, two new combination, and seven new species were described. The discovery of seven novel species indicated that many micro fungi on Para rubber are waiting to be discovered.
Discovery and description of the sexual morph of *Lecanicillium fungicola*

Toshiyuki Tokiwa¹), Dai Hirose²), Kenichi Nonaka¹)

¹) Kitasato Institute for Life Sciences, Kitasato University, Japan
²) School of Pharmacy, Nihon University, Japan

**Purpose:** *Lecanicillium fungicola* (Hypocreales, Cordycipitaceae) is a mycoparasite of Agaricaceae. This species has not had the sexual morph found. We found it for the first time in Japan and report the description of it.

**Methods:** Specimen on *Pleurotus* sp. sporocarp was collected at Mikuni pass, Minamitsuru, Yamanashi, Japan. For observations of the asexual morph, a culture from ascospores of the specimen was incubated on PDA and PCA. Thirty or fifty measurements for each morphologically characteristic [av. 12.24±1.27*4.4±0.29 um, n = 50]. Characteristics in culture colonies on structure were made.

**Results:** The characteristics of morphology is the following. Subiculum is light brown~brown and KOH(-). Ascospores are hyaline, long ellipsoidal to naviculate, 0-1 septa and (9.8-)11.8-13.8*(3.9-)4.3-4.9 um PDA are growing to reach 33-39mm in diam. after 7 days at 25°C, floccose and wax white to yellowish white. Conidiophores are verticillium like. Conidia are long ellipsoidal to cylindrical and (3.9-)4.5-5.9(-9.8)*1.9-2.3(-2.9) um [av. 5.46±1.15*2.26±0.19 um, n = 50]. Chlamydospor is absent.

**Conclusions:** Morphological characteristics of the sexual morph of *L. fungicola* was similar to those of genus Hypomyces which is phylogenetically distinct from *Lecanicillium*. 
**Isolation fungi from carnivorous plants and bioactive evaluation of the secondary metabolites**

Naoya Matsuo\(^1\), Kazunari Sakai\(^2\), Toshiyuki Tokiwa\(^2\), Mayuka Higo\(^2\), Kenichi Nonaka\(^{1,2}\)

\(^1\)Graduate School of Infection Control Sciences, Kitasato University, Japan
\(^2\)Kitasato Institute for Life Sciences, Kitasato University, Japan

**Purpose:** Filamentous fungi produce a wide variety of secondary metabolites, and many bioactive compounds are being sought for drug discovery. However, fungi used to search for compounds are only a small fraction of the total fungi, most of which are soil isolated fungi. Therefore, we focused on carnivorous plants as a new source of fungi. Sarracenia is known to use the enzymes of symbiotic microorganisms to digest insects and use nutrient sources. The unique characteristics of Sarracenia may lead to find unknown fungi and novel bioactive compounds. We report here the result of isolation of fungi and bioactivity tests.

**Methods:** Fungi were isolated from the digestive fluids and pitcher of Sarracenia. These samples were ground, diluted and spread on isolation media. The obtained strains were identified by morphological observation and the DNA analysis using Internal Transcribed Spacer. Isolates were evaluated by antimicrobial and insecticidal activity tests.

**Results and conclusions:** As a result, a total of 40 strains including 16 strains from digestive fluids and 24 strains from pitchers were isolated. As a result of the antibacterial activity test and insecticidal activity test, these isolates from pitcher showed high antibacterial activity against plant pathogenic bacteria *Xanthomonas campestris*. On the other hand, in the insecticidal activity test, the isolates from digestive fluids showed high insecticidal activity. These results suggest that isolates from pitchers are involved in host defense and that from digestive fluids are involved in the digestion of prey.
Good practices in studying fungi: fungaria, isolation, culture and sporulation protocols

Indunil Chinthani Senanayake\textsuperscript{1)}, Kevin David Hyde\textsuperscript{2)}, Rajesh Jeewon\textsuperscript{3)}, Eric HC McKenzie\textsuperscript{4)}, Putarak Chomnunti\textsuperscript{2)}, Ning Xie\textsuperscript{1)}

\textsuperscript{1)}Shenzhen Key Laboratory of Microbial Genetic Engineering, College of Life Science and Oceanography, Shenzhen University, China
\textsuperscript{2)}Center of Excellence in Fungal Research, Mae Fah Luang University, Thailand
\textsuperscript{3)}Department of Health Sciences, Faculty of Science, University of Mauritius, Mauritius
\textsuperscript{4)}Manaaki Whenua Landcare Research, New Zealand

The modern mycology has moved forward with the combination of chemotaxonomy, phylogeny, genetics and molecular biology. The utilization of DNA sequencing for the phylogenetic analysis explains the relationships between fungi and its diversity clearly. However fungal morphological characterization and morphology based taxonomy is fundamental and still essential even though molecular techniques are developed. However there are some conventional methods, as well as newer methods to collect, isolate, describe and preservation of fungi. In this study, literature was reviewed under five major topics as fungal specimen collection and processing, morphological examination and characterization, isolation techniques, sporulation techniques and preparing fungaria specimens. Several new techniques have been tried for fungal isolation to reduce cost, isolation time and contaminations. Two new isolating techniques have been introduced here as film-culture method and broth-culture method. Technical guidelines for morphological examination and isolation of Ascomycota are provided. Additionally we discuss the customary methods and their drawbacks. This study will be a guideline for future mycologists.

Keywords: Fungal ex-situ preservation, Morphological examination, Mycotaxonomy, isolation techniques, Sporulation
A new *Aureobasidium* species isolated from litter samples in Kupang, Indonesia

Kristi Ningrum\(^1\), Dyah Noor Hidayati\(^1\), Diana Dewi\(^1\), Kenichi Nonaka\(^2\), Erwahyuni Endang Prabandari\(^1\), Toshiyuki Tokiwa\(^2\), Mihoko Morii\(^2\), Danang Waluyo\(^1\), Agung Eru Wibowo\(^1\), Kazuro Shiomi\(^2\), Tomoyoshi Nozak\(^3\)

\(^1\)Laboratory for Biotechnology, BPPT, Indonesia  
\(^2\)Kitasato Institute for Life Sciences, Kitasato University, Japan  
\(^3\)University of Tokyo, Japan

**Purpose:** Fungal strain BioMCC.f.PL.142 was isolated from leaf litter sample obtained from Kupang, Indonesia. This strain has antimalarial activity based on enzymatic and cell based assay against *Plasmodium falciparum*, therefore characterization of this strain is very important to do.

**Methods:** BioMCC.f.PL.142 was isolated using moist chamber method. The mycelia on the leaf litter were observed under microscope, transferred into agar medium and incubated at 25\(^\circ\) C for 7 days. The strain was preserved as freezing glycerol stock.

**Results and conclusions:** The result of molecular identification using LSU (d1/d2 region) showed 96% similarity with *Selenophoma australiensis* and *Aureobasidium thailandense*. The phylogenetic analysis was inferred using Neighbor-Joining method (NJ) and the result showed the strain BioMCC.f.PL.142 was closely relative to *S.australiensis*. Morphological and physiological observation revealed a similar colony shape of BioMCC.f.PL.142 with *Aureobasidium sp.*, which resembled one of yeast. Meanwhile, the conidia from this strain were different from those of *S.australiensis* and *A.thailandense*. The conidia of BioMCC.f.PL.142 were crescent shaped, hyaline, conidial stage allantoides and fulcatus-like. Based on those features, we would like to propose the strain of BioMCC.f.PL.142 as a new species of the genus *Aureobasidium*. 
Isolation of fungi from the insect's gut and bioactive evaluation of the secondary metabolites

Naozumi Kondo1), Toshiyuki Tokiwa2), Kazunari Sakai2), Mayuka Higo2), Kenichi Nonaka1,2)
1)Graduate school of Infection Control Sciences, Kitasato University, Japan
2)Kitasato Institute for Life Sciences, Kitasato University, Japan

Purpose: Insecta is huge taxa (approximately 1 million species), and most have a symbiotic relationship with the microbe. Among them, some insects make use of filamentous fungi to produce antimicrobial compounds. So in this study, symbiotic fungi were focused on for the discovery of novel useful bioactive compounds. We report here fungi isolated from the insect's gut and purified a bioactive compound from secondary metabolites.

Methods: Insects were collected from several locations in Japan. As gut samples, feces and intestinal tracts were mashed and diluted to 10^2-10^3 times. The diluted suspension was spread on potato dextrose agar (PDA) with 100 mg/L kanamycin and chloramphenicol and then incubated at 25°C, for 5d. Isolates were identified by morphological observation and DNA sequencing analysis. And isolates were evaluated by antimicrobial and insecticidal tests.

Results and Conclusion: The DNA analysis showed that insect parasites (Clavicipitaceae s.l.) were isolated from insects with leg defects and other defects. And plant-related isolates such as plant pathogens were isolated from plant-eating insects. These results suggest the species isolated by insects vary depending on their health and food habits.

The secondary metabolite of Gliocladiopsis sp. FKI-9511, isolated from Plesiophthalmus nigrocyaneus, had no insecticidal activity against aphid and exhibited broad antibacterial activity. This was purified and the bioactive compound isolated.
Taxonomy and phylogeny of *Muyocopron thailandica* sp. nov.

Sinang Hongsanan¹, Naruemon Huanraluek², Kasun Thambugala³, Kevin Hyde², Ning Xie¹

¹Shenzhen Key Laboratory of Microbial Genetic Engineering, College of Life Sciences and Oceanography, Shenzhen University, China
²Center of Excellence in Fungal Research, Mae Fah Luang University, Thailand
³Industrial Science and Management (International Program), Faculty of Science and Technology, Thammasat University (Rangsit Center), Thailand

*Muyocopron* species are mostly found on dried branches, twigs and leaves of various plants. They are characterised by dark brown to black, flattened ascomata, with a central ostiole, appearing as small black dots or thick stromata, forming superficially on the substrate, without mycelium. Asci are bitunicate, 8-spored and contain ellipsoidal, hyaline ascospores. *Muyocopron thailandica* sp. nov. was collected in northern Thailand, and sequenced directly from the fruiting body. Molecular analyses based on LSU and ITS sequence data indicate that the new species forms a distinct lineage within the *Muyocopron* clade, and is related to *M. lithocarpi*. The new species differs from *M. lithocarpi* by forming on dried twigs, and having ellipsoid to subcylindrical asci, with a very short pedicel. Asci and ascospores of *M. thailandica* are larger than those of *M. lithocarpi*.
New and rapid method for producing stable appressoria of the genus *Colletotrichum*

Yoshiki Takata¹, Shunsuke Nozawa¹, Takuto Hatae¹, Kyoko Watanabe¹,²

¹Graduate School of Agriculture Tamagawa University, Japan
²Tamagawa University Research Institute, Japan

**Purpose:** Morphological features of appressoria is one of the most important criteria for species level classification of the genus *Colletotrichum*. Currently, the common method to study appressoria in *Colletotrichum* is to grow the fungus on SNA media and observe appressoria through the underside of the petri dish. However, this method does not provide stability in appressorial shape, and it could take more than a month for some appressoria to form, and sometimes no appressoria are found. To create a stable and fast method to obtain appressoria.

**Methods:** Six different extraction methods from tomato peels were evaluated by the following procedures: 5 µl of the extraction fluid was placed on a sterilized slide glass and dried, and then 20 µl of a conidial suspension (5 x 10⁴ conidia/ml) were placed on this slide and incubated for 12 to 16 hours at 25 C. For comparison of the previous method and our new methods, appressoria of five strains belonging to different species complexes (*C. higginsianum, C. gloeosporioides, C. orbiculare, C. spaethianum, and C. truncatum*) were evaluated.

**Results:** The results showed that extraction fluid by 80% ethanol (50 ml) from 2 g tomato peels overnight was the most effective for optimal appressoria number and size. The standard deviations (n = 30) of the length and width of appressoria using the new method (0.6 to 1.2 and 0.4 to 0.7, respectively), were significantly less than on SNA media (2.2 to 3.1 and 0.8 to 1.4).
Baseline data for generalist and specialist fungi associated with ants, *Rhododendron* species and *Dracaena* species

Kevin David Hyde  
Center of Excellence in Fungal Research, Mae Fah Luang University, Thailand

**Purpose:** Thailand being a tropical country has significant undiscovered fungal diversity. In the case of microfungi, the numbers are likely to be higher. This is because there are numerous cryptic species as well as undiscovered genera. In this study, we provide baseline data for the fungi on insects, Dracaena and Rhododendron in order to establish the effects of climate change on their fungal communities.

**Methods:** Collections from each of the hosts were made throughout Thailand and Yunnan Province, China

**Results:** To date, we have found 48 fungi on insects including nine species on ants in the genera Aschersonia, Beauveria, Chlorocillium, Cordyceps, Gibellula, Hymenostilbe, Hypocrella, Isaria, Lecanicillium, Ophiocordyceps, Orbiocrella, Paecilomyces, Polycephalomyces, Simplicillium, Sporothrix and Torrubiella. We also found 35 species on Dracaena in the genera Cladosporium, Colletotrichum, Hermatomyces, Ochroconis, Pestalotiopsis, Sarcopodium and Zygosporium. We also collected 25 taxa from Rhododendron including Colletotrichum, Diaporthe, Neopestalotiopsis and Seimatosporium species. In this presentation, we illustrate the morphology of some of the fungi.

**Conclusions:** The ultimate goal of this study is to show the effects of climate change on fungal communities and establish protocols to solve any resulting potential problems.
Purpose: Species of T. benhamiae complex have a worldwide distribution and their host range includes many pet and livestock animals. Nowadays, this complex have become very important due to the epidemic spread of T. benhamiae complex species in pets and consequently also in children and pet owners. No causal mechanism has been found that would explain this increase. A considerable genetic and phenotypic variability has been revealed in these emerging pathogens but the species limits are not always clearly defined.

Methods: A total of 352 clinical isolates from T. benhamiae complex associated with human and animal dermatophytoses were analysed using molecular markers (DNA sequence data and microsatellites), and morphological and physiological methods.

Results and conclusions: Species boundaries in the T. benhamiae clade were resolved by using polyphasic approach. This approach supported recognition of nine species, including three new zoophilic species. Trichophyton benhamiae was split into two varieties; T. benhamiae var. luteum is currently responsible for the European outbreak of zoonotic infections.
Purpose: The present work was done to investigate, characterize and identify powdery mildew fungus that is infecting *Clematis* plants in different regions of Khyber Pakhtunkhwa province, Pakistan. *Clematis* (Ranunculaceae) is an economically important plant as being source of different types of pharmaceutical compounds. In Pakistan, there are 10-11 species of this genus that are mostly present in the temperate areas of the country and are being reported to be infected by powdery mildew disease.

Methods: The plants of *Clematis montana* growing in Ayubia National Park, Abbottabad district, KP, Pakistan have been collected to be infected with powdery mildew fungus. Molecular and morpho-anatomical techniques were used to characterize and identify this powdery mildew fungus.

Results and Conclusions: After careful observations, the fungus is identified as *Erysiphe aquilegiae* that belongs to the largest genus of Erysiphales including about 50% of all species of family Erysiphaceae. This genus is represented by 378 species worldwide with 19 species reported from Pakistan. *Erysiphe aquilegiae* is new record for Pakistan and is an addition to our fungal flora. This work will not only be a baseline for further such studies in selected site but will also help in selection of means to protect this important plant (*Clematis montana*) as this disease can compromise its economic and esthetic value by reducing the plant's floral output and stunting its growth.
Biodiversity of Powdery Mildew Fungi in Cultivated Area of Phetchaburi and Prachuap Khiri Khan Province of Thailand

Sararat Monkhung¹, Panida Duangkaew²

¹Crop Production Technology Program, Faculty of Animal Science and Agricultural Technology, Silpakorn University, Thailand
²Bioscience for Sustainable Agriculture Program, Faculty of Animal Science and Agricultural Technology, Silpakorn University, Thailand

Collection of powdery mildew samples in Phetchaburi and Prachuap Khiri Khan Provinces were conducted. In this study, 129 samples of powdery mildews on 26 plant species were described and identified based on host plants and morphological characteristics. The symptoms of plants infected by these fungi showed mycelia with a whitish, dusty appearance. For the morphological studies, the powdery mildew fungi were classified into 3 genera including, Oidium, Ovulariopsis and Oidiopsis. The 22 plant species classified from 99 samples were infected by Oidium that found on Ocimum sanctum, Scoparia dulcis, Euphorbia hirta, Abutilon persicum, Cardiospermum halicacabum, Heliotropium indicum, Coccinia grandis, Coriandrum sativum, Ipomoea obscura, Cleome rutidosperma, Tamarindus indica, Carica papaya, Mangifera indica, Sesbania grandiflora, Macroptilium lathyroides, Ocimum gratissimum, Zinnia violacea, Vernonia cinerea, Aeschynomene americana, Eupatorium odoratum, Phyllanthus amarus, Vitis vinifera. Three plant species classified from 21 samples were infected by Ovulariopsis that found on Cassia fistula, Euphorbia heterophylla, and Morus alba. In addition, Oidiopsis was found in one plant species classified from 9 samples that found on Capsicum frutescens. Furthermore, this study revealed that the powdery mildews in asexual state were mostly found in this study. However, the powdery mildew infected on Cassia fistula was found both of sexual state and asexual state.
**Purpose:** The high concentration of zinc (Zn) can be toxic to plants. *Miscanthus sinensis* has Zn tolerance by producing chlorogenic and succinic acids to detoxify Zn. Some root fungal endophytes produced compounds to chelate with Zn. The purpose of this study is to elucidate the Zn tolerance mechanism of *M. sinensis*, considering the effects of root fungal endophytes.

**Methods:** The concentrations of heavy metals in soil and each plant tissues were measured by ICP-OES. To clarify chemical Zn tolerance in *M. sinensis*, organic acids and phenolic acids exacted from *M. sinensis* roots were analyzed by GC/MS and HPLC/MS, respectively. To evaluate the detoxification ability of root endophytes via production of Zn-chelating compounds, which would be detoxicants to Zn, media including insoluble Zn were used for the inoculation.

**Results:** The concentrations of Zn in each plant tissues were higher than those detected generally in plants. Succinic acid and chlorogenic acid were detected as Zn-chelating compounds in the roots. Especially, the concentration of chlorogenic acid was high (up to 1.05 mg/g FW). Root fungal endophytes *Penicillium*-like fungi showed the highest activity of producing Zn-chelating compounds will be selected for soil-inoculation test using *M. sinensis* to clarify Zn tolerance in *M. sinensis* via measuring concentration of Zn by ICP-OES.

**Conclusions:** *Miscanthus sinensis* could detoxify high concentration of Zn by producing succinic and chlorogenic acids. Root fungal endophytes *Penicillium*-like fungi, which produced detoxicants via chelation with Zn, could increase Zn tolerance in *M. sinensis*. It suggested that *M. sinensis* could be a potential plant for mine-bioremediation.
Identification of genes involved in early stage of fruiting body development in *Coprinopsis cinerea*

Yuichi Sakamoto\(^1\), Shiho Sato\(^1\), Keishi Osakabe\(^2\)

\(^1\)Iwate Biotechnology Research Center, Japan
\(^2\)Tokushima University, Japan

**Purpose:** Light is one of the crucial environmental factors influencing fungal sexual reproduction. In *Coprinopsis cinerea*, blue light induces simultaneous hyphal knot (earliest stage of fruiting body) formation in mycelia grown on low glucose (0.2%) media within 24h. Genes that are crucial for hyphal knot development after light stimulation were investigated.

**Methods:** Gene expression after 0, 1, 6, 12, 18h after light stimulation was analyzed by RNA-seq. Genes specifically expressed in each time point were mutated by CRISPR/cas9.

**Results and conclusion:** It is revealed that cell adhesion (fas1), fatty acid modification (cfs1, cfs2) and transcription factors were highly expressed at 1h after light stimulation. Disruption of the cfs1 resulted in deficient of hyphal knot induction, suggesting that some of genes expressed in this stage will be involved in induction of hyphal knot. 12 to 18h after light stimulation, the ich1 which is involved in fruiting body development, galectins (cgl1-3), pheromone peptides and farnesyl cysteine-carboxyl methyltransferases (fccm1 and fccm2), hydrophobins were upregulated. Disrupted mutant of the fccm2 by CRISPR/cas9 could form immature fruiting body but could not fully mature. This suggests that some genes expressed in this stage will be involved in fruiting body development from hyphal knot. Therefore, we investigated genes up-regulated 6h after light stimulation. There were fewer genes up-regulated at 6h after light stimulation compare with genes up-regulated at 1h and 12h after light stimulation. We could identify that expression of the hydrophobin genes started to increase at 6h after light stimulation. The hydrophobins would be involved in hyphal aggregation for hyphal knot development.
Decay properties of *Agrocybe cylindrica* isolated from street trident maple (*Acer buergerianum*) trees in Kyoto City

Kazuko Ono, Tsuyoshi Yoshimura, Toshimitsu Hata
Research Institute for Sustainable Humanosphere, Kyoto University, Japan

**Purpose:** There are many trident maples as street tree in Kyoto City. Our recent survey has revealed that some trees are the host of a mushroom *Agrocybe cylindrica*. The decay-fungi infestation of street trees can cause collapse of the trees, and early detection of *A. cylindrica* infestation in street trees and its control might be important to ensure the safety of Kyoto City residents and beauty of the urban landscape. We report the results of laboratory wood decay tests of *A. cylindrica*, isolated from street trident maple trees.

**Methods:** Test wood specimens were hardwood species, *Fagus crenata*, *Populus xuroamericana*, *Acer pictum*, *Kalopanax septemlobus*, *Zelkova serrata*, and *Ulmus davidiana*. Heartwood and sapwood were tested separately. The size of wood specimen was 20 (R) x 20 (T) x 10 (L) mm. The test was carried out in the laboratory for 12 weeks at 26 degrees Celsius in the dark.

**Results:** There were no significant difference in the mass loss rates of decayed specimens for heartwood and sapwood, except for *Z. serrata* and *U. davidiana*. From the results of components analyses of decayed specimens, it was suggested that *A. cylindrica* preferentially degraded cellulose and hemicellulose, even though the fungus was categorized as a white rot. The observation of specimens by SEM also showed that the secondary wall of cell wall of xylem fiber was susceptible for degradation by *A. cylindrica*. 
Chemolithoautotrophic sulfur oxidation in *Fusarium solani f.sp. pisi* NBRC9425 indicates a novel microbial sulfur metabolism

Haibo Xu\textsuperscript{1,2)}, Yoko Katayama\textsuperscript{1)}
\textsuperscript{1)Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Japan}
\textsuperscript{2)Current Affiliation: Graduate School of Agriculture, Kyoto University, Japan}

**Purpose:** Chemolithoautotrophic sulfur oxidation is thought to be an ancient metabolic process carried out exclusively in prokaryotes. Given that *Fusarium solani f.sp. pisi* NBRC9425 grows using $S_0$ as a sole energy source, it is reasonable to doubt the “fact” that eukaryotes are not capable of chemolithoautotrophic sulfur oxidation. Therefore, the aim of this study is to investigate sulfur metabolism in *F. solani f.sp. pisi* NBRC9425.

**Methods:** Chlamydospores from organics-free sulfur-containing medium served as inoculum. Activities of sulfur-oxidizing enzymes were examined after the fungus was cultured in organics-free sulfur-containing medium. The proteome in response to sulfur and maltose was analyzed on two dimensional (2D) electrophoresis gels.

**Results and Conclusions:** After 15 days growth, the fungus oxidized $S_0$ and $S_2O_3^{2-}$ giving 0.19 mM $SO_4^{2-}$. Culture pH decreased from initial 5.0 to 3.9, suggesting that this fungus could oxidize sulfur chemolithoautotrophically. When incubated with various sulfur compounds, culture filtrate did not oxidize sulfur, indicating that sulfur oxidation occurred intracellularly. However, cell-free extract also did not show activity, suggestive of importance of cell integrity in sulfur oxidation. Now that culture filtrate or cell-free extract of prokaryotes oxidizes sulfur, strain NBRC9425 seemed employ a novel sulfur-oxidation strategy. 2D display of soluble proteins of NBRC9425 grown on different energy sources indicated distinct metabolisms.
Genomic perspective on the biology and ecology of *Rhizodermea veluwensis*, which enhanced the heavy metal stress tolerance of *Clethra barbinervis*

Hayato Masuya¹, Keiko Yamaji², Ri-ichiro Manabe³, Moriya Ohkuma⁴, Rikiya Endoh⁴

¹Forestry & Forest Products Research Institute, Japan
²Life and Environmental Science, University Tsukuba, Japan
³Center for Integrative Medical Sciences (IMS), RIKEN, Japan
⁴Japan Collection of Microorganisms (JCM), RIKEN-BRC, Japan

**Purpose:** *Rhizodermea veluwensis* is the monotypic species of the root endophyte belonging to Dermeaceae, Helotiales and recently become known to enhance heavy-metal stress tolerance of *Clethra barbinervis* at mining site in Japan. However, the detailed mechanism of enhancing the tolerance is still of uncertain. For understanding of the biology and ecology of *R. veluwensis*, we obtained its draft genome sequences, analyzed its genes related to the host-fungus interaction, and compared them with those of other root endophytic fungi.

**Methods:** Extracted DNA and RNA of *R. veluwensis* were analyzed by using Illumina Hiseq 2500. Obtained reads were assembled using ALLPATHS-LG. Gene prediction was carried out using MAKER annotation pipeline. Total of 21674 protein-coding genes were predicted using AUGUSTUS. Draft functional annotation was performed using Sma3S, Uniprot-TrEMBL and Uniprot-sprot databases. For genome comparison, genome data of *Botrytis cinerea*, *Oidiodendron maius*, *Hyaloscypha hepaticicola*, and *H. variabilis* were downloaded from JGI. KOG, CAZy database and PHIbase were used for genome comparison.

**Results:** Draft genome sequence of *R. veluwensis* (59,797,709bp, 219 scaffolds) was obtained in this study. 14,299 and 3,481 genes were functionally annotated by Uniprot-TrEMBL and Uniprot-sprot, respectively. Comparative genome analysis for KOG showed that *R. veluwensis* has a greater number of secondary-metabolism-related genes than the other fungi. The comparison of CAZyme genes showed that *R. veluwensis* has a greater number of CAZyme genes than *Botrytis cinerea* and *H. hepaticicola* but less than *O. maius* and *H. variabilis*.

**Conclusion:** *Rhizodermea veluwensis* has a series of secondary-metabolism-related genes, some of which may contribute to adaption of the host on the heavy-metal contaminated environment.
Alternative respiration mechanism produced by soil humic acid in *Aspergillus nidulans*

Tsugumi Miyazaki, Tao Oizumi, Nami Nakazawa, Shunsuke Masuo, Naoki Takaya
Faculty of Life and Environmental Sciences, Microbiology Research Center for Sustainability, University of Tsukuba, Japan

**Purpose:** Filamentous fungi respire by using two terminal oxidase. Cytochrome c oxidase (COX) is a ubiquitous respiratory oxidase in mitochondria, and alternative oxidase (AOX) is considered to be an assistant role for COX. This study investigates the fungal AOX-dependent respiration activity in the presence of humic acid (HA), which is a major organic matter in soil.

**Methods:** The fungus *Aspergillus nidulans* was cultured in the medium containing HA, and its physiological, biochemical, and genetic responses were analyzed.

**Results:** We found that adding HA to liquid culture increased AOX activity in *A. nidulans*. Sterile soil increased the fungal AOX activity, too. We also found that HA increased cellular superoxide dismutase and catalase activity, and decreased reactive oxygen species (ROS) level, which agrees to predicted HA's role in ROS reduction. Growth rate of the fungus was higher in the presence of HA. These results indicated that HA switches respiration mechanism from COX-dependent to AOX-dependent one, and diminishes ROS stress. Gene disruptants of acuK and acuM encoding transcription factors for acetate utilization produced little AOX activity and its transcript in the HA-exposed cells, indicating that they are required for transcription activation by HA. Adding HA partially inhibited a typical autolysis phenomenon observed in the late-stage of the culture, suggesting that HA contributes to maintain the cells in stationary growth phase. Fifteen filamentous fungal species produced more AOX activity in the presence of HA.

**Conclusions:** Filamentous fungi respond and adapt to HA, and probably to soil environment, by regulating their metabolic mechanisms.
Physiology of White Rot Fungus *Trametes polyzona* on Ligninolytic Enzyme Production and Enzymatic Capability in Deactivation of Pharmaceutical Products

Piyangkun Lueangjaroenkit¹, Churapa Teerapatsakul¹, Kazuo Sakka², Makiko Sakka², Tetsuya Kimura², Emi Kunitake², Lerluck Chitradon¹

¹Department of Microbiology, Faculty of Science, Kasetsart University, Thailand
²Graduate school of Bioresources, Mie University, Japan

**Purpose:** Diverse effect on *Trametes polyzona* physiology, focusing ligninolytic enzyme productivity was directed by the fungal morphology as pellet or mycelial clump that occurred under different culture conditions. Ligninolytic enzymes of the fungus promising for deactivation of pharmaceutical products.

**Methods:** Cultivation of a white rot fungi in various submerge condition was done to manage its growth to become mycelial clump or pellet of different sizes. Productivity of ligninolytic enzymes of each fungal form was investigated. Efficiency of the enzymes on pharmaceutical product deactivation was evaluated. The fungus was identified based on morphological and molecular genetic characteristics.

**Results:** The fungus was identified as *Trametes polyzona*. Aeration and fungal morphology were important factor for the strain to produce ligninolytic enzymes, manganese peroxidase and laccase. Different cultivation methods influenced the fungal growth to obtain different morphology as mycelial clump or pellet. Amount of inoculum affected on pellet size, porosity and chlamydospore-like structure formation. Biomass increased with increasing amount of inoculum while pellet diameter decreased, resulted decreasing in porosity. Either clump/pellet form or pellet morphology of which size, porosity and formation of chlamydospore-like structure significantly impacted on *Trametes polyzona* productivity of ligninolytic enzymes. The enzymes effectively deactivated pharmaceutical products in tetracycline, β-lactam, and quinolone classes.

**Conclusion:** *Trametes polyzona* ligninolytic enzymes capable of deactivated pharmaceutical products. Cultivation methods affected *Trametes polyzona* to obtain diverse morphology of either mycelial clump, pellet of different sizes and porosity or formation of chlamydospore-like structure. The fungal morphology and aeration conducted the fungal physiology on ligninolytic enzyme productivity.
Ligninolytic System of A White Rot Fungus *Trametes polyzona* with Novel Enzymes Promising for Bioremediation of Dye

Lerluck Chitradon¹, Piyangkun Lueangjaroenkit¹, Churapa Teerapatsakul¹, Kazuo Sakka², Makiko Sakka², Tetsuya Kimura², Emi Kunitake²

¹Department of Microbiology, Faculty of Science, Kasetsart University, Thailand
²Graduate school of Bioresources, Mie University, Japan

**Purpose:** Ligninolytic system of *Trametes polyzona* was proposed its high efficiency in dye degradation in enzymatic and genetic levels. The fungal enzyme system explained a success in dye bioremediation in mediator-free system.

**Methods:** Purification and characterization of three main ligninolytic enzymes were done. Genes encoding the two manganese peroxidases (MnPs) and a laccase were cloned and identified. Phylogenetic relationships of MnPs and laccase were analyzed by their deduced amino acid sequence similarities.

**Results:** Two MnPs and a laccase of *Trametes polyzona* played important roles as main enzymes in dye degradation. The degradation abilities were driven without mediator. Complete degradation of Remazol Brilliant Blue R (25mg/L) was within 10-30 min by either enzymes. Laccase completely degraded Remazol Navy Blue and removed 75% Remazol Red in 7 days. Remazol Navy Blue and Remazol Brilliant Yellow were more than 70% removed by MnPs. Cloning of mnp1 and mnp2 revealed distinct deduced amino acid sequence with classification as new members of short-type hybrid manganese peroxidase in subfamily A2, Class II fungal secretion heme peroxidase. The new MnPs had novel properties in stability against organic solvents and metal ions, which triggered their activities at certain concentrations.

**Conclusion:** Ligninolytic system of *Trametes polyzona* was proved to be important for dye degradation and had high efficiency under the mediator-free system. The system consisted of three main enzymes, two MnPs and one laccase that showed high abilities and individually interesting properties. Herewith, a new insight into two new MnPs with novel properties of high stability against organic solvents and metal ions was found and offered an advantage of using *Trametes polyzona* in environment contaminated with such reagents.
Ribosomal DNA phylogenies reveal that *Isthmolongispora* is polyphyletic and proposal new species of this genus

Le Thi Hoang Yen¹, Yasuhisa Tsurumi², Kaoru Yamaguchi², Duong Van Hop¹, Katsuhiko Ando²

¹Institute of Microbiology and Biotechnology, Vietnam National University, Vietnam
²Biological Resource Center, National Institute of Technology and Evaluation, Japan

Species of *Isthmolongispora* was described by Matsushima 1971, which have sympodially proliferating conidiophore and conidia with isthmus connection, *Isthmolongispora minima* was type species. There were 12 species have been described, *I. intermedia* have three to four cells conidia. *I. minima, I. basitruncata, I. ampulisformis, I. rotunda* and *I. geniculata* Nawawi 1988 have two cells conidia. *Isthmolongispora quadricellularia* was 5-6 cells conidia. *Isthmolongispora valiabilis* was 4-13 cells. *Isthmolongispora quadricellularia* was 5-6 cells conidia. *Isthmolongispora lanceata* was 14-22 cells conidia. Among 12 known *Isthmolongispora* species, conidia cells of *I. asymmetrica* were unknown and *I. briamifera* have two arms. During the investigation of Viet Nam microbe, eleven *Isthmolongispora*-like isolated were isolated from fallen leaves. Morphological identification they were belonged to: *I. valiabilis* (3 strains), *I. ampulisformis* (5 strains), *I. geniculata* (one strain), *I. rotunda* (one strain) and a novel species: *Isthmolongispora flexousa* (3-4 cells, bending conidia). When determine the phylogeny position of these fungi base on 18S and 28S rDNA sequence analysis, the results showed that these *I. minima*- like were placed in 3 difference class: *I. minima* group (included *I. valiabilis* and *I. minima* P037) was sit in Leotiomycetes; the new species *I. flexousa* and *I. rotunda* were sited in Sodariomycetes and *I. ampulisformis* group (included *I. ampulisformis* and *I. geniculata*) was sit in separated single clad which should be a new class of the Acomycocetes.

Keywords: Aquatic habitats, Freshwater fungi, Biogeography, Phylogeny, taxonomy.
Wild Mushrooms Diversity and Indigenous knowledge from North West Himalaya of Jammu and Kashmir State, India

Sanjeev Kumar, Yash Pal Sharma
University of Jammu, J&K, India

With the realization of implications of wild mushrooms, a large number of researchers engaged themselves in the survey, distribution and myco-ecological aspects of this natural resource wealth belonging to this group from different parts of the world.

Ethnomycological Studies on the Bugkalot Indigenous Community in Alfonso Castaneda, Nueva Vizcaya, Philippines

Mark Louie S. Torres\textsuperscript{1)}, Delia C. Ontengco\textsuperscript{1)}, Edwin R. Tadiosa\textsuperscript{2)}, Renato G. Reyes\textsuperscript{3)}

\textsuperscript{1)}The Graduate School, University of Santo Tomas, Philippines
\textsuperscript{2)}Philippine National Herbarium, Botany Division, National Museum of the Philippines, Philippines
\textsuperscript{3)}Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Philippines

Purpose: Bugkalots, a well-known ethnic group in the Northern Luzon, Philippines are believed to use various species of mushrooms as part of their daily lives. However, there are no available in-depth studies on the knowledge and culture of the Bugkalots when it comes to utilizing mushrooms. Hence, this study was conducted to provide an initial data on the ethnomycological knowledge of the Bugkalots.

Methods: In order to document the knowledge, belief, practices and utilization of macrofungi by the Bugkalots in Alfonso Castaneda, Nueva Vizcaya, a survey and interview approach were used. The collected specimens were preserved and identified based on their morphological features with comparison to relevant literatures.

Results: A total of 38 species of macrofungi has been reported by the Bugkalots. However, only 30 species were collected and identified during the sampling period. Out of these macrofungi, only 10 species were used as food (\textit{Auricularia auricula-judae}, \textit{Coprinellus sp.}, 2 species of \textit{Lentinus tigrinus}, \textit{Lentinus sp. 1}, \textit{Lentinus sp. 2}, \textit{Pleurotus dryinus}, \textit{Polyporus sp. 1}, \textit{Polyporus sp. 2} and \textit{Schizophyllum commune}) and 5 species were used as medicine (\textit{Ganoderma applanatum}, 2 species of \textit{Ganoderma lucidum}, \textit{Polyporus picipes} and \textit{Polyporus sp. 4}). Their specific use, mode of preparation and administration is documented in this paper. This is the first ethnomycological study conducted on the Bugkalot community in the Philippines.

Conclusion: A total of 38 local species of macrofungi has been reported by the Bugkalots wherein 30 species of which were collected and identified morphologically. Of these macrofungi, only 15 species were either utilized as food or medicine. Bugkalots possessed a great knowledge on many different macrofungal species as these become part of their daily lives. However, these mushrooms including the inedible ones must be given attention in future studies for possible utilization by the tribal community.
Kuehneola species which infected Rosa plants are revised to Phragmidium species, and a new rust pathogen of Rosa

Yun Liu, Yingmei Liang, Makoto Kakishima
Beijing forestry University, China

*Kuehneola* (Phragmidiaceae, Pucciniales, Basidiomycota) currently includes two species which parasitism on *Rosa* plants (Rosaceae). The genus has been distinguished from *Phragmidium* by white and delicate-walled teliospores. Whether these morphological features are reliable as the basis for distinguishing the two genera is unknown. The primary aim of the present study was to clarify species of *Kuehneola* which infected *Rosa* plants status and the characters of *Phragmidium* genus. Based on biogeography, morphological characteristics and 28S and ITS sequence data of type specimens and fresh specimens, *K. japonica* and *K. warburgiana* are recombined as *P. japonicum* and *P. warburgiana* respectively. Analyses also indicate that *P. japonicum* is not microcyclic as previously recognized but macrocyclic. Only the classification of the *Kuehneola* on the rose has been revisited here, further examination of species boundaries and host ranges of the fungi formerly classified in *Kuehneola* is warranted. Besides, a new species found on *Rosa* with Type 6 spermogonia, a type previously known on *Rosa* only in *P. warburgiana* was recognized as *Phragmidium leucoaecium* in the study by phylogenetics.
15 shades of grey
- a multi-gene phylogeny and taxonomic review of *Pseudotomentella tristis*, integrating ecological and geographical data

Sten Svantesson¹,²,³), Karl-Henrik Larsson⁴), Urmas Koljalg⁵), Tom W. May³), Patrik Cangren¹,²), R. Henrik Nilsson¹,²), Ellen Larsson¹,²)

¹University of Gothenburg, Sweden
²Gothenburg Global Biodiversity Centre, Sweden
³Royal Botanic Gardens Victoria, Australia
⁴University of Oslo, Norway
⁵University of Tartu, Estonia

**Purpose and Methods:** *Pseudotomentella tristis* s.l. is a commonly collected, grey, corticioid, ectomycorrhizal and probably insect-dispersed fungus with a very wide geographical distribution and a very large ecological amplitude - DNA-sequences and basidiomata attributed to *P. tristis* have been encountered in habitats and with hosts ranging from the Swedish tundra with *Salix polaris* to the neotropics of Mexico with *Abies religiosa*. Aiming to clarify their taxonomy, systematics, morphology, ecology and geographic distribution, we studied the type specimens of *P. tristis* and its seven morphologically similar taxa, as well as 147 recently collected specimens. We produced species trees in the software STACEY and ASTRAL III and gene trees in BEAST 2 and PhyML, based on ITS, LSU, Tef1α and mtSSU sequences generated from basidiome DNA and complemented with ITS data from the UNITE sequence database.

**Results and Conclusions:** We found *P. tristis* s.l. to contain 15 molecularly and morphologically distinct species. We described ten species as new to science and discovered *P. atrofusca* together with *P. rhizopunctata* to form a sister clade to the remaining species in the *P. tristis* group. These two species, unlike the remaining species in the group, are dimitic. We revealed the previously described *P. umbrina* to indeed be a common species with a wide, Holarctic range and a very large ecological amplitude, while all other species were found to be considerably less common and seem to have smaller ecological niches.
The Phylogenetic Relationship of the Boletaceae collected from Northeastern Thailand

Soravit Chaimongkol¹-², Nattawut Rungjindamai¹, Phongsawat Khamsuntorn², Sujinda Sommai², Satinee Suetrong², Umpawa Pinruan², Sayanh Somrithipol²

¹Department of Biology, Faculty of Science, King Mongkut’s Institute of Technology Ladkrabang (KMITL), Thailand
²National Center for Biotechnology and Genetic Engineering (BIOTEC), Thailand

Purpose: The Boletaceae are a family of mushrooms within the Boletales (Agaricomycetes, Basidiomycota). Some of them live symbiotically as ectomycorrhiza in tree plantations and coniferous forests in tropical and mid latitudes. While some are edible mushrooms which are widely consumed as a gourmet food. They are mostly collected exclusively from the wild. The objectives of this study were (1) To collect mushrooms of the Boletaceae from Northeastern Thailand and (2) To study their phylogenetic relationship.

Methods: The 75 specimens were collected from four locations including three community forests and one silvicultural research station of the Royal Forest Department in the Northeastern Thailand. They were studied macro-and microscopically and their important characteristics were photographed.

Results: Thirty eight taxa were preliminary identified based on text books. Of these, 21 taxa were selected for molecular study. DNA was extracted directly from the fresh and dried specimens. Five genes including ITS, LSU, ATP6, RPB2 and TEF1 were amplified and phylogenetic trees were constructed. The results shows that some of them may be new species.

Conclusions: This study provides the further knowledge of fungal diversity in Thailand and the Boletaceae has great economic potential to develop into a controlled commercial scale production.
Monokaryotic fruiting body and clamp cell formation in *Mycoleptodonoides aitchisonii* (Bunaharitake)

Rini Riffiani, Wada Takayuki, Norihiro Shimomura, Takeshi Yamaguchi, Tadanori Aimi
Tottori University, Japan

Two types of sexual reproduction systems exist in basidiomycete mushrooms: heterothallic and homothallic systems. The term heterothallic refers to mating between two separate monokaryons carrying compatible mating type that is required for the formation of clamp cells and complete fruiting bodies. However, monokaryotic fruiting body formation was previously reported in *Schizophyllum commune*, *Sistotrema brinkmanii*, and *Coprinopsis cinerea*. Therefore, it is unclear whether dikaryotization is necessary for the formation of clamp cells and/or complete fruiting bodies.

**Purpose:** Here, I describe monokaryotic clamp cell formation, fruiting body formation and meiosis in *Mycoleptodonoides aitchisonii*.

**Methods:** Several parameters like the morphological and cytological characterization of fruiting bodies, clamp cell formation in monospore isolates and monokaryotic fruiting were examined.

**Results and conclusions:** A single dikaryotic *M. aitchisonii* strain, TUFC50005, and 20 monokaryons derived from the 50005 strain, which exhibited a wide spectrum of monokaryotic fruiting and monokaryotic clamp cell formation. Most strains formed primordia, or young fruiting body-like structures, but only one of the monokaryons, strain TUFC50005-4, formed a complete fruiting body, even though it had only one nucleus and produced only two basidiospores after meiosis. We demonstrated that dikaryotization was not required for clamp cell formation, fruiting body formation, and meiosis in this mushroom. This is one of the first reports to show that mating and nuclear fusion are not essential for mushroom development.
Multigene Phylogeny of *Panus* sensu stricto (Polyporaceae)

Jaya Seelan Sathiya Seelan¹, David Hibbett², Nelson Menoli³, Young Woon Lim², Edward Grand⁴, Abel Lupalla², Romina Gazis⁵

¹Universiti Malaysia Sabah, Malaysia
²Seoul National University, Korea
³Instituto Federal de Educacao, Ciencia e Tecnologia de Sao Paulo, Brazil
⁴Mahidol University, Thailand
⁵Clark University, United States

**Purpose:** The genus *Panus* Fr. (Polyporaceae, Basidiomycota) includes saprotrophic, lamellate, lignicolous, and wood-decaying (white rot) mushroom forming fungi. This genus is widely documented from tropical and temperate forests and is taxonomically controversial. Here, the generic concept Corner (1981) is followed for *Panus* sensu stricto.

**Methods:** We obtained 219 ITS, 120 nrLSU, 28 RPB2 and 20 TEF1 sequences, focusing on *Panus* sensu stricto, and performed phylogenetic analyses using maximum likelihood and Bayesian analysis.

**Results and conclusions:** These results showed that the ITS, LSU, RPB2 and TEF1 concatenated phylogeny suggests that the diversification of *Panus* sensu stricto has been more extensive in tropical regions. Our analyses showed that *Panus* sensu stricto comprises 12 major clades. Pegler's (1983) sections, *Panus* and *Velutini* are not monophyletic. Based on molecular data and on the size of the pseudosclerotium, *Panus fulvus* and *Panus fulvus* represent two different lineages. Species delimitation within the genus *Panus* were resolved with more informative markers like elongation factor (TEF-α1) and RPB2 are produced herein with supported phylogeny.
Phylogenetic Placement of *Collybia reinakeana* P. Henn. Philippine Isolates Based on internal transcribed spacer Nucleotide Sequences

Minerva Capon Arenas¹,², Renato Gutierrez Reyes³, Ariel Joseph J. Barza³, Ryo Sumi⁴, Nobuo Mori⁴, Fumio Eguchi⁵

¹Far Eastern University - Manila, Philippines
²De La Salle University - Manila, Philippines
³Central Luzon State University, Philippines
⁴Nikken Sohonsha Corporation, Japan
⁵Tokyo University of Agriculture, Japan

Members of Tricholomataceae (Basidiomycota) include fungi of nutriceutical value. Their taxonomy remains untenable, partly due to their remarkable phenotypic diversities, overlapping distributions, and species that are ill-defined. The Philippine endemic *Collybia reinakeana* P. Henn belongs to this assemblage. Locally known as kabuteng calao, *C. reinakeana* is an edible mushroom. This species is distinguished by having a convex pileus, amazing aggregate fruiting bodies, lacks annulus and volva, and gills that bear white circular to spherical spores. Based on of phylogenetic evidence, *Collybia* appears to reduced its species membership, while most of its originally circumscribed species were transferred into separate genera. Moreover, the phylogenetic and taxonomic placement of *C. reinakeana* have yet to be known. Six pure cultures of *C. reinakeana* fruiting bodies collected from several sites in Luzon, Philippines and were prepared using standard laboratory procedure and were used to infer its phylogenetic placement based on internal transcribed spacer (ITS). Four strains fell within the Callocybe clade whereas the other three isolates were closely related to *Macrocybe gigantea*. It appears that *C. reinakeana* includes at least two species conglomerate. It is therefore desirable that the taxonomic placement of *C. reinakeana* should be thoroughly investigated.
Studies of Representatives of the Novel Red and Black Lingzhi from Vietnam

Le Xuan Tham¹, Nguyen Le Quoc Hung¹, Phan Quoc Chinh¹, Pham Ngoc Duong², Nguyen Thi My³, Le Thanh Nhan⁴

¹Department of Science & Technology, Vietnam
²National Park of Cat Tien, Vietnam
³Hung Loc Agricultural Research Center (HARC), Vietnam
⁴University of Agro-forestry, Vietnam

Basidiospores of H. longipes typically haddowioid, globose - subglobose to ellipsoid, not truncate, bitunicate. Exospore strongly thickened, with clearly thick pilar layer of interwalls; Endospore with rather prominent apiculus, punctate - reticulate, splitting along with the exospore longitudinally into ridges, partly connected by transverse membranes. So high similarity in macro- and micromorphological features, particularly specific basidiospores double crested of this taxon suggest that there are some variants conspecific in H. longipes, pantropical taxon covers both subspecies or biogeographical races of unique H. longipes, including the second one named H. aetii as a variety and exclude some taxa as H. guizhouense and H. neurosporum.

Basing on morphological characters of fruit bodies there are controversial arguments on the treatments of taxonomy of a rare Black Lingzhi fungus Magoderna subresinosum, however, basidiospores ganodermoid type with germpore (hyaline aperture - apex) and particularly the hilum as a fine verrucose tiny appendice located on the bottom, opposite to the apex, at which almost researchers seemed to glance at or neglect, and consider the amaurodermoid type, and consequently to treat acceptably yet. Setting the genus Magoderna accomodated 3 species Steyaert (1972) however, only the type M. subresinosum should be accepted and a new synonym M. parasiticum Corner suggested and retain two others as initially Amauroderma infundibuliforme and A. vansteenesii, as due to their basidiospores of typical amaurodermoid.

Data of rDNA analysis (sequences of ITS regions) and even multigenes led to distinctive monospecies genus with the type H. longipes and with exclusion of a case suggested from China and M. subresinosum due to its basidiospore type of haddowioid and ganodermoid as a special lineage of evolution grouped closest taxa Tomophagus, Magoderna, Humphreya and Amauroderma and speciated from the Ganoderma as a core in the Ganodermataceae Donk.
**Diversity of the genus *Calocybella* in tropical India**

K.P. Deepna Latha, Patinjareveettil Manimohan  
Department of Botany, University of Calicut, India

**Purpose:** *Calocybella* Vizzini, Consiglio & Setti (Lyophyllaceae, Agaricales, Basidiomycota), is a lyophylloid genus recently erected based primarily on molecular phylogeny. The genus was originally characterized by collybioid basidiocarps, a reddening context, basidiospores showing *Rhodocybe*-like verruculose ornamentation, and clamped hyphae. Since then, however, some subsequently described species of the genus were not showing some of these morphological features necessitating a reappraisal of the morphological circumscription of the genus. Although originally described from Europe, several of the subsequently described species of *Calocybella* are from tropical India. Here, we present an account of all *Calocybella* species known from India including a species new to science.

**Methods:** Conventional morphology-based taxonomic methods and molecular phylogenetic analyses were employed for this study.

**Results:** The diagnostic features, photographs of the basidiocarps in their natural habitat and the microscopic structures, and an ITS-based phylogram generated from Maximum likelihood (ML) and Bayesian Inference (BI) analyses depicting their placement of the Indian species are provided. A key to all known species of *Calocybella* also is given.

**Conclusions:** The study indicated that the diversity of the genus *Calocybella* is quite high in tropical India.
Diversity of the genus *Entoloma* in Kerala State, India

K.N. Anil Raj¹, Patinjareveettil Manimohan²

¹Mahatma Gandhi Government Arts College, Mahe, Union Territory of Pondicherry, India
²Department of Botany, University of Calicut, India

**Purpose:** *Entoloma* P. Kummer (Entolomataceae, Agaricales, Basidiomycota) represents one of the larger genera of Agaricales with more than 1500 species distributed worldwide. *Entoloma* species are characterized by a pinkish spore print and angular basidiospores. An overview of the diversity of *Entoloma* in Kerala State, India is presented here.

**Methods:** Conventional morphology-based techniques were used for the floristic study. In addition, the ITS and LSU sequences obtained from some species were utilized in BLAST searches.

**Results:** Our study revealed a total of sixty-seven species of *Entoloma* belonging to the following nine subgenera: *Cyanula* (thirty-three species), *Alboleptonia* (ten species), *Pouzarella* (eight species), *Nolanea* (six species), *Entoloma* (four species), *Inocephalus* and *Leptonia* (two species each), and *Claudopus* and *Omphaliopsis* (one species each).

**Conclusions:** The species of the subgenus *Cyanula* were found to outnumber all other groups. The most widely distributed *Entoloma* species in Kerala also belongs to the subgenus *Cyanula* (*Entoloma niranjanum*). The most remarkable outcome of the present study is the discovery that about 61% (forty-one out of the sixty-seven) of the *Entoloma* species collected and studied during this study are new to science. Several species exhibited a very restricted distribution. Another feature observed during this study is the low number of fruiting bodies of any species observed in any one location at any time.
Purpose: Epidemiological data showed increasing incidence rates of mushroom poisoning cases in Thailand. This study aimed to identify mushroom samples from 187 clinically reported cases during 2012 to 2019 based on DNA barcoding and also determine their toxins.

Methods: Clinical mushroom samples were identified using DNA sequence data under the selected phylogenetic criterion and DNA barcoding analysis. Mushroom toxins were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) with multiple reaction monitoring (MRM) as well as liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS).

Results and conclusions: Our results revealed that gastrointestinal irritant mushroom poisoning was most frequently encountered, followed by other types of mushroom poisoning, including neurotoxic, cytotoxic, myotoxic and metabolic/endocrine toxicity. More than 80% of poisoning cases occurred in rainy season extending from May to August. Most commonly found wild mushrooms were *Amanita brunneitoxicaria*, *A. exitialis*, *A. fuliginea*, *Cantharocybe virosa*, *Chlorophyllum molybdites*, *C. globosum*, *Entoloma sp.*, *Inocybe sp.*, *Leccinellum sp.*, *Russula subnigricans* and *Xerocomus sp.* These included three main lethal species; *A. brunneitoxicaria*, *A. exitialis* and *R. subnigricans*. The toxins discovered from the clinical samples were alkaloid muscarine, alpha-amanitin, beta-amanitin, phallacidin, phalloidin, allenic norleucine, coprine & cycloprop-2-ene carboxylic acid. These cases of mycetism occurred mainly due to misidentification and misconceptions about indigenous knowledge of the nontoxic and toxic mushrooms.

Keywords: DNA barcoding; lethal species; mycetism; Thailand
Ligninolytic enzyme production from white-rot fungi by using oil palm decanter cake as substrate to high value add products

Pisit Thamvithayakorn1), Cherdchai Phosri2), Rungpetch Khaengraeng3), Anthony JS Whalley4), Nuttika Suwannasai5)

1)Department of Biology, Faculty of Science, Srinakharinwirot University, Thailand
2)Department of Biology, Faculty of Science, Nakhon Phanom University, Thailand
3)Environmental Science Programme, Faculty of Science and Technology, Pibulsongkram Rajabhat University, Thailand
4)School of Pharmacy and Biomolecular Science, Liverpool John Moores University, United Kingdom
5)Department of Microbiology, Faculty of Science, Srinakharinwirot University, Thailand

Purpose: White-rot fungi (WRF), mostly basidiomycetes, are able to degrade major wood components, especially lignin because of the ability to produce ligninolytic enzymes such as laccase, manganese peroxidase (MnP) and lignin peroxidase (LiP). These enzymes have been widely used in several industrial and biotechnological applications. The objective of this study was to screen WRF from the northeast of Thailand for ligninolytic enzyme production by using palm oil mill waste as substrate, oil palm decanter cake (OPDC).

Methods: A total of 150 pure culture isolates from 264 samples were screened for ligninolytic enzyme production by using an agar plate assay with three different indicators (guaiacol or ABTS or RBBR). The positive isolates were then selected to grow on OPDC and identified by using ITS sequences. The quality of ligninolytic enzyme production was measured.

Results: Twenty-six fungal isolates exhibited high enzyme activities with rapid growth. They were then identified to species level based on morphological and molecular characteristics using internal transcribed spacer sequences. The majority belonged to Polyporaceae (Coriolopsis, Ganoderma, Microporus, Nigroporus and Trametes) followed by Cerrenaceae, Hericiaceae, Incrustoporiaceae and Stereaceae. There were 9 identified species and 6 unidentified species. From the results, Pseudolagarobasidium sp. PP17-33 showed high activities of laccase (315.56 U/L), MnP (680.00 U/L) and LiP (2.41 U/L).

Conclusions: This study is the first report of WRF producing ligninolytic enzymes from OPDC. Fungal strains obtained will be good candidates for ligninolytic enzyme production, lignin degradation and suitable for biotechnological applications in the future.
Phylogeny and taxonomy of *Ceriporia* and other related taxa

Che-Chih Chen¹, Chi-Yu Chen¹, Young Woon Lim², Sheng-Hua Wu¹,³

¹Department of Plant Pathology, National Chung Hsing University, Taiwan
²School of Biological Sciences and Institute of Microbiology, Seoul National University, Republic of Korea
³Department of Biology, National Museum of Natural Science, Taiwan

**Purpose:** The polypore genus *Ceriporia* belongs to *Polyporales* of *Basidiomycota* and encompasses around 80 species which are saprotrophs or endophytes in forest ecosystems. Despite its high species diversity, few *Ceriporia* species have been incorporated into phylogenetic studies. To better understand the limits of *Ceriporia* and its phylogenetic relationship with other related taxa, we expand the molecular and taxonomic sampling, including new sequences, and new samples from East Asia.

**Methods:** We used morphology and multi-marker phylogenetic analyses based on sequences of the nuc rDNA ITS1-5.8S-ITS2 (ITS), D1-D2 domains of nuc 28S rDNA (28S), and RNA polymerase II largest subunit (rpb1). Two datasets were used: (i) The ITS+28S+rpb1 dataset was used to investigate the systematic positions of *Ceriporia* species within families of *Polyporales*; (ii) The ITS+28S dataset, with a larger sampling of species and specimens, was used to infer interspecific relationships and taxonomy in clades I and II recovered in the 3-marker analyses.

**Results and conclusions:** Our results show that *Ceriporia* is polyphyletic, and distribute across clades I-III in *Irpicaceae* and a lineage (*C. alachuana*) in *Meruliaceae*. In clade I, some species previously considered classified in *Ceriporia*, are now recovered in *Meruliopsis*, resulting in four new combinations: *M. albomellea*, *M. crassitunicata*, *M. nanlingensis*, and *M. pseudocystidiata*. Clade II, having dominant *Ceriporia* species, represents *Ceriporia* sensu stricto. Clade III includes *C. cystidiata* and *C. sulphuricolor*. Three species are new to science: *C. arbuscula* and *M. parvispora* from Taiwan; *M. leptocystidiata* from North East Asia. Three species are new records: *C. mellita* and *M. nanlingensis* from Japan and Taiwan; *M. taxicola* from Taiwan.
Unveiling of sequestrate bolete genus *Rossbeevera* in China

Md. Iqbal Hosen, Tai-Hui Li
State Key Laboratory of Applied Microbiology Southern China, Guangdong Institute of Microbiology, China

**Purpose:** *Rossbeevera*, a sequestrate ectomycorrhizal genus of Boletaceae, which comprises 12 species, are mainly distributed in East Asia and Australasia. The purpose of this study is to investigate the species diversity of *Rossbeevera* in China.

**Methods:** *Rossbeevera* samples were collected from China. Both morphological and molecular approaches of the four genes (ITS+28S+tef1-α+ rpb2) were used for the species delimitation of *Rossbeevera*.

**Results:** *Rossbeevera* collections from China, comprised six species, of which two are new to science, and two are new records to China. Phylogenetically, both new species are closely related to *R. paracyanea* but distinctive in their morphology.

**Conclusion:** This is the first comprehensive study to explore the *Rossbeevera* fungal resources in China, species diversity within *Rossbeevera* in East Asia and their evolutionary relationships will be addressed during the congress.
Taxonomic reexamination of *Auricularia* specimens deposited in the National Museum of Nature and Science, Japan

Mami Kusamoto, Takashi Shirouzu
Graduate School of Bioresources, Mie University, Japan

**Purpose:** The genus *Auricularia* is a group of jelly fungi in Agaricomycotina, Basidiomycota. *Auricularia* species are significant wood decomposers in forest ecosystems and are important bioresources as cultivated mushrooms especially in Asia. The classification of *Auricularia* spp. has traditionally been based on morphological criteria, but in recent years the species taxonomy has been revised to reflect their phylogenetic relationships. Consequently, a taxonomic reexamination of Asian *Auricularia* species is necessary. In this study, we aimed to taxonomically review the Japanese *Auricularia* specimens deposited in the National Museum of Nature and Science, Japan.

**Methods:** Three specimens of *Auricularia* spp. (TNS-F 433, TNS-F 427, and TNS-F 51392) identified in previous studies were selected from the herbarium of the National Museum of Nature and Science. For morphological observations, the dried specimens were kept in humid conditions overnight. Pieces were cut out from the center of basidiocarps and observed under a biological microscope.

**Results and Conclusions:** The specimens TNS-F 427, TNS-F 433, and TNS-F 51392 were morphologically similar to *Auricularia minutissima*, *A. thailandica*, and *A. villosula*, but these specimens could not be identified to the species level. The lengths of basidiospores and abhymenial hairs of the TNS specimens were shorter than those of *A. auricula-judae* collected in Europe, suggesting that there is no *A. auricula-judae s. str.* among these specimens. These results support the hypothesis that *A. auricula-judae s. str.* is distributed only in Europe. The results of this study suggest that further taxonomical reexamination is needed for Japanese *Auricularia* specimens.
Purpose: Singapore does not have a comprehensive checklist of present day macro-fungal diversity. We want to invite collaborators working on different groups of macrofungi to collect as well as make use of our old collections.

Methods: The SING Herbarium has databased all of its material. Here we present statistics on our herbarium collections, highlighting the strengths of the current collection and identification methods. Most sites of natural vegetation in Singapore are easy to get to as the country is small and yet, as a part of the Malay Peninsula, fungal diversity is high and research is vital for a better understanding of the distribution of various taxa. Due to the lack of expertise, we rely on the limited keys of this region as well as social media for the identification of our collections.

Results and conclusions: Singapore is an important type locality for many species of macrofungi, especially for those collected by Edred John Henry Corner between 1929 and 1945 and later described by him. There is a rich potential for more unsubscribed species. We aim to make information on the macrofungi of Singapore more accessible for taxonomists through the publication of a macrofungi checklist of Singapore, including all new collections. We have a very long way to go to make such a checklist fully comprehensive because we know that without collaborative work, this undertaking will not be possible.
Purpose: Wild mushrooms accumulate high concentrations of radiocesium ($^{137}$Cs). Their $^{137}$Cs activity levels are known to vary according to taxon and the $^{137}$Cs levels in their substrates. However, the $^{137}$Cs activity levels often fluctuate widely among individual fruiting bodies, even within the same species collected at the same location. To clarify the factors affecting the variation in $^{137}$Cs activity among individual fruiting bodies, we investigated the relationships among $^{137}$Cs, stable cesium ($^{133}$Cs), and exchangeable bases (rubidium ($^{85}$Rb) and potassium ($^{39}$K)) contents in mushrooms, litter, and soils.

Methods: We collected 24 fruiting bodies of a mycorrhizal mushroom species, Boletus hiratsukae, their neighboring soils (at depths of 0-5, 5-10, and 10-15 cm), and litter at two plots in Tsukuba, Ibaraki, Japan. The $^{137}$Cs activity was measured using an SeGe coaxial detector and the concentrations of $^{133}$Cs, $^{39}$K, and $^{85}$Rb in mushrooms and soils were determined by inductively coupled plasma-mass spectrometry.

Results: There was a strong correlation between the $^{137}$Cs and $^{133}$Cs concentrations in mushrooms. We detected significant negative correlations between the $^{39}$K concentration in soil and the $^{137}$Cs and $^{133}$ Cs concentrations in mushrooms, while there was no correlation between the $^{137}$Cs concentration in soils and that in mushrooms.

Conclusions: Our results showed that the variations in $^{137}$Cs concentrations of B. hiratsukae mushrooms collected at the same site were explained by the $^{133}$Cs concentrations in the mushrooms. We concluded that the concentration of soil-exchangeable $^{39}$K regulates the absorption of $^{137}$Cs by wild mushrooms.
Taxonomy and Phylogeny of *Marasmius* (Basidiomycota, Agaricales) Section Marasmius from North Eastern Thailand

Nopparat Wannathes¹, Nakarin Suwannarach²,³, Jaturong Kumla²,³, Saisamorn Lumyong²,³,⁴

¹Faculty of Science and Technology, Pibulsongkram Rajabhat University, Thailand
²Department of Biology, Faculty of Science, Chiang Mai University, Thailand
³Center of Excellence in Microbial Diversity and Sustainable Utilization, Chiang Mai University, Thailand
⁴Academy of Science, Thai Royal Society of Thailand, Thailand

**Purpose:** *Marasmius* Fries is a well-known saprobe agaric with worldwide distribution. In Thailand, the diversity studies were carried out mainly in northern areas. In order to expand our understanding of the diversity of the genus in north eastern areas, then taxonomy and phylogeny of *Marasmius* section *Marasmius* in Khao Yai National park were conducted in 2018.

**Methods:** Classification and identification were performed based on morphological characters and the sequence data of internal transcript spacer (ITS) of ribosomal DNA.

**Results and conclusions:** Thirteen species of *Marasmius* section *Marasmius* were recorded. Seven species viz. *M. albulus*, *M. brunnneisetosus*, *M. obscuro-aurantiacus*, *M. pseudoruforotula*, *M. sordidoflavus*, *M. subarmeniacus* and *M. tangerinus* are described as new to science.
Construction of a model system to analyze the decomposition process of bamboo culm adopting mushrooms in Agaricomycotina

Takehiro Ochi\(^1\), Tatsuya Fukuda\(^{1,2}\), Akira Suzuki\(^2\)

\(^1\)Graduate School of Environment and Information Studies, Tokyo City University, Japan
\(^2\)Faculty of Knowledge Engineering, Tokyo City University, Japan

**Purpose:** We focused on construction of a model system to analyze the decomposition process of bamboo culm adopting mushrooms in Agaricomycotina.

**Methods:** We used five white-rot fungi and two litter-inhabiting fungi, and selected a brown-rot fungus as a control. The growth rate of the fungi on PDA medium and lignin decomposition ability by Bavendamm reaction and Remazol Brilliant Blue R test were examined. Among them, *Pleurotus djamor var. roseus* was selected based on the evaluation of the above criteria. The bamboo powder of *Phyllostachys reticulata* culm powder was moistened at 71%, and 400 to 405 g of the powder was filled in a PP bag followed by sterilization at 120\(^\circ\)C for 1 hour. Five mycelial blocks punched out from a mycelium grown on PDA medium were inoculated onto the bamboo powder. They were incubated at 25\(^\circ\)C and analyzed its decomposition.

**Results and Conclusions:** The mycelium covered the whole surface of the medium in about 2 to 3 weeks in all cultures. Fruiting bodies were formed within 1 month of incubation. It indicates that *Pl. djamor var. roseus* has a high decomposition ability of the culm. Weight loss of the bamboo powder culture was 14\% after 3 months of incubation. Dry weight of \(\alpha\)-cellulose, hemicellulose, and lignin decreased 25.1\%, 11.8\%, and 0.8\%, respectively, suggesting that lignin decomposition initiated within 3 months. Our results suggest that the bamboo culture by *Pl. djamor var. roseus* would be a suitable model system to analyze the decomposition process of bamboo culm.
Russula omiensis sensu lato comprises at least four species!

Yoshito Shimono\textsuperscript{1)}, Taiga Kasuya\textsuperscript{2)}

\textsuperscript{1)Graduate School of Bioresources, Mie University, Japan  
\textsuperscript{2)Department of Biology, Keio University, Japan}

\textit{Russula omiensis} was originally described from Shiga Pref., central Honshu, Japan and its basidiomata usually grows in evergreen broad-leaved forests dominated by Castanopsis and Quercus spp. at low temperature from Late November to April. Caps of \textit{R. omiensis} are dark reddish purple to yellowish purple with wine or dark in the center, and sometimes red and olive in color. Characteristics of this species are varied cap colors, very acrid lamellae and long fruiting period during low temperature climate. In Japan, Russula species sharing these characters have been treated as \textit{R. omiensis} in lump though morphological variation of \textit{R. omiensis} has been recognized. Therefore, Russula species currently identified as \textit{R. omiensis} in Japan probably include several distinct species. Nevertheless, taxonomic reexamination and molecular phylogenetic studies on \textit{R. omiensis} sensu lato have not been conducted yet. To rearrange the species taxonomy of \textit{R. omiensis} sensu lato based on morphology and molecular phylogeny, specimens of the present species and several additional materials of Russula were collected from Japan, and morphological and molecular data were newly generated. Molecular phylogenetic analyses based on ITS region revealed the polyphyly of specimens morphologically identified as \textit{R. omiensis} sensu lato. They were divided into at least four clades, which were morphologically and ecologically distinguishable. As above, these four clades were well supported by morphological and ecological features and therefore, each clade should be treated as independent species.
Three new species and one new record of spinose *Mycena* (sections Longisetae and Spinosae) from Taiwan

Chiung-Chih Chang¹,³, Wen-Neng Chou², Chi-Yu Chen³, Hsiao-Wei Kao⁴

¹Biodiversity Research Center, Academia Sinica, Taiwan
²Department of Biology, National Museum of Natural Science, Taiwan
³Department of Plant Pathology, National Chung Hsing University, Taiwan
⁴Department of Life Sciences, National Chung Hsing University, Taiwan

**Purpose**: Species of the agaric genus *Mycena* (*Basidiomycota*) in sections *Longisetae* and *Spinosae* are characterized by having hairs or spines on their pileus, and usually grow on plant litters in tropical and subtropical forests. These two sections encompass around 18 and 7 species worldwide, respectively. However, the described species-level diversity in Taiwan may vastly underestimate their actual diversity due to limited field investigation. This study aims to discover species diversity of spinose *Mycena* in Taiwan.

**Methods**: Specimens were collected from tropical forests of Southern Taiwan and an arboretum of Central Taiwan. Micro- and macro- morphologies of specimens were examined. Phylogenetic analyses are inferred from both maximum likelihood and Bayesian methods, based on sequences of the nuc rDNA ITS1-5.8S-ITS2 (ITS).

**Results and conclusions**: Based on morphological and phylogenetic evidences, *Mycena fengguans*, *M. subcyanocephala* and *M. turandotiana*, assigned to section *Spinosae*, were proposed as new species. *M. brunneisetosa*, belonging to section *Longisetae*, is a new record from Taiwan. *M. fengguans*, growing on decaying branches, is characterized by having pubescent primordia and fruitbodies, clavate cheilocystidia with cylindrical excrescences, and thin-wall caulocystidia with narrowed apex. *M. turandotiana*, growing on decaying branches, is characterized by having dark gray to grayish-white pileus, pubescent stipe with radiating fibrils in base, capitate, cylindrical cheilocystidia, and abundant halocystidia on stipe, pelius, and the edge of lamellae. *M. subcyanocephala*, growing on rotted wood, is characterized by having tomentulose fruitbodies, white to blueish pileus, cylindrical to spindle shape pileiocystidia and caulocystidia, and capitate cheilocystidia. A key to all *Mycena* species in section *Spinosae* is given.
Unexpected cryptic species diversity of undescribed, white sequestrate *Russula* spp. ("Koishi-take") found from Japan

Takamichi Orihara

Kanagawa Prefectural Museum of Natural History, Japan

**Purpose:** Sequestrate fungi, which include truffles and truffle-like fungi, have evolved in various mushroom-forming fungal lineages in Basidiomycota and Ascomycota. The cosmopolitan mushroom-forming genus, *Russula*, also includes multiple lineages of sequestrate fungi across the genus. However, the diversity of sequestrate *Russula* spp. in Asia is poorly known thus far. In the last decade I have collected various sequestrate *Russula* fruitbodies throughout Japan, especially a number of white, compact, truffle-like ones domestically known as "Koishi-take (pebble-mushroom)." This study aims to clarify the diversity within this sequestrate *Russula* and its allies and to infer their geological origin based on phylogenies.

**Methods:** Fruitbodies of the sequestrate *Russula* ("Koishi-take" and its relatives) were collected from subtropical, temperate and subarctic *Fagaceae* forests throughout Japan and were used for phylogenetic analyses based on nuclear rRNA gene. Phylogeographical analyses were conducted with the Mesquite software. Morphological observation was based on standardized methods.

**Results and conclusions:** The sequestrate *Russula* (Koishi-take) specimens formed a sister clade (= the "Lithogaster" clade) to the clade that included North American "*R. adulterina*. *Cystangium idahoensis* in *Russula s. str.* Despite the high morphological homogeneity of the "Lithogaster" clade members, they were unexpectedly diverged into five species- or subspecies-level lineages. Only one of the five lineages, which were collected in temperate-subarctic *Fagaceae* forests, were morphologically distinct from other lineages. The clade had the highest species-level diversity in temperate evergreen *Quercus-Castanopsis* forests in the Kyushu - Northern Ryukyu region of Japan, and the phylogeographic analysis suggested that they might have been originated there.
Biodiversity and Taxonomy of the Russulaceae in Northeastern Thailand

Wittayothin Yingkunchao1,2), Nattawut Rungjindamai1), Phongsawat Khamsuntorn2), Sujinda Sommai2), Satinee Suetrong2), Umjawa Pinruan2), Sayanh Somrithipol2)
1)Department of Biology, Faculty of Science, King Mongkut’s Institute of Technology Ladkrabang (KMITL), Thailand
2)National Center for Biotechnology and Genetic Engineering (BIOTEC), Thailand

Purpose: The family Russulaceae contains two important genera including Lactarius and Russula. These mushrooms are easily recognized by fairly large basidiocarps, brightly coloured upper cap surface and the gills are straight, arranged in a regular pattern. These ectomycorrhizal mushrooms are widely distributed in nature which some are edible but some are poisonous. Therefore taxonomic study and identification of these genera are extremely important.

Methods: The objectives of this study were (1) To collect and document mushrooms of the Russulaceae from different parts of Northeastern Thailand and (2) To clarify the taxonomic position of the Russulaceous fungi.

Results: A total of 45 specimens were collected from four locations in Northeast of Thailand. Morphological characteristics of these mushrooms are microscopically studied. Based on types of fruiting bodies, there were two different groups. To confirm their identity, their genomic DNA was extracted and two genes (ITS and LSU) were amplified. The phylogenetic relationship of these mushrooms were discussed.

Conclusions: This shows that Thailand has great diversity of the Russulaceae and various species of Lactarius and Russula are reported.
Damage of urban living trees by wood decay fungi in Gangwon Province, Korea

HeeSuk Lee, DaeHo Kim, JongKyu Lee
Tree Pathology and Mycology Laboratory, Division of Forest Science, Kangwon National University, Korea

Wood decay on living trees in street and park areas could increase the likelihood of failure and cause the risk. Major urban trees in Gangwon province were surveyed from May to September in 2018. Fruiting bodies of wood decay fungi and/or decayed woods were collected, the fungi were isolated, and then identified by extracting DNA for molecular analyses. The dominant species were Irpex lacteus, Schizophyllum commune, Trametes versicolor, T. hirsuta, Umbelopsis isabellina, Pycnoporus sanguineus, and Coprinellus radians. Damage rates of living trees by wood decay fungi were 38.5% (273/710) of cherry tree (Prunus sect. Cerasus) and 9.1% (31/341) of Zelkova tree (Zelkova serrata) surveyed. Fruiting bodies were formed on 63.7% (174/273) of the decayed cherry tree, but on 29% (9/31) of the decayed Zelkova tree. For cherry tree, damage rate of tree planted in the street was 40.5%, while that in the public park was 30.3%. For Zelkova tree, the former was 8.6%, while the latter was 12.2%. Decayed parts were mainly occurred in the upper trunk followed by the lower trunk and thick branch in cherry and Zelkova trees. Damage by the decay fungi were the most common in the tree with the range of 11~20cm diameter at breast height, followed by 21~30cm and below 10cm. Cell wall disintegration of the decayed woods were observed under scanning electron microscope after sectioning with freezing microtome.
Macrolepiota in Korea: New records and new species

Ki Hyeong Park1), Hae Jin Cho1,3), Changmu Kim2), Shinnam Yoo1), Young Woon Lim1)
1) School of Biological Sciences and Institute of Microbiology, Seoul National University, South Korea
2) Microorganism Resources Division, National Institute of Biological Resources, South Korea
3) Forest Plant Industry Department, Baekdudaegan National Arboretum, South Korea

Purpose: The genus Macrolepiota (Agaricales, Basidiomycota) is easy to recognize at the genus level because of big, fleshy basidiocarps with squamules covering the pileus; a single or double annulus; and big, thick-walled basidiospores with a germ pore. However, morphological identification is often unreliable in Macrolepiota due to similar morphological features among species. Due to the uncertainty of previous morphological identification in the genus Macrolepiota, it is necessary to re-examine Korean Macrolepiota using molecular data.

Methods: We re-examined 34 Macrolepiota specimens collected from 2012 to 2018 in Korea using a reverse taxonomic approach, whereby species identification was first done based on the internal transcribed spacer (ITS) region analysis, followed by morphological confirmation.

Results: We identified the presence of four species: M. detersa, M. mastoidea, M. procera, and M. umbonata sp. nov. Two species (M. detersa and M. mastoidea) were previously unrecorded from Korea and M. umbonata is a new species. Detailed descriptions of all four species and taxonomic key are provided in this study. Macrolepiota procera and M. umbonata are distributed through the country, but M. detersa and M. mastoidea are distributed only in limited areas.

Conclusion: According to our results, the combination of ITS locus and morphology proved to be a robust approach to evaluate the taxonomic status of Macrolepiota species in Korean. Additional surveys are needed to verify the species diversity and clarify their geographic distribution.
Luminescence properties of ‘non-luminous’ mushrooms by the treatment of hispidin, a luciferin precursor

Masashi Naito¹, Yuichi Oba¹ ²
¹Graduate School of Bioscience and Biotechnology, Chubu University, Japan
²Department of Environmental Biology, Chubu University, Japan

Purpose: Currently about 100 species of bioluminescent fungi were recognized in the world. All bioluminescent species emit light at mycelial stage, and some of them do so at fruiting body stage. The luminescence has basically been recorded by chance at night observation in the wild, thus potential diversity has probably been overlooked. In this study, we searched potential luminous fungi species using the methods based on the bioluminescent reaction mechanism.

Methods: We used hispidin, which is a precursor of luciferin commonly used in fungal bioluminescence reaction, as a probe to screen the potential bioluminescent fungi. 200 µM of hispidin aqueous solution containing 1% DMSO was dropped on the fruit body of freshly collected in wild, and observed the light emission by eyes and high sensitive camera.

Results and conclusions: By using this method, we newly identified three bioluminescent species, which has previously been known as non-luminescent. These three species have luminous mycelium but non-luminescent fruiting body. As a result, the total number of luminescent fungi in Japan is 45 species at present.
Patterns of genome evolution underlying differential wood-decay mechanisms in Polyporales

Mate Viragh1), Zsolt Meren1), Balazs Balint1), Hayat Hage2), Shingo Miyauchi3), Anne Favel4), Otto Miettinen5), Igor V. Grigoriev1), Marie-Noelle Rosso2), Francis Martin3), Laszlo G. Nagy1)
1) HAS, Biological Research Centre, Szeged, Institute of Biochemistry, Hungary
2) INRA, Aix-Marseille Univ, UMR1163, Biodiversité et Biotechnologie Fongiques, BBF, France
3) INRA, University of Lorraine, Laboratory of Excellence Advanced Research on the Biology of Tree and Forest Ecosystems (ARBRE), UMR 1136, France
4) INRA, University of Lorraine, Laboratory of Excellence Advanced Research on the Biology of Tree and Forest Ecosystems (ARBRE), UMR 1136, France
5) Botanical Museum, University of Helsinki, Finland

Purpose: The Polyporales is a diverse group of saprotrophic Agaricomycetes playing a major role in terrestrial carbon cycle. The order includes white- and brown-rot species that use different strategies to decompose woody plant material. To explore changes in the genetic toolkit leading to a great variety of differential wood-decay mechanisms in Polyporales, we aimed to reconstruct historical patterns of genome evolution, with special emphasis on gene families related to wood-decay.

Methods: To investigate genome-wide catalogs of gene families of Polyporales, we first generated a dataset containing the whole proteomes of 107 agaricomycetes fungi. Taxa were sampled so as to cover all major wood decayer Agaricomycetes clades. Protein sequences were clustered into gene families and then aligned. Next we inferred maximum likelihood gene trees and used a concatenated alignment of single-copy orthogroups with at least 70% taxon occupancy to infer a species tree. Then we carried out gene tree-species tree reconciliation and reconstructed gene duplication/loss histories in across all families using the COMPARE pipeline.

Results and conclusions: Our reconstructions of the evolution of 107 fungal genomes including 47 Polyporales species revealed historical genome expansion and contraction events along the phylogeny. Gene duplication/loss history shows distinct patterns in the Polyporales, many of which with clear connections to lifestyle transitions in the order. Consistent with previous studies, our analysis revealed differential evolution of wood-decay related gene families in brown- and white-rot lineages, but also several novel families that show lifestyle-associated gene duplication/loss patterns in the Polyporales.
Amanita virosa is a well-known fungus which has lethal, whitish basidiomata, and it was originally described from Europe. In Japan, lethal Amanita species sharing whitish basidiomata have been treated as “A. virosa” in lump though morphological variations of Japanese “A. virosa” has been recognized. Therefore, Amanita species currently identified as “A. virosa” in Japan probably include several distinct species. Nevertheless, taxonomic reexamination and molecular phylogenetic studies on Japanese “A. virosa” have not been conducted yet. To rearrange the species taxonomy of Japanese “A. virosa” based on morphology and molecular phylogeny, specimens of whitish amanitas which were morphologically treated as “A. virosa” were collected from Japan, and morphological and molecular data were newly generated. Molecular phylogenetic analyses based on ITS region and LSU revealed the polyphyly of specimens morphologically identified as “A. virosa”. They were divided into at least six clades, which were morphologically and ecologically distinguishable. Among them, three were identical to previously known species in Japan including A. oberwinklerana, A. pallidorosea and A. virosa. In Japan, distribution of A. virosa is probably restricted to subalpine and alpine conifer forests. Another three clades were identical to A. rimosa, A. suballiacea and A. subpallidorosea, which are newly recorded species in Japan.
Note of Five Unrecorded Mushrooms containing three rare species from Juwangsan in Korea

Sun Lul Kwon\textsuperscript{1)}, Seokyoon Jang\textsuperscript{1)}, Young Mok Heo\textsuperscript{1)}, Changmu Kim\textsuperscript{2)}, Jae-Jin Kim\textsuperscript{1)}

\textsuperscript{1)}Division of Environmental Science & Ecological Engineering, College of Life Science & Biotechnology, Korea University, Korea
\textsuperscript{2)}Microorganism Resources Division, National Institute of Biological Resources, Korea

\textbf{Purpose:} Mountain Juwang is located arid region and has unique geological conditions consisted of tuff. Though the unusual characteristic of Juwangsan, the existence of uncommon fungal flora is expected. Thus, the aim of this study is to investigate the macro-fungi within a Juwangsan and report unrecorded fungal species from Korea.

\textbf{Methods:} The macro-fungi were collected from July to September in 2018 from three different locations of Juwangsan. The macro-fungi were identified by molecular DNA analysis and morphological analysis. Phylogenetic analysis was performed using the internal transcribed spacer (ITS) region.

\textbf{Results and conclusions:} About 301 mushrooms were collected from Juwangsan and five unrecorded species candidates were identified by molecular DNA analysis: \textit{Psathyrella sulcatotuberculosa}, \textit{P. phegophila}, \textit{Calocybe decolorata}, \textit{Mycena pearsoniana}, and \textit{Crepidotus brunnescens}. Among them, \textit{P. sulcatotuberculosa}, \textit{P. phegophila}, and \textit{C. brunnescens} are known as rare species which rarely reported from Europe. We provide detailed descriptions and phylogenetic analysis of five unrecorded species through this study. The detection of three rare species can anticipate to enhance the macro-fungal diversity and contribute to conserving mushroom species from Korea.
Delimitation of cryptic species within *Hypholoma fasciculare* complex based on parallel MiSeq sequencing of mitochondrial and nuclear loci

Hirotoshi Sato¹, Ryoma Ohta², Noriaki Murakami²

¹Kyoto University, Japan
²Tokyo Metropolitan University, Japan

This study aims to delimit cryptic species within *Hypholoma fasciculare* complex in Japan based on DNA taxonomy. From September 2008 to November 2016, 100 fruiting bodies of the *H. fasciculare* complex were collected in 28 areas in Japan. The large and small subunit of the mitochondrial rDNA (mtLSU and mtSSU), the internal transcribed spacer region of the nuclear rDNA (ITS), and the 20 single-copy genes selected from FunyBase (http://genome.jouy.inra.fr/funybase/) were amplified. The Illumina MiSeq platform for sequencing with 2 x 250 bp read length. Mitochondrial sequences indicated that *H. fasciculare* complex contains four haplotypes. The monophyly of each haplotype was not strongly supported in the maximum likelihood (ML) tree of ITS sequences, but it was strongly supported in that of combined dataset of ITS and 20 single-copy genes. The analysis of molecular variation (AMOVA) for the single nucleotide polymorphisms (SNPs) of the combined nuclear dataset indicated that the genetic variation observed between haplotypes was much higher (77.96%; Phi=0.780; P=0.001) than that observed between local populations within haplotypes (2.07%; Phi=0.094; P=0.001), that between samples within local populations (0.67%; Phi=0.0337; P=0.349) and that within samples (19.29%; phi=0.193; P=0.001). Those results suggest that four haplotypes observed in *H. fasciculare* complex represent reproductively isolated species. Our findings suggest that molecular phylogeny inferred from single nuclear locus (e.g., ITS) is not necessarily sufficient for delimiting sibling species of macro-fungi. Parallel sequencing of mitochondrial loci and multiple nuclear loci using MiSeq and subsequent SNP analyses would allow for more accurate detection of species boundaries.
Calcium signaling and stress tolerance in the model filamentous fungus *Neurospora crassa*

Ranjan Tamuli, Dibakar Gohain, Avishek Roy, Ajeet Kumar, Darshana Baruah, Christy Noche K Marak, Rekha Deka, Vijya Laxmi, Ananya Barman, Ravi Kumar, Anand Tiwari, Serena Ngiimei D
Indian Institute of Technology Guwahati, India

**Purpose:** We studied the calcium (Ca2+) signaling process in the model filamentous fungus *Neurospora crassa*, which possess a complex and unique Ca2+ singling system.

**Methods:** We employed a range of genetic and molecular biology approach including phenotypic analysis of knockout mutants, quantitative real-time PCR analysis, protein and microscopic analysis to investigate the functions of the genes involved in Ca2+ signaling.

**Results and Conclusions:** We previously showed that the crz-1 transcription factor upregulates expression of the Ca2+ sensor for the tolerance to Ca2+ stress in *N. crassa*. Here, we showed that the crz-1 also binds to the promoter of heat-shock protein (hsp)-80 gene to provide thermotolerance in *N. crassa*. The CRZ-1 protein is dephosphorylated, resulting in its nuclear localization, by the Ca2+/calmodulin (CaM) dependent phosphatase calcineurin and we are investigating the transcription factor controlling the calcineurin and the CaM expressions. Furthermore, we also identified a novel role of sPLA2 in cellulose degradation. In a parallel study, we also identified key roles for some zinc transporters in *N. crassa*. Therefore, Ca2+-signaling plays an important role in tolerance to Ca2+ and heat shock in *N. crassa*. 
Tracing the natural lineages of *Saccharomyces* yeasts in Asia

Isheng Jason Tsai, Tracy J Lee, Huei-Mien Ke, Yuching Liu, Hsin-Han Lee, Wei-An Liu
Biodiversity Research Center, Academia Sinica, Taiwan

**Purpose:** *Saccharomyces cerevisiae* is one of the most important model organisms in genetics, cellular and molecular biology. However, outside laboratory strains, its natural history and ecology remains largely unknown. Several recent large-scale population genomic studies have revealed that 'wild' *Saccharomyces cerevisiae* harbor much higher diversity than domestic populations. These studies proposed an 'out-of-China/Taiwan' origin of the yeast as evidenced by a significantly higher divergence within Chinese and Taiwanese natural lineages, compared to domesticated isolates from other parts of the world.

**Methods:** Current collection and genome sequence of natural isolates are still limited, owing to a lack of systematic sampling and sequencing effort. Therefore, we carried out sample collection and species identification since the beginning of 2016. So far, we have collected more than 500 samples from various locations and sequenced 55 *Saccharomyces cerevisiae* isolates.

**Results:** Around half of the isolates showed different levels of aneuploidies. We constructed a phylogeny consisting 287 isolates, among which five Taiwanese isolates formed a monophyletic group with six Chinese isolates, which were found to be the most diverged lineage to date. Among the most diverged lineage, we identified genomic regions with high FST between Taiwanese and Chinese populations. These natural isolates also exhibit clear population structures, and 13 of them displayed mosaic ancestries. More frequent introgressions were observed between natural lineages in Taiwan.

**Conclusions:** These information will allow us to further elucidate mechanisms underlying the species' evolutionary history.
Identification of the genome-wide expression patterns of small RNAs and mRNAs in monokaryotic and dikaryotic mycelia of *Pleurotus eryngii*

Junjun Shang, Dapeng Bao
Institute of Edible Fungi, Shanghai Academy of Agricultural Sciences, China

**Purpose:** The dikaryotic mycelia of *Pleurotus eryngii* arise by cell fusion of two monokaryotic mycelia containing different mating type alleles. We aimed to sequence the small RNA and the mRNA transcriptomes, and profile the small RNA and mRNA expression patterns in monokaryotic and dikaryotic mycelia of *P. eryngii*.

**Methods:** Illumina sequencing was used to generate the transcriptomes of the *P. eryngii* dikaryotic strain 'Xinghan' and its constituent monokaryotic strains '181' and '183', with three biological replicates for each strain.

**Results:** We obtained 18.6 Gb and 118.7 Gb raw data from small RNA and mRNA libraries, separately. The small RNA reads were compared with the known conserved miRNAs by BLASTing against the miRBase. A total of 946 putative miRNA-like RNA (milRNA) candidates were screened out based on the homologies. Differential expression analysis revealed that there were 196 up-regulated milRNAs in 'Xinghan' and 39 up-regulated milRNAs in '181' and '183'. Totally 13,003 unigenes were obtained from the mRNA sequencing and assembling. We found 447 differentially expressed genes (DEGs) between the dikaryotic strain and the monokaryotic strains. Some up-regulated genes in 'Xinghan' might be specific to the dikaryotic strain because of their extremely low expression levels in the monokaryotic strains. The DEGs between dikaryotic and monokaryotic mycelia were compared with the known sex development related genes in the model fungus *Aspergillus nidulans*, and 15 of the DEGs were found to be homologous to sex development related genes in *A. nidulans*.

**Conclusions:** Through this comparative transcriptomic analysis, we systematically revealed the differences of the small RNA and mRNA expression patterns between monokaryotic and dikaryotic mycelia of *P. eryngii*. The differential expression of milRNAs indicates that the dikaryotic mycelia might require more complex regulation of gene expression.
The amanitin-biosynthetic pathway in *Amanita*, *Galerina* and *Lepiota*

Hong Luo
Kunming Institute of Botany, Chinese Academy of Sciences, China

**Purpose:** Amatoxins, such as alpha-amanitin, are a group of cyclic peptide toxins biosynthesized by *Amanita* spp. In the fungal order Agaricales, some distantly related species in the genera *Galerina* and *Lepiota* are known to produce the same toxins. Roughly 90% of the death caused by mushroom poisonings is due to ingestion of these poisonous species. This study aims to gain insights into the biosynthetic pathway in the three agaric genera.

**Methods:** Through genomics and other omics studies, details of the architectures of the amanitin-biosynthetic pathway in the three genera were outlined. Genetic and biochemical approaches were adopted for function analyses of candidate toxin-biosynthetic genes.

**Results and Conclusions:** The results showed that these phylogenetically disjunct species use a related ribosomal mechanism on biosynthesizing the toxins. In addition, evidence suggested that the acquisition of the pathways in the three genera was an event based on horizontal gene transfer (HGT). They bear significant distinctions in terms of genomic arrangement, gene composition, and gene order and placement. The genomes of deadly *Galerina* and *Lepiota* showed they are capable of manufacturing only one or few cyclic peptides, while lethal *Amanita* species have the capacity of producing up to 40 potential cyclic peptides. The genomic architectures of the pathway facilitated the search for and the functional analysis of candidate toxin-biosynthetic genes. Via genetics, 6 new toxin-related genes were determined. Among them, a cytochrome P450 gene was shown to be involved in the toxin biosynthesis. Based on genetic and biochemical approaches, this gene was determined to have roles in oxidization of Ile and/or Pro residues in the cyclic peptide toxins.
Tolerance Induction of Polyhexamethylene Biguanide on *Purpureocillium lilacinum* Strains

Yikelamu Alimu1), Yoko Kusuya1), Naofumi Shigemune2), Kouichi Hosoya3), Takako Yamamoto2), Satoshi Nagai2), Hiroki Takahashi1), Takashi Yaguchi1)

1)Division of Bio-resources, Medical Mycology Research Center, Chiba University, Japan
2)R&D-Safety Science Research, Kao Corporation, Japan
3)R&D-Intellectual Property, Kao Corporation, Japan

**Purpose:** Recently, we have found a fungus to contaminate the product containing polyhexamethylene biguanide (PHMB), and this isolate was identified as *Purpureocillium lilacinum* based on morphology and phylogeny. The aim of this study is the elucidation of a resistant mechanism against PHMB on *P. lilacinum*.

**Methods:** At first, we induced the resistant strains against PHMB from the type strain of *P. lilacinum* by repeated cultivation in a medium containing high concentrations of PHMB. Then we analyzed the DNA sequences by Illumina sequencing in order to explore the presence of genetic mutations in the induced strains. Further, we made the *P. lilacinum* uracil auxotrophic strain, and the pyrG gene as a selection marker tried to knock out the mutant gene at the induced strain by CRISPR-Cas9 genome editing technique.

**Results:** Initially, we got the resistance induced strains from type strain cultured in medium containing high concentrations of PHMB. The induced strain growth rate on PHMB medium was accelerated and the MIC value was also increased. According to analyze the DNA sequences data we found a nonsynonymous point mutation in the mutant gene. Furthermore, the mutant gene has successfully knocked out from the induced strain by the novel CRISPR-Cas9 gene transformation method. The growth rate test and MIC in PHMB condition show that, when mutant gene does not exist, both of the values were dramatically decreased.

**Conclusions:** Knocking out the mutant gene on the induced strain, the resistance on the PHMB was extraordinary reduced. This gene is one of the important resistant factors in *P. lilacinum*. 
Isolation of genes differentially expressed during the fruitbody development of *Pleurotus ostreatus*

Masahide Sunagawa, Takeshi Nishimura

Forestry and Forest Products Research Institute, Japan

To analyze genes involved in fruit body development of *Pleurotus ostreatus* (FMC456), mRNAs from three different developmental stages: vegetative mycelium, primordium, and mature fruit body, were isolated and reverse-transcribed to cDNAs. One hundred and fifty random PCR amplifications were performed with the cDNAs.

**Materials and methods:** *Pleurotus ostreatus* (FMC456) was cultivated in a sawdust-medium containing beech sawdust and rice bran 3:1 (v/v). During the cultivation of FMC456, samples from three stages of development, i.e., mycelium, primordium (3-7 mm in diameter), and mature fruit body, were obtained. First-strand cDNA synthesis was performed in a reaction mixture containing 50 mM Tris-HCl (pH 8.5), 40 mM KCl, 5 mM MgCl2, 2 mM DTT, 850 μM each dNTP, 95 units of RNAase Inhibitor, 0.2 mM random primer, and 40 units of Superscript II Reverse Transcriptase. 10-mer RAPD primers were used to PCR amplify the second-strand cDNA. PCR was carried out as 45 cycles of the following thermal cycle: 30s at 95°C, 1 min at 50°C, and 2 min at 72°C.

**Results:** To detect changes in transcripts during fruit body development of *Pleurotus ostreatus* (FMC456), mRNAs were isolated from three stages of development. Then, reverse-transcribed cDNAs were used as templates for the following PCR. A total of 150 PCR amplifications were performed with 10-mer RAPD primers. Each PCR product separated by the agarose gel was resolved into 1 to 9 distinct DNA bands. A total of 482, 484, and 483 cDNA fragments were identified in the mycelium, primordium, and mature fruit body, respectively. The electrophoresis patterns of the PCR-amplified cDNA were confirmed as reproducible in two or three independent experiments.

**Acknowledgment:** This work was supported by JSPS KAKENHI Grant Numbers 18K19241
Stress responses to antifungal azoles and their regulation in *Neurospora crassa*

Xianyun Sun\(^1,2\), Chengcheng Hu\(^1,2\), Xi Chen\(^1,2\), Kangji Wang\(^1,2\), Yajing Yin\(^1,2\), Wei Xue\(^1,2\), Shaojie Li\(^1,2\)

\(^1\)State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, China
\(^2\)University of Chinese Academy of Sciences, China

To adapt to antifungal azole stress, fungi are able to change the expression of many genes. However, the regulatory mechanisms of transcriptional adaption to azoles in filamentous fungi are poorly understood. In this study, by RNA-seq analysis, we found that 488 genes were transcriptionally upregulated and 427 genes were transcriptionally downregulated by more than 2 folds in response to ketoconazole treatment in *Neurospora crassa*. Functional enrichment analysis showed that cellular protein metabolism, steroid metabolism and response to salt stress were the mainly bioprocesses in response to azole stress. These responsive genes in *N. crassa* were compared with previously reported in *Saccharomycete cerevisiae*, *Cryptococcus neoformans*, *Fusarium graminearum*, and *Trichophyton rubrum*. Genes related to ergosterol biosynthesis, drug efflux, and cellular stress responses are the common azole responsive genes in all five fungal species. The functions of some responsive genes were analyzed by drug sensitivity test for the corresponding gene knockout mutants. Five new transcription factors and one kinase regulating azoles stress responses were found in *N. crassa*. CCG-8 regulates the intracellular accumulation of azoles and several key genes required for basal resistance to ketoconazole stress, including the azole target encoding gene erg11. ADS-4 regulates several key genes participating in antifungal azole resistance, including genes coding the azole efflux pump CDR4 and the sterol C-22 desaturase ERG5. ADS-5 regulates ads-4 and a number of stress responsive genes. ADS-1 regulates cdr4 transcription and ergosterol biosynthesis. The serine/threonine kinase STK-17 mainly regulates ergosterol biosynthesis and drug accumulation in response to antifungal azole stress. As a negative regulator, CSP-1 represses the transcription of cdr4, stk-17, and several erg genes. Based on these results, a possible signal network for adaptive responses to azole stress in *N. crassa* was proposed.
Phylogenetic relationship of *Microporus* species in East and Southeast Asia

Bee Kin Thi¹, Yuko Ota², Tsutomu Hattori³

¹Forest Research Institute Malaysia, Malaysia
²College of Bioresource, Nihon University, Japan
³Forestry and Forest Products Research Institute, Japan

**Purpose:** *Microporus* spp. are widely distributed in temperate East Asia and Paleotropical regions. They play an important role as wood decomposers in the forest ecosystems. However, species recognitions within the genus *Microporus* were inadequate due to their variable morphologies. In this study, phylogenetic relationships of *Microporus* spp. in East Asia (including cool-temperate forests, warm-temperate forests, and subtropical forests) and South East Asia (tropical forests) were investigated to evaluate the species delimitation.

**Methods:** Deoxyribonucleic acid (DNA) was extracted from the fruiting bodies or mycelia of *Microporus* species. Molecular sequence data from two different regions, namely nuclear ribosomal DNA (nucLSU) and protein-coding genes (RPB2), were generated from the study. Phylogenetic analyses were performed with maximum parsimony and Neighbor-joining method.

**Results:** The phylogenetic trees based on nucLSU and RPB2 showed that species in *Microporus* consist of a monophyletic group within the family Polyporaceae. A total of 43 collections of *Microporus* from different forest types in Japan and Malaysia were grouped into seven different clades.

**Conclusion:** This study indicated that the biogeographical distribution range is an important feature for species delimitation of *Microporus* spp. in addition to using the morphological characteristics.
CRISPR/Cas9-driven simultaneous gene mutations in both nuclei of dikaryotic strain of *Pleurotus ostreatus*

Fuga Yamasaki, Takehito Nakazawa, Masahiro Sakamoto, Yoichi Honda
Kyoto University, Japan

**Purpose:** We recently reported an efficient genome editing to introduce gene mutations in a monokaryotic strain of *Pleurotus ostreatus* using CRISPR/Cas9. However, it would be required to obtain dikaryotic strains with mutations in the targeted genes in both nuclei for molecular breeding of cultivated mushrooms. In this study, we attempted to introduce gene mutations into both nuclei of a dikaryotic strain of *P. ostreatus* by CRISPR/Cas9 at one transformation experiment with the aim of developing an efficient methodology for molecular breeding of *P. ostreatus*.

**Methods:** We selected msh4 and mer3 as target genes to be disrupted. We previously reported that single-gene disruption of these genes impairs basidiospores production/formation in *P. ostreatus*. Plasmids containing the hygromycin B-resistant gene and expression cassettes for Cas9 and gRNA were introduced into the dikaryotic strain, PC9x#64. Among the obtained hygromycin B-resistant transformants, strains with clamp cells were selected. Genomic PCR was then performed to check if mutations were introduced in the target genes of both nuclei of the transformants.

**Results and conclusions:** Frequencies of introducing msh4 and mer3 single-gene mutations in both nuclei were 35% and 63%, respectively. Dikaryon with single mutations in msh4 or mer3 of both nuclei were selected and fruiting bodies were developed. Few basidiospores were produced in some of these fruiting bodies. These results suggest that gene mutations can be efficiently introduced in both nuclei of mushrooms by our CRISPR/Cas9 system.
Effects of hirA disruption on extracellular cellulase and xylanase activities and histone H3K4 dimethylation in *Pleurotus ostreatus*

Hongli Wu, Takehito Nakazawa, Ryota Morimoto, Shivani Shivani, Masahiro Sakamoto, Yoichi Honda
Graduate School of Agriculture, Kyoto University, Japan

**Purpose:** White-rot fungi efficiently degrade lignin in wood biomass. It was recently shown that mutations in the gene hirA, which encodes a putative histone chaperone that probably plays an important role in DNA replication-independent nucleosome assembly, cause defects in the ability of the white-rot fungus *Pleurotus ostreatus* to degrade wood lignin. Contrarily, transcriptome analysis showed that cellulolytic and xylanolytic enzyme genes were significantly up-regulated in hirA disruptants. Based on these our recent results, this study aims to explore the impact of hirA disruption on extracellular enzyme activities and histone modification in *P. ostreatus*.

**Methods:** Two hirA disruptants, hirAd#1 and hirAd#2, and their parental strain, 20b, were grown on beech wood sawdust medium. Extracellular CMCase (carboxymethyl cellulase) and xylanase activities were examined after 13- and 20-days cultivation. Chromatin immunoprecipitation (ChIP) analysis was carried out using mycelial cells grown for 13 days. In this study, anti-histone H3 and histone H3 N-dimethylated at K4 (H3K4diMe) antibodies were used.

**Results:** The extracellular CMCase and xylanase activities were shown to be higher in hirA disruptants than in 20b. ChIP analysis showed that H3K4diMe level was higher in 5'- upstream regions of some of cellulolytic enzyme genes up-regulated in hirA disruptants than in 20b.

**Conclusions:** The impact of hirA disruption on extracellular enzyme activities were consistent with changes in transcripts accumulation of corresponding enzyme genes. ChIP analysis suggests that histone H3 dimethylation at K4 is possibly associated with transcriptional regulation of the celluolytic enzyme genes in *P. ostreatus*.
**Purpose:** Fluorescent proteins serve as an efficient tool in observing organelle morphology and dynamics. Although changes in mitochondrial morphology during fruiting development has been suggested in agaricomycetes, it has yet to be observed in living cells. Visualizing organelles like endoplasmic reticulum (ER) and Golgi-equivalents (GEs) would be helpful in studying protein secretory mechanisms/pathways, which has not been well studied in agaricomycetes. The aim of this study is to visualize organelle dynamics using fluorescent proteins in hyphal cells and fruiting bodies of the agaricomycete *Pleurotus ostreatus*.

**Methods:** Plasmids containing expression cassettes for various EGFP and mCherry fluorescent fusion proteins were introduced into *P. ostreatus* strain PC9. Fluorescence in various parts of hyphal cells and fruiting body was observed via fluorescence microscopy.

**Results and Conclusions:** EGFP fused with N-terminal 100 and 99 amino acids of Cit1A and Cit1B, respectively, were shown to localize in mitochondria of hyphal cells, gill, stipe, and basidiospores; the morphology was dependant on the cell type. The mCherry attached with peroxisomal targeting signal at C-terminus was observed only in hyphal cells suggesting its absence in fruiting bodies. Sec24 fused with EGFP at C-terminus, Sec24-EGFP, was likely localized at GEs in every observed cell. Sec13-EGFP was likely observed in the ER and GEs of the hyphal cells and at the nuclear pore outer rings, suggesting its association with nucleoporins. It was absent in stipe cells. The fluorescent fusion proteins used in this study would be useful in future cell biological studies on development and protein secretion in *P. ostreatus*. 
Deletion analysis of the basidiomycete *Coprinopsis cinerea* cel6A promoter suggested new cellulose-responsive element

Dong Xuan Nguyen\(^1\),\(^2\), Takehito Nakazawa\(^1\), Masahiro Sakamoto\(^1\), Yoichi Honda\(^1\)

\(^1\)Graduate School of Agriculture, Kyoto University, Japan
\(^2\)Biotechnology Center of Ho Chi Minh City, Vietnam

**Purpose:** Mechanisms underlying transcriptional regulation of lignocellulolytic enzyme genes in wood-decaying basidiomycetes have not been well studied owing to the difficulty in conducting sophisticated molecular genetic study. *Coprinopsis cinerea* is one of model basidiomycetes for molecular genetic studies. In this fungus, cel6A gene encoding a cellobiohydrolase was shown to be upregulated in the presence of cellulose (Yoshida et al. 2009, Biosci. Biotechnol. Biochem.), one of major wood components. In order to gain an understanding of how lignocellulolytic enzyme genes are regulated in basidiomycetes, the cellulose-responsive element in Cccel6A promoter was investigated.

**Methods:** Deletion analysis of the promoter was done with the luminous shrimp luciferase gene (NanoLuc) as the reporter using a random integration approach in *C. cinerea* strain 326.

**Results:** Plasmid containing NanoLuc driven by the 799-bp cel6A promoter was introduced into *C. cinerea*. The luciferase activity of the obtained transformants was 43-fold higher on minimum medium containing microcrystalline cellulose (Avicel) as carbon source (MMA) than on MM containing glucose (MMG). Deletion of 22 bp (nucleotide positions from -799 to -778 upstream of the start codon) significantly decreased luciferase activity on MMA, but not on MMG. The cellulose-responsive element proposed in *Aspergillus nidulans* [5'-CC(A/T)6GG-3'; Yamakawa et al. 2013, BBRC] was not found in this 22-bp region.

**Conclusions:** The results suggest new cellulose-responsive element, which would provide useful knowledge about mechanisms underlying regulation of lignocellulolytic enzyme genes in basidiomycetes. We are currently conducting detailed deletion and mutation analyses of this promoter using a gene knock-in approach.
Comparative transcriptome analysis identified candidate genes involved in mycelium browning in *Lentinula edodes*

Hwa-Yong Lee, Suyun Moon, Hojin Ryu  
Department of Biology, Chungbuk National University, Korea

**Purpose:** To understand the molecular mechanisms underlying this critical developmental process in *Lentinula edodes*, we characterized the morphological phenotypic changes in a strain, Chamaram (strain # 07-84), associated with abnormal brown film formation.

**Methods:** To investigate the brown film formation characteristics in the mycelial tissue, the strain Chamaram, was cultured on oak sawdust medium. The sawdust media inoculated with Chamaram spawn were cultured under three conditions: (1) continuous darkness for 100 days to form the white mycelial film, (2) continuous darkness for 40 days and followed by under 16 h light/8 h dark cycle for 60 days to form the normal brown mycelial film, and (3) 16 h light/8 h dark cycle for 100 days to induce the partial brown mycelial film. These mycelial films were observed by FE-SEM and RNA-sequencing was performed.

**Results and conclusions:** Compared to white mycelial film, the hyphae in normal brown film was elastic and the hyphae in partial brown film was slender and observed sawdust powder. The formation of brown film in sawdust medium for *L. edodes* assumed to be associated with light sensing via photoreceptors such as FMN- and FAD-bindings, signal transduction by kinases and GPCRs, melanogenesis via activation of tyrosinases, and cell wall degradation by glucanases, chitinases, and laccases. This study analysed the expression patterns of light-induced browning-related genes in sawdust medium for *L. edodes*. The result of this study will provide information for further investigations of browning formation mechanisms in sawdust cultivation for *L. edodes*.
The virulence factors CoNpc1 and CoNpc2 are required for intracellular sterol transport and appressorial penetration of Colletotrichum orbiculare

Sayo Kodama, Naoki Kajikawa, Fumi Fukada, Yasuyuki Kubo
Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Japan

Purpose and methods: Fungal morphogenesis depends on accurate cell cycle progression. GTPase activating protein complex CoBub2-CoBfa1 interacts with a downstream factor, GTPase CoTem1, and is required for G1/S progression and pathogenesis in the cucumber anthracnose fungus Colletotrichum orbiculare. To elucidate the signal cascade of CoTem1, we screened physical interaction factors with CoTem1 by Yeast Two-Hybrid system and identified a phosphatidylglycerol phosphatidylinositol transfer protein CoNpc2. The Niemann-Pick type C (NPC) proteins NPC1 and NPC2 are sterol-binding proteins required for the export of lipoprotein-derived sterol from lysosomes in mammals.

Results: The CoNpc2 co-localized with CoNpc1 and a late endosome marker CoRab7 at vacuoles and granular body. Furthermore, sterol stained by filipin III was observed along the conidial and appressorium membrane in the wild type, whereas filipin staining in conpc1 and conpc2 mutants was recognized in the conidial vacuole, suggesting that NPC proteins are involved in sterol transport in C. orbiculare. The conpc1 and conpc2 mutants formed normal appressoria, however, penetration hyphae were not observed, thus the lesion formation of the host plant was markedly reduced. By contrast, the conpc1 and conpc2 mutants formed lesions similar to the wild type on wounded cucumber leaves, suggesting that NPC proteins are not required for invasive growth. Furthermore, TEM observation revealed immature appressorial cone of conpc2 mutant that lead to the defect in penetration peg formation on host plant surface.

Conclusion: Taken together, CoNpc1 and CoNpc2 function as a sterol transporter required for appressorium-mediated host cuticle penetration.
Purpose: To analyze genetic diversity and discriminate between *Lentinula edodes* varieties, we developed simple sequence repeat (SSR) markers that can complement internal genetic and external phenotypic traits.

Methods: To design reliable SSR markers from reference whole-genome sequencing data for *L. edodes*, we produced read data by re-sequencing genomic DNA extracted from 33 tested strains developed in East Asian countries (15 accessions originating from Korea, 12 accessions originating from Japan, and 6 accessions originating from China). Sequencing reads were then mapped to selected SSR motif regions of the reference genome.

Results and conclusions: Amongst all SSR motifs, 205 motifs that showed the largest conservation rate and diversity were selected, and sixteen genomic DNA SSR markers were developed. The number of alleles ranged from 3-14 and the major allele frequency was distributed from 0.17-0.96. The values of observed and expected heterozygosity ranged from 0.00-0.76 and 0.07-0.90, respectively. The polymorphic information content value ranged from 0.07-0.89. A dendrogram, based on 16 SSR markers clustered by the paired hierarchical clustering method, showed that 33 *L. edodes* varieties could be divided into three major groups and successfully identified. These SSR markers will contribute to the efficient breeding of this species by providing diversity in *L. edodes* varieties. Furthermore, the genomic information covered by the markers can provide a valuable resource for genetic linkage map construction, molecular mapping, and marker-assisted selection in *L. edodes*. 
Parasexuality in the root endophytic fungus, *Glutinomyces brunneus*

Noritaka Nakamura\(^1\), Chihiro Tanaka\(^2\), Yuko Takeuchi-Kaneko\(^2\)

\(^1\)Forest Research and Management Organization, Japan
\(^2\)Graduate School of Agriculture, Kyoto University, Japan

**Purpose:** Parasexual cycle has been hypothesized to play a key role in genetic exchange in some root endophytic fungi whose sexual structures have not been discovered. This manner of genetic process, however, has not been reported in these fungi including *Glutinomyces brunneus*, a member of the hyaloscyphaceous root endophytic fungi. Our aim was to examine parasexual ability of *G. brunneus*.

**Methods:** We developed mutant strains which were resistant to each of the two chemicals (Benomyl and Hygromycin B) by UV radiation and the Agrobacterium-mediated transformation. After 2 weeks of co-cultivation on 2% malt extract agar, the strains were transplanted onto new plates which contained both chemicals and dual-resistant strains were selectively cultivated.

**Results:** Significantly larger number of the dual-resistant strains were obtained when the co-cultivation was conducted between Benomyl- and Hygromycin B-resistant strains. All the analyzed dual-resistant strains had the Hygromycin B-resistant gene *hph* and the identical benomyl-resistant point mutation in the beta-tubulin gene, supporting transmission of the resistant genes between vegetative hyphae. Both the original and the dual-resistant strains had monokaryotic hyphae but 4 out of 8 dual-resistant strains contained both the wild and the mutated beta-tubulin genes. These results implied gradient loss of extra chromosomes of diploid nuclei after anastomosis and diploidization. This phenomenon was observed between the stains which shared the same precursors.

**Conclusion:** We concluded that the parasexual process was present in *G. brunneus*, but it was limited between genetically closely related individuals.
Optimization of protoplast generation and polyethylene glycol-mediated transformation of the pepper anthracnose pathogen *Colletotrichum scovillei*

Jong Hwan Shin, Hyun Hoo Park, Kyoung Su Kim
Division of Bio-Resource Sciences and BioHerb Research Institute, Kangwon National University, Korea

*Colletotrichum acutatum* is a species complex causing anthracnose disease in a wide range of crops. We isolated a *Colletotrichum* species from an infected pepper in Gangwon Province of South Korea. The isolate was identified as *C. scovillei* using combined sequence analyses of the nuclear ribosomal internal transcribed (ITS) region, partial sequences of the chitin synthesis 1 (CHS-1), β-tubulin (TUB2), actin (ACT), , histone 3 (HIS3), and an intron sequence of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH). *C. scovillei* is a member of the *C. acutatum* species complex and recently shown to cause anthracnose disease on pepper in South Korea. In this study, we optimized the transformation system of *C. scovillei*. The maximum release of protoplasts was produced from young hyphae of *C. scovillei* in an enzymatic digestion of 2% lysing enzyme and 0.8% driselase in 1M NH₄Cl for 3 h incubation. The optimal concentration of hygromycin B as a selection marker was 200 µg/mL in regeneration media. Next, we tested PEG-mediated transformation of *C. scovillei* protoplasts using 19 different loci. The average number of candidates for gene deletion mutants was 17.1% in the PCR screening. By southern blot analysis, we confirmed that at least one transformant among 2-5 PCR-screened positive transformants per locus had single copy integration.
Agrobacterium-mediated transformation of Agaricus bisporus with Iron-responsive transcription factor

MinSeek Kim\textsuperscript{1)}, Cheol-Won Yoon\textsuperscript{2)}, Hyeon-Su Rho\textsuperscript{1)}

\textsuperscript{1)}Division of Applied Life Science, Gyeongsang National University, Korea
\textsuperscript{2)}Division of Life Sciences, Korea University, Korea

The button mushroom Agaricus bisporus is an edible mushroom native to grasslands in Europe and North America and is one of the most commonly and widely consumed mushroom species throughout the world. Most of studies on this mushroom have been focused on its breeding, cultivation and related diseases. In this study, we have tried to use A. bisporus as a mean of biological factory to produce valuable matters. As a model study, we generated transformants that over-express siderophore that is known to iron-chelating compound and having anti-carcinogenic properties in recent study. hapX gene, which regulates siderophore biosynthesis, was inserted to binary vector pBGgHg. For constitutive expression of hapX, promoter was replaced to A. bisprous GPD promoter. Then pBGgHg-hapX was transformed to four hundred of A. bisporus gill tissue fragments through Agrobacterium tumefaciens-mediated Transformation(ATMT). Obtained transformants were then confirmed by DNA sequencing and mRNA expression. After then, stability was checked with serial subculture. Finally selected 3 transformants were cultured on compost PDB, and siderophore in culture medium was analyzed by CAS assay, Atkin's assay and HPLC.
The mitogen-activated protein kinase CsPMK1 regulates development and pathogenicity of *Colletotrichum scovillei*

Teng Fu, Kyoung Su Kim
Division of Bio-Resource Sciences and BioHerb Research Institute, Kangwon National University, South Korea

The phytopathogenic fungus *Colletotrichum scovillei* is a common agent to cause severe anthracnose disease of pepper (*Capsicum annuum*). To establish invasion in host cells, the *C. scovillei* develops specialized structures called appressoria to penetrate plant surface. The appressorium-mediated infection regulated by signaling pathways have been found to play indispensable roles in several plant pathogenic fungi. The conserved mitogen-activated protein (MAP) kinase PMK1 is known to orchestrate appressorium development during infection. To study the PMK1 orthologue in *C. scovillei*, a homologous replacement method was used to delete the gene CAP_011033.1 (named CsPMK1), which was predicted to encode a protein sharing 99% identity with PMK1 from *Magnaporthe oryzae*. Deletion of CsPMK1 resulted in a mutant showing normal mycelial growth and conidiation, and impaired conidial germination on both artificial surfaces and host plant epidermis, compared to wild type. This result indicates that CsPMK1 is related to conidial germination. However, germinated conidia of ∆Cspmk1 failed to produce appressoria on the same surfaces, which suggests that CsPMK1 is required for appressorium formation. The plant pathogenic assays displayed that ∆Cspmk1 was unable to cause lesion on both wounded and unwounded healthy pepper fruits, which implies that CsPMK1 may be associated with penetration and invasion in host plant. Localization of CsPMK1: GFP fusion protein showed that a strong GFP signal was detectable in developing conidia and appressoria, which is corresponded to the phenotypes of ∆Cspmk1. Collectively, these results suggest that CsPMK1 regulates infection-related morphogenesis of *C. scovillei*. 
Purpose: *Orientophila* (Teloschistaceae, Teloschistales) is characterized by crustose thallus with or without lobes, paraplectenchymatous cortex, zeorine ascomata, polardiblastic ascospore. Since the establishment of this genus, nine taxa have been described from Asian coasts. Although the genera was established based on tree topologies generated in previous molecular studies, morphological features to distinguish related genera is still not explored and needed revision. The main objectives of the present study were to establish a taxonomic framework within *Orientophila*.

Methods: A total of nine specimens were used for morphological observations and phylogenetic analyses. Phylogenetic analyses based on ITS, partial LSU, and partial mtSSU regions were conducted using ML and Bayesian methods.

Results: We found several cryptic species that were morphologically similar to *O. dodongensis*. These species could be separated on the number of apical cell, branching pattern of paraphysoid, and size and septum thickness of ascospore, and these features seem to be informative for species delimitation within this genus.

Conclusions: An expanded generic concept of *Orientophila* is considered to accommodate species having non-seashore habitats, non-saxicolous species that were traditionally especially emphasised as a useful character for genetic circumscription in lichenized fungi. Although the thickness and differentiation of thallus were unstable characteristics depending on different conditions (i.e., substrate and environment), their peridial features, such as the existence of the anthraquinones layer, positions of algae, the contexture of the peridium were always stable at even on different conditions. These anatomical feature could be useful for generic circumscriptions.
Additions to the diversity of Tubeufiales fungi in Thailand

Saranyaphat Boonmee, Kevin D. Hyde, Yong-Zhong Lu
Center of Excellence in Fungal Research, Mae Fah Luang University, Thailand

Tubeufialean fungi are functional decomposers and woody microorganisms that prefer living in temperate zones with high humidity. Recent research indicates that they have the potential to act as bio-control agents in plant disease control. The fungal order Tubeufiales is an important group of Dothideomycetes represented by a monotypic family, whose members are mainly saprobes. Despite their importance they have been relatively poorly studied worldwide and are hardly studied at all in Thailand. We deal with all 42 genera of Tubeufiaceae (Tubeufiales) as currently recognized. During our study, fresh collections from Thailand were made in an attempt to re-collect and isolate type species, discover unknown species, to provide epitype where necessary. In addition this will contribute to the sequence data of Tubeufiales in GenBank, which will help establish the sexual and asexual relationships based on phylogenetic evidence.
Two new species of *Pestalotiopsis* isolated from healthy loquat flowers in Japan

Takaki Nakashima¹, Syunsuke Nozawa², Yoshiki Takata², Kiichi Kaneko¹, Keisuke Uchikawa³, Kyoko Watanabe¹,²

¹Under Graduate School of Agriculture, Tamagawa University, Japan
²Graduate School of Agriculture, Tamagawa University, Japan
³Shimabara Regional development Bureau, Japan

**Purpose:** The genus *Pestalotiopsis* is ascomycetous belongs to the Amphisphaeriaceae. We found two new species which were isolated from healthy loquat flowers grown in Nagasaki Prefecture, Japan.

**Methods:** For taxonomic classification of both strains [TAP19N001, TAP19N002], molecular and morphological analysis were conducted.

**Results:** Phylogenetic trees [NJ, MP and MP trees] including reported species of the ITS + β-tubulin + tef1 regions placed each of the two new strains an independent species in *Pestalotiopsis*: s. str. Conidial morphology was compared based on 30 spores. Conidia of both strains were pyriform, slightly curved, and four septate with three median colored cells. TAP19N001 measurements were as follows: 12.5-19x3.5-5.5 μm spore size, three median cells are 7.5-11.5 μm length, and the number of appendages was 1-3, and 11.5-20.5 μm in length. Morphologically, the most similar species to this strain was *P. guepinii*; but conidia of TAP19N001 were smaller than those of *P. guepinii*. TAP19N002 were: 19.5-22x4-6 μm in size, three median cells are 11-13 μm in length, the number of appendages is 1-3, and 9-17 μm in length. Most similar species to this strain is *P. humus*: morphologically, but the maximum length 6 μm of the appendages of TAP19N002 is longer than *P. humus*.

**Conclusions:** These results provide each strain was new species belonging to the genus *Pestalotiopsis*: s. str
Four endophytic ascomycetes new to Korea

Dong Jae Lee¹, Jae Sung Lee¹, Hyang Burm Lee², Young-Joon Choi¹
¹Department of Biology, Kunsan National University, Korea
²Division of Food Technology, Biotechnology and Agrochemistry, Chonnam National University, Korea

Purpose: Ascomycota is the largest phylum of the Fungi, including approximately 6,600 genera. They were often isolated from soils, indoor air, and freshwater environments, but also from plants as pathogen or endophyte. In this study, four species of Ascomycota (two of Cladosporium, one of Daldinia and Nigrospora) were collected from the leaves of four woody plants (Camellia japonica, Ginkgo biloba, Quercus sp., Vitis vinifera).

Methods: Cultural characteristics were investigated on five different media (PDA, V8A, CMA, MEA, CZA) 3 days after inoculating at 25 C in darkness. Both BLASTn search and phylogenetic analysis were performed using the internal transcribed spacer (ITS) rDNA sequences, in addition to tef1 gene sequences for Cladosporium species.

Results and Conclusions: Based on cultural, morphological, and phylogenetic data, they were identified as Cladosporium anthropophilum, Cladosporium pseudocladosporioides, Daldinia eschscholtzii, and Nigrospora chinensis. Previously, some members of Cladosporium and Nigrospora have been recorded as endophytes inhabiting the leaves and stems of various plants. Also, Daldinia eschscholtzii was a wood-inhabiting endophyte or wood-decaying fungus. This is the first report of these four ascomycetes in Korea. Active and extensive surveys of fungal diversity in Korea over the past decade have dramatically increased the number of indigenous and unrecorded fungi in Korea.
The cAMP/PKA signaling pathway is one of the most important signal transduction pathways. The PKA (cAMP-dependent protein kinase A) plays an important role as downstream effector in this pathway. The PKA was found to be involved in the morphogenesis and virulence in phytopathogenic fungi. Many filamentous fungi possess two genes encoding the catalytic subunits of PKA. However, their specific and redundant functions have not yet been elucidated in Bipolaris maydis. In this study, to investigate roles of PKA in this fungus, we characterized two PKA catalytic subunit genes, pka1 and pka2, and generated disruption mutants of these gene, ∆pka1 and ∆pka2. ∆pka1 strains showed severely defected phenotypes in hyphal growth, pathogenicity and sexual development, whereas ∆pka2 strains showed similar phenotypes as the wild-type. To generate ∆pka1∆pka2 double mutants, we adapted two strategies, sexual hybridization with ∆pka1 and ∆pka2, and successive transformation by protoplast-PEG method. By sexual hybridization, an offspring colony of ∆pka1∆pka2 double mutant was not obtained. We carefully studied the genotypes of ascospores in asci using a tetrad analysis technique with a micromanipulator. The ascospores with ∆pka1∆pka2 genotype showed immediate melanization after germination, and stopped hyhal growth within 15 hours. Our observation suggested the possibility that double deletion of pka1 and pka2 is lethal. However, by protoplast-PEG method, we obtained ∆pka1∆pka2 candidate strains. The results of their crossing with the wild-type showed occurrence of the suppressing mutation for ∆pka1∆pka2 double mutant lethality in these strains. Currently, we are characterizing the causal mutation by genome comparison.
Cla4 PAK-like kinase regulates asexual/sexual development, pathogenicity and polarity in *Bipolaris maydis*

Yuki Kitade¹, Takuya Sumita¹, Kosuke Izumitsu², Chihiro Tanaka¹
¹Graduate School of Agriculture, Kyoto University, Japan
²Graduate School of Environmental Science, The University of Shiga Prefecture, Japan

PAK (p21-activated protein kinases) -like kinases are master regulators of development and morphogenesis, which are highly conserved among eukaryotes. The broad functions of PAK-like kinases in fungal development and pathogenicity have not been elucidated. In this study, to clarify broad functions of PAK-like kinases in growth, asexual/sexual reproduction and pathogenicity, we identified and characterized two PAK-like kinases, Ste20 and Cla4 in *Bipolaris maydis*, a causal agent of southern corn leaf blight. A single mutant of each Ste20 or Cla4 gene was viable, while the double mutant was not available, suggesting the possibility that these genes share essential roles in this fungus. ∆*cla4* strains showed severely defected phenotypes in growth, conidiation, and pathogenicity, while ∆*ste20* strains showed similar phenotypes with the wild-type. Mating tests clarified that Ste20 is dispensable for maternity, while Cla4 is essential for maternal pseudothecium development and also involved in ascospore development in paternal pseudothecium. In summary, Cla4 rather than Ste20 is critical for growth, asexual/sexual reproduction and pathogenicity. Next, in the detail examination of the growth defects in ∆*cla4* strains, we found the alternation of branching pattern in ∆*cla4* strains, elevated frequency of tip splitting. In fluorescent microscopy using FM4-64, temporal loss and subsequent split of vesicle assembly were observed at the hyphal tip in ∆*cla4*, supporting the importance of Cla4 in polarity. Through this study, we clarified conserved functions of Cla4-type in growth, conidiation and pathogenicity among filamentous fungi, and novel functions of Cla4-type in sexual reproduction and polarity.
Biocontrol and applied extension of antifungal bacterium
Burkholderia lata CAB13001 for anthracnose control on pepper

Soo Sang Hahm, Kwang Seop Han, Byung Ryun Kim, Mi Kyung Kwon, Sun Gye Lee
Chungchengnam-do Agricultural Research and Extension Service, Korea

**Purpose:** The anthracnose caused by *Colletotrichum acutatum* was found to be high virulent to the fruits and leaves on pepper. To control the pepper anthracnose, antifungal bacterium, CAB13001 strain which was isolated from natural soil, was selected as useful antifungal agent.

**Methods:** Antifungal bacterium was selected as the strain that inhibited pathogen's growth on PDA (Potato Dextrose Agar) and by testing in vitro bioassay. And also, the CAB13001 strain identified 16S rDNA sequencing.

**Results and Conclusions:** Their antagonistic activity against pathogens as *Sclerotinia cepivorum*, *Botrytis cinerea* and *Stemphylium* sp. was remarkable superior as well as *Colletotrichum acutatum*. In vitro bioassay using the green pepper fruit, CAB13001 strain suppressed the lesion development of Anthracnose disease, and its control value compared to the untreated one was 82.4% on pepper fruit in field test. Therefore, it can be applied to control Sclerotinia rot, Leaf blight, Gray mold and White rot disease of various vegetables including garlic, shallow, onion and lettuce based. By the way, as analysis of the nucleotide sequence of the gene 16S rDNA, this antagonistic bacterium was identified *Burkholderia lata*. 
Antifungal Activity of Philippine Endemic Plants Leaf Extracts on Fungal Growth and Spore Germination of *Aspergillus niger*

Melanie Martos Garcia, Maria Luisa Macalos Antao, Chloe Lynn Montilla Gotera, Rizzartein Samblaceno, Paulyn Jane Hermoso, Russel Valencia  
Davao Doctors College, Biology Program, Philippines

Aspergillus niger is a filamentous fungi known to cause the disease called black mold. Black mold is a ubiquitous occurrence on certain fruits and vegetables. In this study, the researchers wished to test the antifungal activity of the leaf extracts of the following endemic plants: Premna odorata Blanco, Petersianthus quadrialatus Merr, and Shorea astylosa Foxw., against the fungus Aspergillus niger. The leaf extracts that were obtained through ethanolic extraction were subjected to a rotary evaporator to obtain the pure extract. The pure leaf extracts of each endemic plant were diluted with distilled into 50% concentrations. Observations were done in 7 days for the colony growth and 6 hours for spore germination. Results revealed that the Yakal extract exhibited the most inhibition on colony growth with a mean growth of 0.54 cm and on spore germination among extracts with a mean value of 8.72%. The Alagao extract, however, showed no significant difference from the distilled water on colony growth and on spore germination. Since the Yakal extract showed the most antifungal potential among the leaf extracts, it is recommended to decrease its concentration to further test its maximum antifungal capability. However, since the Alagao and the Toog extract showed the least, and moderate potentials respectively, it is recommended to increase their concentrations to determine their effectivity.

Keywords: Mycology, Antifungal, Premna odorata, Petersianthus quadrialatus, Shorea astylosa, Aspergillus niger, Experimental, Davao City, Philippines
Pathogenic Sordariomycetes in China: *Allobotryotrichum blastospora*, gen. et sp. nov. on *Saccharum* (Poaceae)

Mubashar Raza\(^1,2\), Lei Cai\(^2\)

\(^1\)University of Chinese Academy of Sciences, China
\(^2\)State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, China

Fungi are under-explored with respect to novel species. A new genus, *Allobotryotrichum*, typified by *A. blastospora* sp. nov., is proposed based on distinct morphological characters and phylogenetic replacement. This genus collected from Guangxi Province (China) on sugarcane roots and characterized by its short conidiophore partitioned with septum, hyaline and broad irregular conidiogenous cells usually containing drop-like masses, hyaline to pigmented conidia with smooth or appearing to have concave dimple and sessile. Molecular phylogenetic analyses using rDNA internal transcribed spacer regions (ITS), large subunit (LSU), beta-tubulin (TUB2) and (RPB2) gene revealed that this genus sister to the genus *Myceliophthora* and *Thielavia* (Chaetomiaceae), but morphologically it is distinct from *Myceliophthora* and *Thielavia* in sterile setae and production of asexual morph, respectively. Morphologically, *Allobotryotrichum* should be compared to *Botryotrichum* (Chaetomiaceae) in its tuft of setae and globose or subglobose conidia, but they were phylogenetically distant. Photogenicity test confirmed that this genus is pathogenic to sugarcane plant.
Identification of an unknown *Colletotrichum* species found on *Schima mertensiana* in Bonin Islands, Japan

Hanh Hong Truong¹, Tomohiro Yamanaka², Tsuyoshi Ono³, Hayato Masuya⁴, Yuuri Hirooka¹

¹Department of Clinical Plant Science, Hosei University, Koganei, Japan
²Niihari Breeding Station, Musashino Seed Co., Ltd., Japan
³Ogasawara Subtropical Branch of Tokyo Metropolitan Agriculture and Forestry Research Center, Japan
⁴Department of Forest Microbiology, Forestry and Forest Products Research Institute, Japan

**Purpose:** The understanding of *Colletotrichum* diversity especially in tropical and subtropical regions plays an important role in more novelties. In this study, an unknown species of *Colletotrichum* isolated from anthracnose symptoms on leaves of *Schima mertensiana* (Siebold and Zucc.) Koidz at Bonin (Ogasawara) Islands, an archipelago of volcanic islands approximately 1000 km from Tokyo, was identified and characterized.

**Methods:** Isolates of the fungus were obtained by single-spore isolation method. Its morphological examination was performed on PDA at 25°C degree after seven days. Molecular phylogenetic trees of *Colletotrichum gloeosporioides* species complex were produced based on ITS, ACT, β-tubulin, CHS-1 and CAL.

**Results:** Our unknown species bare big straight conidia with rounded at the base and apex observed from black acervuli. Based on our phylogenetic analyses, this fungus was clearly distinct from all existed species within *Colletotrichum gloeosporioides* species complex. Our morphological comparison between the fungus and *C. henanense*, which is phylogenetically the closest species, showed that they are distinct from conidial size on PDA (12.5-19.0 x 5.0-6.5 μm in our fungus vs 8.0-17.0 x 3.0-5.5 μm in *C. henansense*).

**Conclusions:** We conclude that our fungus is a new species of *Colletotrichum*, and it might be possible that the fungus is endemic because it has been only isolated from *S. mertensiana* in Bonin Islands.
Importance of phylogenetic maintenance of scientific names of fungi in a culture collection

Takayuki Aoki, Shihomi Uzuhashi, Hideki Kito, Fukuhiro Yamasaki, Miyuki Yamazaki, Hiromi Nakajima, Mamoru Satou
Genetic Resources Center, National Agriculture and Food Research Organization, Japan

**Purpose:** Culture collections may accumulate various fungal species for long-term preservation and for distribution to the users. These fungal strains stored are labeled ordinally with scientific names that were given at the time of their deposition. Scientific names labeled to the strains may become handled by the users for various purposes. However, classification of fungi, as the basis of these scientific names, may become altered gradually or sometimes drastically during the progress of their taxonomy. In the NARO Genebank, Microorganisms Section (MAFF), phytopathogenic fungal strains isolated mainly from Japanese agricultural fields have been accumulated for more than 35 years. Scientific names labeled to the strains were basically those given by their depositors. Based on the progress/alteration of the taxonomic systems, the fungal names applied at the time of deposition may often become out-of-dated. Moreover, recent alteration of fungal nomenclature, i.e., ICNafp, claimed unification of fungal teleomorphic and anamorphic names, based on the one-fungus-one-name rule. Then, the deposited strains should be reidentified and their scientific names should be confirmed/updated based on the current classification to secure their accuracy.

**Methods:** Considering these backgrounds, the MAFF Collection is conducting comprehensive sequencing analyses of barcode gene regions of fungal strains preserved. By conducting Sanger sequencing of DNA, more than 13,000 strains out of ca. 21,000 fungal strains stored were molecularly analyzed and more than 4,000 strains were taxonomically verified.

**Results and conclusions:** Scientific names of the strains newly identified are indicated in the Microorganisms Database, together with the names recorded at the time of their deposition.
New species of the genus *Gnomoniopsis* isolated from Chestnut rot with notes on its life-cycle

Ayaka Minoshima¹, Takeaki Nishimura¹, Toyozo Sato², Donald M Walker³, Allison K Walker⁴, Atsuko Sasaki⁵, Yuuri Hirooka¹

¹Department of Clinical Plant Science, Hosei University, Japan
²Department of Agro-Food Science, Niigata Agro-Food University, Japan
³Department of Biology, Tennessee Technological University, USA
⁴Department of Biology, Acadia University, Canada
⁵Division of Fruit Production and Postharvest Science, Institute of Fruit Tree and Tea Science, NARO, Japan

**Purpose:** Rotted chestnuts (*Castanea* sp.) harvested were found in several areas of Japan. From the chestnuts, an unknown *Gnomoniopsis* species was repeatedly isolated. The goal of this study was to identify this fungus and to know its life-cycle.

**Methods:** The fungus was isolated from the rotted fruits, young fruits, male flowers, overwintered burrs and galls made by *Dryocosmus kuriphilus* during continuous survey in chestnut fields. Its morphological examination was performed on PDA at 25°C after 10 days. Molecular phylogenetic trees were produced based on five loci.

**Results and Conclusions:** The fungus is characterized by conidiomata with cream conidial droplets. Conidia are ellipsoid to oblong and 4.5-6.4 x 1.6-2.7 µm in size. We compared these morphological characters with *G. smithogilvyi*, which causes chestnut rot or brown rot in Oceania, Europe and United States. The conidial size easily distinguished the fungus from *G. smithogilvyi*. Our phylogenetic trees also showed that the fungus was distantly separated from *G. smithogilvyi* within the genus *Gnomoniopsis* and made a monophyletic clade. The fungus was not only isolated from harvested fruits but several parts of the chestnuts. These results suggest that the fungus produces conidia on overwintered burrs in early spring, then the conidia spread to male flowers, female flowers and the galls in late spring. Extra conidia are probably produced on these infected tissues, subsequently spread to young fruits, and finally the infected fruits rotted in autumn. Additional observation and examination using several tools such as molecular analyses are needed to demonstrate the hypothesis.
The first approach to taxonomical and phylogenetic properties of grapevine leaf rust (GLR) fungi in Vietnam

Izumi Okane1), Huy Duc Nguyen2), Cham Thi Mai Le3), Yoshitaka Ono4), Yuichi Yamaoka1)
1)Faculty of Life and Environmental Sciences, University of Tsukuba, Japan
2)Faculty of Agronomy, Vietnam National University of Agriculture, Vietnam
3)Division of Microbial Biotechnology, Biotechnology Center of Ho Chi Minh City, Vietnam
4)Faculty of Education, Ibaraki University, Japan

Purpose: In Vietnam, Trinh et al. (2001) reported 40 species of rust fungi, followed by Kaneko et al. (2007) reporting additional 13 species. Since the two publications, no systematic research has been conducted. Therefore, building the rust fungus inventory in Vietnam is scanty. It is, thus, natural consequence that rust fungi threating economically important crops, e.g., grapevines, coffee trees, and so on, are largely unknown. Wine grape plantation has become popular in central Vietnam. Despite of wide occurrence of grapevine leaf rusts (GLR), the causative agents of GLR have not been investigated. We aimed to explore biological properties of the GLR fungi in Vietnam and to determine their taxonomical and phylogenetic of status.

Methods: In 2017, uredinial stages were found on the leaves of cultivated grapevines in Vietnam Nat. Univ. of Agriculture and surrounding areas. Four fungal specimens were examined in morphology and molecular phylogeny.

Results and conclusions: The specimens were morphologically identified to Phakopsora. Urediniospores were 13-23 X 8-17 µm in size. The spore wall was 0.8-1.9 µm thick. Peripheral paraphyses were 40-69 X 7-14 µm in size. The wall was 0.5-2.5 µm dorsally and 0.8-3.5 µm apically. In a phylogenetic analysis using genomic rDNA ITS2 regions and LSU rDNA D1D2 regions, Vietnamese specimens were included in the Thai GLR fungus population, which was phylogenetically separated from other Southeast Asian and East Asian populations of GLR fungi. Further studies will reveal phylogenetic and taxonomic relationships between Asian GLR fungus populations and their geographic distributions.
Re-classification of *Melanopsichium inouyei* producing galls on buds of *Machilus* spp.

Saho Shibata¹, Makoto Kakishima²,³, Yuuri Hirooka¹

¹Hosei University, Japan
²Beijing Forestry University, China
³University of Tsukuba, Japan

**Purpose:** *Melanopsichium inouyei* transforms shoot buds of *Machilus japonica* and *Ma. thunbergii* (Lauraceae) into galls. This fungus was first collected in Tosa, Japan and described as *Uredo inouyei* by Hennings (1900). About thirty years later, Hino and Nagaoka (1931) reported a fungus collected from Miyazaki as a new smut species, *Cintractia machili*, without morphological comparison with *U. inouyei*. Now these two species have been treated under *Me. inouyei* by Ling (1953) on the basis of their morphological characters. The taxonomic concept of this genus and its relatives is, however, unstable because their morphological characters are relatively simple and detail molecular approaches have not been done. The objectives of this study are to reclassify this fungus using current detailed morphological and molecular analyses.

**Methods:** We obtained a fresh specimen on *Ma. japonica* from Miyazaki, the type locality of *C. machili*. Its morphological examination was performed with light microscope. Molecular maximum likelihood trees were produced based on ITS and LSU.

**Results and Conclusions:** Based on our morphological observation, the fungus bear abundant basidia with aseptate, elliptic basidiospores. The basidiospores that could germinate but not bud on culture were morphologically somewhat similar to ustospores. Our molecular phylogenetic analyses using ITS and LSU regions showed that the fungus fell into *Clinoconidium* clade (Cryptobasidiaceae, Exobasidiomycetes), not *Melanopsichium* (Melanopsichiaceae, Ustilaginomycetes). A new combination, *Clinoconidium inouyei*, is therefore needed.
Phylogeny and taxonomy of a rust fungus newly found on Lygodium in Thailand

Katsura Ohmachi¹, Izumi Okane², Yoshitaka Ono³,⁴, Jintana Unartngam⁴, Chanjira Ayawong⁵, Pattama Janruang⁶

¹Graduate school of Life and Environmental Sciences, University of Tsukuba, Japan
²Faculty of Life and Environmental Sciences, University of Tsukuba, Japan
³Faculty of Education, Ibaraki University, Japan
⁴Department of Plant Pathology, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Thailand
⁵Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Thailand

Purpose: A rust fungus was found on a primitive fern, Lygodium flexuosum, in Thailand. As the only rust fungus on Lygodium spp., Puccinia lygodii was previously known in the Americas. This study aimed at clarifying the phylogeny and taxonomy of the newly found Lygodium fungus in Thailand.

Methods: We carried out morphological observation of its uredinial stage by light and scanning electron microscopy and molecular phylogenetic analyses based on ITS and LSU regions. We also compared the Thai fungus with P. lygodii.

Results and conclusions: Uredinia of the Thai fungus were Milesia-type with peridium and occurred on the abaxial surface of a host leaf. Urediniospores wall was echinulate without smooth areas. Morphological features of uredinial stage of the Thai fungus were similar to that of P. lygodii in having Milesia-type uredinia and echinulate urediniospores. However, the Thai fungus was distinguished from P. lygodii that has smooth areas on the surface of urediniospores. In addition to morphological experiments, we were able to determine ITS and LSU partial sequences of the Thai fungus. Molecular phylogenetic analyses revealed that the Thai fungus positioned in a Milesina clade, one of the fern rust genera. The Thai fungus positioned distantly from P. lygodii in a phylogram generated. We concluded that the Thai fungus should be classified in the genus Milesina.
Foliar pathogenic fungal species associated with *Allium fistulosum* (Welsh onion) in Sanxing, Taiwan

Wang Chun-Hsiang1, Yen Chia-Yun1, Hiran Anjana Ariyawansa1, Tsai Yi-Chen2, Hsieh Wen-Tung2, Hung Ting-Hsuan1,3,5, Wu Meng-Ling4, Yu Pin-Hsin5

1Department of Plant Pathology and Microbiology, College of Bio-Resources and Agriculture, National Taiwan University, Taiwan
2Hualien District Agricultural Research and Extension Station, Taiwan
3Research Center for Plant Medicine, National Taiwan University, Taiwan
4Division of Forest Protection, Taiwan Forestry Research Institute, Taiwan
5Friendly Foundation, Taiwan

**Purpose:** To study the occurrence of leaf blight diseases of *Allium fistulosum* (Welsh onion) and identify the pathogens involved.

**Methods:** A survey was conducted during 2018 and 2019 in Sanxing Township, Taiwan. Samples with leaf blight symptoms were collected randomly from Welsh onion fields. Single spore isolation was done to obtain the pure culture of the fungal pathogens and their morphological characteristics were observed. In total, 36 fungal isolates were collected from leaf blight and phylogenetic analysis based on six genes (ITS, gapdh, tub2, cal, act1, and tef-1α) was performed to further confirm the phylogenetic placement of causative agents.

**Results:** According to the morphological and molecular data, those isolates belong to *Colletotrichum circinans*, *Colletotrichum spaethianum* and *Stemphylium vesicarium*. Pathogenicity tests conducted in the main Welsh onion cultivar ‘SiaoLyu’ using the spore suspension method indicated that all fungal species were pathogenic to *Allium fistulosum*. All taxa tested caused leaf blight in Welsh onion leaves although virulence among species varied from high to moderate.

**Conclusions:** General, *Stemphylium vesicarium* was the most widespread species associated with leaf blight of *Allium fistulosum* in Sanxing Township, Taiwan.
Analysis of Microbial Community during *Astragalus membranaceus* (Hwanggi) Cultivation Period

Jae Hyoung Yi, Young Moon Mo, Jung Su Jung, Jae Hee Won, Ye Ji Yoon, Gi Wook Lee
Ginseng and Medicinal Plant Research Institute, Gangwondo Agricultural Research and Extension Services, Korea

**Purpose:** Hwanggi was a major herb in Gangwon Province. Hwanggi increased its efficacy in 3 year cultivation, but the incidence of root rot and wilt disease was also increased. It was known that the causative fungi of the root rot disease were Phytophthora sp., and the causative fungi of wilt disease was Fusarium sp. Until now, the causative fungi of Hwanggi has been identified by cultivating the bacteria isolated from infected individuals or soil. However, microorganisms that did not grow in a specific medium were difficult to identify.

**Methods:** Microbial community analysis is a new technology to identify microorganisms that are difficult to separate by conventional culture method using next generation sequencing method. Therefore, microbial community analysis was used to analyze the microbial diversity and the causative microorganisms distribution in the Hwanggi field.

**Results:** In the 1-year-old and 2-year-old field of Hwanggi, there was no difference in the distribution of fusarium sp. between the diseased and non-diseased sites. However, in the 3-year-old Hwanggi field, the distribution of fusarium sp. was 8.3 times higher than that of the non-diseased sites. Microbial diversity was found in various species in the non-diseased sites. When the disease occurred, the causative organisms dominated and the microbial diversity was lowered.

**Conclusions:** Using the microbial community analysis method, it is possible to secure a healthy field when selecting the Hwanggi field. If the database was complementary, further studies of the Phytophthora sp. were required.

(Acknowledgement) This work was carried out with the support of “Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ0136252019)” Rural Development Administration, Republic of Korea.
The spatial distribution of *Serpula* spp. in the decayed woods of Sawara cypress and the decay development process

Toshihiro Umebayashi, Ryusei Haraguchi, Toshihide Hirao, Toshihiro Yamada
The University of Tokyo Chichibu Forest, The University of Tokyo, Japan

**Purpose:** Butt rot is induced in many adult trees of Sawara cypress (*Chamaecyparis pisifera*) plantations. As the main pathogen, we detected *Serpula himantioides* on the butt-rot Sawara cypress wood in Japan. Understanding the spatial distribution of *Serpula* spp. in the decayed wood needs to reveal the developmental process of the butt rot. In this study, we explored the spatial distribution of *Serpula* spp. and compared with other fungi in decayed Sawara cypress woods using real-time PCR and amplicon sequence analysis.

**Methods:** We used healthy and decayed wood samples of Sawara cypress. We extracted genomic DNA from their woods, and conducted real-time PCR of fungal rDNA 18S gene and amplicon sequence analysis of fungal rDNA ITS2 region.

**Result and conclusions:** There were some fungi in the decayed wood, and many sequence reads assigned to *Serpula* spp. were detected not only in the decayed regions, but also in the border of the decayed regions. Our results suggest that the decay caused by *S. himantioides* is developing in the front of advanced decayed area. We also revealed the *Serpula* spp. distribution in non-decayed heartwood.
Rapid characterization of wood-decaying fungal communities using the nanopore sequencing system

Toshihiro Yamada, Ryusei Haraguchi, Toshihide Hirao
The University of Tokyo, Japan

**Purpose:** Detection and identification of tree pathogenic fungi are essential for forest pest management and tree protection, especially of hidden wood decay without fruiting body. Conventional molecular diagnostic methods to detect and identify are reliable but laborious and time-consuming. Recent high-throughput DNA sequencing technologies potentially provide efficient tools for characterizing the ecology and functioning of pathogenic fungi. Here, we examined the applicability of a portable nanopore-based sequencing system to the detection and identification of wood-decaying fungi in the field.

**Methods:** Healthy and decayed wood samples were collected from *Chamaecyparis pisifera* wood, and genomic DNA was extracted from those samples. Based on the samples, a short-read Illumina amplicon sequencing of the rDNA ITS2 was conducted. Then, the qualitative (taxonomic richness) and quantitative (taxonomic relative abundance) recovery of taxa by long-read amplicon sequencing of fungal rDNA and shotgun metagenomics were tested using the Oxford Nanopore MinION platform.

**Results and conclusions:** The short-read sequencing found that OTUs of the genus *Serpula* dominated in fungal communities of decayed wood. The nanopore sequencing also succeeded in detecting dominant OTUs affiliated in the genus *Serpula*, although taxonomic richness was low and taxonomic relative abundance was biased in comparison with the results from the short-read sequencing. These results show that the portable nanopore sequencing system is potentially applicable to characterizing the significant components of wood-decaying fungi, implying an advantage for routine diagnosis of forest pathogens.
Pathogenicity and molecular diversity of *Fusarium solani* from different cucurbits in Taiwan

Benjapon Sritongkam\(^1\), Wen-Hsin Chung\(^1,3\), Pei-Hsin Chung\(^2\)
\(^1\)Department of Plant Pathology, National Chung Hsing University, Taiwan.
\(^2\)Taichung District Agricultural Research & Extension Station, Council of Agriculture Executive Yuan, Taiwan
\(^3\)Innovation and Development Center of Sustainable Agriculture (IDCSA), Taiwan

**Purpose:** *Fusarium solani* (Teleomorph: *Nectria haematococca*) is the most worldwide distribution fungus caused in several economic plants. It was considered to be plant pathogen and saprophyte. Among the pathogenic *F. solani*, the *F. solani f. sp. cucurbitae* (FSC) is important pathogen to cause crown rot and fruit rot of several cucurbit plants. Two races of FSC have been reported that race1 cause crown rot, root rot, stem rot and fruit rot on cucurbit plant and race2 only cause fruit rot. The purpose in this study is to carry out the pathogenicity and virulence of *F. solani* isolates from different cucurbits and analyze its characteristics.

**Methods:** A total of 34 isolates of *F. solani* from stem and fruit of pumpkin, cucumber, luffa, bitter gourd and muskmelon were identified following morphology and ITS rDNA. For pathogenicity test, these isolates (1*10^6 spore/ml) were inoculated on stem with 2 to 3 true leaves of plants and fruits based on wound inoculation. The total DNA extraction followed to use 5-7 days old mycelia and be extracted by DNA extraction Kit. The races identification was used the specificity primers of Fsc1-EF1/Fsc1-EF-2 and Fsc2-EF1/Fsc2-EF3. The ITS rDNA and TEF-1\(\alpha\) gene were analyzed for the relationship between theses isolates.

**Results and conclusions:** The pathogenic test showed that the *F. solani* isolates from different cucurbits might have cross pathogenicity on different cucurbits. Races analysis demonstrated that most of isolates could not be detected by specific primers. The phylogenetic analyses indicated that *F. solani* isolates could not be formed as one molecular phylogenetic group. These results revealed that the *F. solani* isolates from different cucurbits are not monogenic pathogen in Taiwan.
Characteristics of secreted in xylem (SIX) gene in *Fusarium oxysporum* from different cucurbits in Taiwan

Wen-Hsin Chung\(^1,2\), Ching-Chen Chung\(^1\), Chao-Jen Wang\(^1\)

\(^1\)National Chung Hsing University, Taiwan
\(^2\)Innovation and Development Center of Sustainable Agriculture (IDCSA), Taiwan

**Purpose:** *Fusarium oxysporum* is important fungal pathogen causing Fusarium wilt in many crops. More than 50 formae speciales have been recorded. The secreted in xylem (SIX) gene is considered as important effector gene to cause wilting symptom in host plants. Previous reports indicated that cross-infection has been observed between different cucurbits. The purpose of this study is to carry out the characteristics of secreted in xylem (SIX) gene in different formae speciales from cucurbits in Taiwan.

**Method:** Thirty-two *F. oxysporum* isolates from cucumber, loofah, bitter gourd and melon were analyzed in this study. The mycelia were used to extract DNA after 5 days cultured on PDA media. The primers and PCR condition for amplifying SIX gene were followed and modified according to previous studies. Moreover, the PCR products will be sequenced by Tri-I Biotech Co. and analyzed by MEGA7. For comparing with other *F. oxysporum* isolates in SIX gene, 2 non-pathogens, 15 human pathogens and 3 non-systemic pathogens of *F. oxysporum* were added in this study.

**Results and Conclusions:** The results showed that the SIX gene of non-pathogens, human pathogen and non-systemic pathogens of *F. oxysporum* could not be amplified by specific primers. Moreover, the pathogenic *F. oxysporum* from cucumber, loofah and bitter gourd could be separated into different molecular groups based on SIX 6 sequences. However, the isolates from melon could not form a single group. These results demonstrated that SIX gene has diversity between different formae speciales from cucurbits in Taiwan.
Phylogenetic species and biological characteristics of mango anthracnose pathogens in Taiwan

Chih-Li Wang¹,³, Wei-Lun Lin¹, Chung-Han Duan²

¹Department of Plant Pathology, National Chung Hsing University, Taiwan
²Pesticide Application Division, Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Taiwan
³Innovation and Development Center of Sustainable Agriculture, National Chung Hsing University, Taiwan

Purpose: The study was aimed to identify phylogenetic species of mango anthracnose pathogens in Taiwan, and to compare their morphology, virulence and fungicides sensitivity.

Methods: Ninety-six Colletotrichum isolates were collected from diseased fruits, asymptomatic twigs and peduncles of Irwin, Jin-Hwang and native cultivars. Isolates of C. gloeosporioides species complex (CGSC) and C. acutatum species complex (CASC) were analyzed. Representative isolates of CGSC and CASC were further identified by phylogenetic analyses with concatenated sequences of multiple genes. Irwin mango fruits and leaves inoculations were used to compare the virulence of phylogenetic species. Eight fungicides media were used to assay the fungicide sensitivity of phylogenetic species.

Results and conclusions: C. asianum is a dominant species from diseased fruits, asymptomatic twigs and three mango cultivars. C. fructicola and two minor species were also identified. A possible new species (temporarily named Casp) of CASC was dominant in peduncles. In virulence assays, C. asianum and C. fructicola caused significantly bigger lesions than the other phylogenetic species on fruits, but only C. asianum significantly caused bigger lesions on leaves. In fungicide sensitivity tests, all species except MML025 and Casp showed high resistance to thiophanate-methyl and carbendazim. All species were resistant to azoxystrobin and kresoxim-methyl, but sensitive to tebuconazole, difenoconazole and prochloraz. To accelerate the identification, a specific primer, Casia-F2 / Casia-R2 was designed to amplify a band of about 300 bp. from C. asianum. This study may provide the basis for exploring the biological differentiation among phylogenetic species, and for studying the ecology of the mango pathogens.
**Distribution of mating types of Phytophthora colocasiae in Japan**

Koji Kageyama\(^1\), Wenzhuo Feng\(^1\), Ayaka Hieno\(^1\), Kayoko Otsubo\(^1\), Haruhisa Suga\(^2\)

\(^1\)River Basin Research Center, Gifu University, Japan
\(^2\)Life Science Center, Gifu University, Japan

**Purpose:** In 2014, Phytophthora leaf blight of taro suddenly occurred in taro main production areas, Kagoshima, Miyazaki and Ehime prefectures of Japan. Furthermore, the disease has expanded to Saitama and Chiba prefectures since 2017. The pathogen, heterothallic Phytophthora colocasiae, is reported to be mainly A2 type all over the world. In preliminary test of Japanese isolates, we found self-fertile isolates as well as A1 type isolates. In this study, we investigated the distribution of the mating type in Japan.

**Methods:** The mating culture was performed on 20% V8 juice agar (V8A) medium using A1 (He-A1) and A2 (He-A2) type strains obtained in the preliminary test. The single culture were also prepared to determine a self-fertilization. The pathogenicity test was conducted on the detached taro leaf. The mycelial plug from the colony margin on V8A medium were placed on the leaf and measured the length of the lesion from the inoculated agar plug.

**Results:** All of 40 isolates in Chiba and Saitama were A2 type. On the other hand, 188 isolates from Kagoshima, Miyazaki and Ehime included He-A1 (8 isolates), He-A2 (108 isolates) and self-fertile (SF; 72 isolates). Out of 32 fields in which multiple isolates were examined, He-A1 and He-A2, He-A2 and SF, and He-A1, He-A2 and SF isolates were simultaneously obtained in two, 16 and five fields, respectively. The virulence was compared among the mating types, He-A1, He-A2, and SF. Although a variation in the virulence was found among the isolates, He-A1 isolates tended to be low virulent.

**Conclusion:** He-A1 and SF isolates as well as He-A2 isolates were found in Japan. Furthermore, different mating types co-existed in the same fields. The results indicate that oospore will be a survival structure in field soils and taro tuber seeds. An invasion of He-A1 and SF isolates or an occurrence of a mutation to SF isolates might contribute to a quick expansion over the main taro production areas.
Pathotype dynamics and genetic variability of *Colletotrichum scovillei* causing pepper anthracnose in Taiwan

Zong-Ming Sheu, Ming-Hsieh Chiu, Reuy-Jean Chang, Jaw-Fen Wang, Lawrence Kenyon

*World Vegetable Center, Taiwan, R.O.C*

**Purpose:** Fruit anthracnose caused by *Colletotrichum acutatum* sensu lato has been known the main pathogen constraints pepper production in Asia including Taiwan. This study aims to clarify the species following the recent taxonomy updates and analyze the temporal and spatial distributions on the pathotype and genetic diversity of Taiwan populations.

**Methods:** A total of 168 isolates collected from main production areas of Taiwan were analyzed. The virulence of all isolates was tested by inoculating fruits of two differential host genotypes. Forty-four strains, arbitrarily selected from diverse geographic origin and collection time, were used for sequencing of the nuclear ribosomal internal transcribed spacer (ITS) and AFLP assay. Twelve representing strains were further chosen for fungal identification through multi-locus phylogenetic analyses using combined sequences of the ITS, the β-tubulin (TUB2), actin (ACT), calmodulin (CAL) and GAPDH genes.

**Result and Conclusions:** *C. scovillei* was identified and 3 pathotypes within the species were determined. Among these strains, 77 and 98 strains were CA1 and CA2 pathotypes, respectively. Only 11 strains were CA3 and all of them showed weak virulence on both genotypes. CA2 were more virulent than CA1 and caused susceptible reactions on a pepper genotype derived from PBC932, which was resistant to CA1. CA2 was first found in 1997 but soon replaced CA1 in many locations of Taiwan. Since 2000, CA2 predominated in southern (78%) and eastern (96%) Taiwan; however, the CA1 was dominant in central Taiwan (59%). AFLP analysis showed that CA1 were genetically diverse, whereas CA2 were homogenous, mostly clonal.
Detection and absolute quantification of *Serpula himantioides* in wood of *Chamaecyparis pisifera* and soil of *C. pisifera* plantation by real-time PCR

Ryusei Haraguchi, Toshihide Hirao, Toshihiro Yamada, Toshihiro Umebayashi
The University of Tokyo, Japan

**Purpose and methods:** In *Chamaecyparis pisifera* plantations in Japan, it is presumed that butt-rot caused by *Serpula himantioides* has spread out. Proportion of heavily damaged trees exceeds 50% in some stands in the University of Tokyo Chichibu Forest. To examine the distribution of *S. himantioides* in decayed wood, we have designed specific primers derived from the rDNA ITS region and tried to detect *S. himantioides* in wood of *C. pisifera* and soil of *C. pisifera* plantation by quantitative real-time PCR with intercalator method.

**Results and conclusions:** Real-time PCR assays with the specific primers were positive for *S. himantioides* and negative for *S. lacrymans* of the same genus and *Coniophora puteana* of the same family. For absolute quantification, a standard curve was constructed by plasmid inserted with a fragment of rDNA ITS region of *S. himantioides*, where strong linearity was validated in the range of $10^1$ to $10^{10}$ copies. The rDNA copy number of *S. himantioides* was highest in slightly decayed wood, and was rather low in adjacent advanced decayed wood. *S. himantioides* was also detected in part of the soil of *C. pisifera* plantation.
Rust Fungi in Chiang Mai province, Thailand

Sukanya Haituk, Ratchadawan Cheewangkoon, Panatda Kankavee
Department of Entomology and Plant pathology, Faculty of Agriculture, Mai University, Thailand

Rust fungi (Basidiomycota, Pucciniomycetes) consist of the most species rich group of obligate, plant pathogenic fungi. They include many important plant pathogens. These fungi have unique systematic characteristics which differ from all fungal groups. Therefore, this study aims to identify and observe the critical morphology of these fungi in Chiang Mai province. Fifty symptomatic samples of rust fungi characterized by different pustules and rusty yellow spores occurring on various plants in Chiang Mai, Thailand, were collected during March 2015 to March 2016. Microscopic features of pustules, uredium, urediniospores, telium and teliospores for all specimens were examined using the stereo and compound microscopes. Slides were preserved in lactic acid. Results revealed that symptoms appeared on host plants as bright yellow or yellow-orange powdery sori on the upper and lower leaf surface. Pustules, occurring on round to ellipsoidal necrotic spots up to 1-3 mm diam., were erumpent, and ranged from yellow to orange or brown to dark brown. Rust caused by uredinium and telium was small, bright yellow or yellow-orange, pale brown to brown or hyaline. Urediniospores were ellipsoidal, subglobose or obovoid, yellow to pale yellowish brown, echinulate or verrucose. Teliospores were characterized by 2 celled-spores divided by a horizontal septum, with each cell being echinulate and having a germ pore and pigmented wall. The rust fungi were identified morphologically and divided into six families, namely, Pucciniaceae, Coleosporiaceae, Phakopsoraceae, Chaconiaceae Sphaerophragmiaceae and Uropyxidaceae. Ten genera were identified, including, Aecidium, Crossopsora, Coleosporium, Hemileia, Phakopsora, Puccinia, Maravalia, Nyssopsora, Tranzschelia, and Sphaerophragmium.

Keywords: Rust fungi Pucciniales morphology and Chiang Mai Province
New Cercosporid on *Gliricidia sepium* from Thailand

Nisachon Tamakaew, Patchareeya Withee, Panpraorn Phitchayawasin, Thitima Wongwan, Ratchadawan Cheewangkoon
Chiang Mai University, Thailand

Several species of Cercosporid fungi have been associated with leaf and fruit spot diseases of plants, of which two are regarded as highly pathogenic. Most of them are leaf-spot making plant pathogens with special phytopathological relevance. The main aim of this study was to provide a comprehensive database and identification of Cercosporid fungi on Mata Raton (*Gliricidia sepium*) in Chiang Mai province. Two samples with symptoms were collected from Chiang Mai University in January 2019. In this study, a multigene phylogenetic analysis (LSU, ITS, and RPB2) was performed with incorporating ex-type strains in Mycosphaerellaceae. In this study, a new genus sister to the Neophloeospora was introduced. The polyphyly of the Cercosporid fungi was observed among *Cercosporidum, Pluripassalora*, and *Pseucoercospora*. For some species, the both sexual and asexual morphs were obtained by different culture conditions.
Pathogenicity of five species of Botryosphaeriaceae isolated from Tectona grandis (teak); the pathogenic potential of Lasiodiplodia species

Mingkwan Doilom, Kevin D. Hyde, JianChu Xu
Kunming Institute of Botany, China

Purpose: We determined the pathogenicity of botryosphaeriaceous taxa associated with stem cankers and die-back of Tectona grandis and also surveyed the potential for pathogenicity of saprobic Botryosphaeriaceae from teak using Koch's Postulates.

Method: An inoculation trial is conducted using five different species from four genera in the Botryosphaeriaceae viz. Barriopsis tectonae, Dothiorella tectonae, Lasiodiplodia brasiliense, L. pseudeotheobromae and Sphaeropsis eucalypticola. Excised twigs of T. grandis were inoculated by placing agar plugs 5 mm3 of each actively growing colony on wound. Lesion development was recorded after 7 days of inoculation.

Result and conclusion: Lasiodiplodia pseudeotheobromae (strains MFLUCC 12-0772 and MFLUCC 12-0796) associated with stem cankers and die-back lesions are significantly pathogenic on T. grandis excised twigs. Lasiodiplodia pseudeotheobromae is however, associated with canker in only one natural forest site, and die-back in only one plantation site of the 35 sites surveyed. Thus, at this stage we concluded that L. pseudeotheobromae is a significant pathogen causing stem cankers and die-back of teak but it is not commonly found in teak plantations. The disease caused by Barriopsis tectonae, Dothiorella tectonae and Sphaeropsis eucalypticola are not statistically significantly different from the control and therefore are not considered as pathogens of teak; they are likely to be endophytes and/or saprobes. Lasiodiplodia brasiliense (strain MFLUCC 11-0414) and L. pseudeotheobromae (strain MFLUCC 12-0053) which are isolated from dead branches and twigs, respectively, are significantly pathogenic on T. grandis and are likely causal agents of lesions. Lasiodiplodia brasiliense (strain MFLUCC 11-0414) which was isolated as a saprobioc fungus, produced the longest lesions.
Rapid diagnosis using specific primer for *Passalora sequoiae*, a needle blight disease of Japanese cedar

Yuho Ando, Hayato Masuya
Forestry and Forest Products Research Institute, Japan

**Purpose:** Passalora needle blight is one of the serious disease in the nursery of Japanese cedar (*Cryptomeria japonica*). This disease is caused by *Passalora sequoiae* (Mycosphaerellaceae; Ascomycota) which is thought to be an invasive species from North America. With the recent increase in production of cedar saplings for re-afforestation, it is concerned that this disease will be prevalent. Therefore, accurate and rapid diagnosis method is required to control this disease. However, this disease is sometimes misdiagnosed as some other diseases with similar symptoms. The purpose of this study is to develop a series of rapid diagnosis protocol using species-specific primers.

**Methods:** Five species (*P. sequoiae*, *Pseudocercospora cryptomeriicola*, *Pse. pini-densiflorae*, *Cercospora exosporioides*, *Mycosphaerella laricis-leptolepidis*) that infest conifers were used to design the specific primer. Total DNA were extracted, and rDNA ITS region was sequenced. A dataset created from obtained sequence with those of other Mycosphaerellaceae species deposited in NCBI. Specific primers were designed from the aligned sequence dataset. Eight primers were designed on the basis of the unique sequences shown in rDNA ITS region of *P. sequoiae* compared with other fungi. Specificity of designed primers were checked by PCR amplification and electrophoresis.

**Results and conclusions:** In amplification using the specific primers in all combinations, *P. sequoiae* was specifically detected in one primer pair. By using this primer set, we could succeed specific and rapid detection from cultures and even from conidial masses and diseased leaves. Diagnosis method in this study can contribute to control and management of this disease.
Wood decay fungi isolated from heart rots on Japanese larch plantation trees in Nagano, Japan

Hiromu Hashitani1), Yuko Ota1), Tsutomu Hattori2), Kana Yamashita2), Toshihiro Yamada3), Yasuhisa Nishioka4), Kenichi Yanagisawa4), Kenichiro Toda4)

1) College of Bioresource Science, Nihon University, Japan
2) Forestry and Forest Products Research Institute, Japan
3) The University of Tokyo Chichibu Forest, Japan
4) Nagano Prefecture Forestry Research Center, Japan

Purpose: Decay damage of Japanese larch increases with tree age and differs depending on the growing environment. Many larch plantations in Japan have reached logging age. Therefore, it is important to know the current state of wood decay to control decay damage in the next generations. In this study, we isolated wood decay fungi from heart rot and determined the decay damage rate in Japanese larch trees in plantations at three sites.

Methods: Japanese larch trees were logged and collected from plantations at the following three sites in Nagano prefecture: Kawakami (115-yr old), Ina (55-yr old), and Sakuho (68-yr old). The decay damage rate was calculated as the proportion of decayed trees out of the total number of trees examined. Disks were collected from some of decayed trees and wood decay fungi were isolated from heart rot in the disks. Wood decay fungi were identified based on their rDNA ITS sequences and the morphology of their mycelia on PDA.

Results and Conclusions: Six species of wood decay fungi (Sparassis crispa, Phaelous schweinitzii, Porodaedalea chrysoloma, Oligoporus balsameus, Antrodia carbonica, and Coniophora sp.) were isolated from heart rot. The decay damage rates were 31%, 18%, and 18% in plantations at Kawakami, Ina, and Sakuho, respectively. Phaelous schweinitzii was frequently isolated from Japanese larch trees at all sites. Porodaedalea chrysoloma was frequently detected from Japanese larch trees in Kawakami and Ina, but not from those in Sakuho, suggesting that heart rot fungi in Japanese larch may depend on specific environmental or host conditions.
New disease of Sequoia sempervirens in Japan caused by the *Pestalotiopsis* spp.

Kiichi Kneko¹, Shunsuke Nozawa², Yoshiki Takata², Takaki Nakashima¹, Masaru Kanda³, Kyoko Watanabe¹,²

¹Under Graduate School of Agriculture, Tamagawa University, Japan
²Graduate School of Agriculture, Tamagawa University, Japan
³Green Doctors, Japan

**Purpose:** Pinnate compound leaves of *Sequoia sempervirens* which had turned to brown and withered were collected in Japan in March 2019. This symptom resembles the leaf disease of *S. sempervirens* caused by *Cercospora exosporioides*, but differs in that the disease which occurred in 2019 produced many acervuli on the back of the leaves. Conidia are typical morphologies of *Pestalotiopsis* fungi. The aim of this study is to diagnose this unknown disease.

**Methods:** Two monocultural strains (TAP19K001, TAP19K002) were obtained from the leaves which showed symptoms. Pathogenicity tests were conducted on the healthy leaves with mycelial discs. The pathogens were identified based on molecular and morphological analyses.

**Results:** Both strains had pathogenicity to healthy leaves with wounds and were re-isolated from the leaves showing symptoms. Conidia of TAP19K001: 14-24x6-7.5 μm in size; three median cells; mainly versicolorous, 12-17 μm; apical appendages, 6.5-19.5 μm. These characteristics are similar to *P. palmarum*. TAP19K002: 14-26.5x3.5-6 μm; three median cells, concolourous, 12-17 μm; apical appendages, 6-17 μm. Identity of nucleotide sequences of ITS+β-tubulin+TEF1 between these strains was 99.6%. NJ tree based on these sequences showed both strains were placed in the *P. chamaeropis* clade with 94% BS value. However, the conidial morphologies of both strains differed from those of *P. chamaeropis*.

**Conclusion:** This disease that affects the leaves of *S. sempervirens* was caused by an unidentified species belonging to the genus *Pestalotiopsis*. There is no record of this disease in Japan.
Phosphorescent quantum dots/ethidium bromide nanohybrids based on photoinduced electron transfer for DNA detection

Bi Lin
Shanxi Institute Of Medicine And Life Science, China

Mercaptopropionic acid-capped Mn-doped ZnS quantum dots/ethidium bromide (EB) nanohybrids were constructed for photoinduced electron transfer (PIET) and then used as a room-temperature phosphorescence (RTP) probe for DNA detection. EB could quench the RTP of Mn-doped ZnS QDs by PIET, thereby forming Mn-doped ZnS QDs/EB nanohybrids and storing RTP. Meanwhile, EB could be inserted into DNA and EB could be competitively desorbed from the surface of Mn-doped ZnS QDs by DNA, thereby releasing the RTP of Mn-doped ZnS QDs. Based on this mechanism, a RTP sensor for DNA detection was developed. Under optimal conditions, the detection limit for DNA was 0.045 mg L⁻¹, the relative standard deviation was 1.7%, and the method linear ranged from 0.2 to 20 mg L⁻¹. The proposed method was applied to biological fluids, in which satisfactory results were obtained.
Forest vegetation types reflect elevation and climatic effects on endophyte communities in Taiwan

Yu-Ling Huang
Department of Biology, National Museum of Natural Science, Taiwan

Purpose: Fungal endophytes are fungi inhabit in plants without causing any symptoms. Class 3 endophytes usually grow in the aboveground tissues and globally distribute in all known plants. Taiwan is an island located at the boundary of subtropics and tropics, and the large elevation differences result in diverse plant communities. Hence, the highly diverse endophytes on the island could be expected. This study aimed to examine the endophyte diversity in Taiwan and to understand the relationships among endophytes, plants and environments.

Methods: Foliar endophytes were isolated from representative gymnosperms and Rhododendron spp. across different vegetation types in the forests of Hehuanshan and Taipingshan in Taiwan. Based on the similarity of ITS sequences and the preliminary phylogenetic analyses, the diversity and community structures of endophytes in different locations, altitudes, vegetation types and host plants were assessed. In addition, the representative isolates were queried to the public databases to discover the potentially unknown species of fungi in Taiwan.

Results: All isolates belong to Ascomycota, and most of them are Sordariomycetes. The ecological analyses show the isolation frequency and diversity of endophytes decrease as the elevation increases, and the taxonomic composition, isolation frequency and diversity of endophytes differ as a function of host families and vegetation types. In addition to the host effects, vegetation types explain the integrated effects of elevation and climatic gradients on endophyte community structures.

Conclusions: This study represents the close relationships of host plants, environments, and endophyte communities. Fungal endophytes are important resources for discovery the fungal diversity of Taiwan.
Community analysis of fungi and bacteria related to tree root decomposition in Japanese forest soil

Nanako Ishiyama¹, Sakae Horisawa¹, Yoshimi Sakai²
¹Graduated school of engineering, Kochi University of Technology, Japan
²Kyushu Research Center, Forestry and Forest Products Research Institute, Japan

Purpose: The tree root play various roles, such as nutrient source for underground ecosystem, carbon stock and so on. These roles will retain until the tree root are completely degraded. Wood degradation above the ground is caused by termites and wood rot fungi, however, it is not clear yet what kinds of organisms degrade tree root under the ground, and its period. We analyzed fungal and bacterial communities which degrade buried in forest soil to search for wood degrader.

Methods: Wood species of Cryptomeria japonica were buried in five forest in Japan. They were buried at three kinds of level, on the ground, 5-10 cm, and 20-30 cm depth. Some of them were collected half or one year after and total DNAs were extracted. 16s rDNA and Internal transcribed spacer (ITS) region was amplified from extracted DNA by PCR, respectively. These PCR products were sequenced by using Miseq (illumina) and result sequences were searched using NCBI/DDBJ/EMBL database.

Results: The microbial communities in the sample specimens from the test sites were similar diverse between the depth of ground level and experimental periods, except for one site, in which the community had lower diversity of the community mainly consisted of Megacollybia and Bacilli.

Conclusions: In the early degradation, microbial communities are rich in variety. We have to study the succession of the communities along with the progress of further decomposition, as a future work. This work was supported by JSPS KAKENHI Grant Number JP12345678Grant-in-Aid for Scientific Research B (19H03012).
Purpose: Airborne substances, having a close relation with various dust including Kosa, are transported from the Asian continent to Japan by the air flow. Airborne microbes which have resistant to UV light and dryness can be transported alive over long distances and detected at several thousand meters above the ground. In forest ecosystems, various microorganisms play important roles in material cycles. The inflow of various airborne substances including microbes transported a long distance should be investigated from the view point of understanding the material circulation within the ecosystem. In this study, the microbial diversity in rain water was analyzed.

Methods: Rainwater was collected from test site forests on the Sea of Japan side (Ishikawa) and the Pacific side (Ibaraki) and then microbes in the rainwater were collected by membrane filter. Total DNA was extracted from them and barcode DNA fragments such as 16s rDNA and ITS region were amplified by PCR. The sequences of these DNA fragments were analyzed by NGS.

Results: Results showed that difference of the fungal diversity between both sides of the mountain range was detected while the bacterial diversity was similar. Currently, we are trying to detect and quantify functional genes.

Conclusion: The present study suggested that the diversity of microorganisms in rainwater can be detected and analyzed by DNA barcoding technique and NGS.

This study was supported by the River Fund of The River Foundation, Japan.
**Does water fungal eDNA assemblage reflect surrounding terrestrial mushroom community?**

Yoriko Sugiyama¹), Shunsuke Matsuoka²), Yoshito Shimono³), Masayuki Ushio⁴), Hideyuki Doi²)

¹) Graduate School of Human and Environmental Studies, Kyoto University, Japan
²) Graduate School of Simulation Studies, University of Hyogo, Japan
³) Graduate School of Bioresources, Mie University, Japan
⁴) Hakubi Center, Kyoto University, Japan

**Purpose:** As a tool for both terrestrial and aquatic fungal community investigation, the usefulness of water environmental DNA (eDNA) has been proposed. However, there are still limited knowledge on how much does water eDNA assemblage represent the surrounding terrestrial fungal community. Here, by comparing two-years data of mushroom observation and water eDNA assemblage, we aimed to test the representiveness of water eDNA assemblage to the surrounding mushroom community.

**Methods:** The study was conducted in “Inochi no Mori”, a restored biotope in Kyoto, Japan. From December 2016 to November 2018, we conducted water sampling and mushroom collection every month. After the fungal metabarcoding with MiSeq, those MOTUs (molecular operational taxonomic units) belonging to the known mushroom-forming families were extracted. Then the mushroom-forming fungal assemblages from water eDNA were compared with the observed mushroom community.

**Results:** In total, 308 suspected mushroom-forming fungal MOTUs were obtained from water eDNA. As mushroom, 157 morphospecies were observed, among which 155 were sequenced and clustered into 158 MOTUs. Among these 158 mushroom MOTUs, 82 MOTUs were shared with water. However, community dissimilarity values between sampling events showed a significant correlation between the observed mushroom and eDNA assemblage (mantel r = 0.34, p = 0.001), suggesting similar temporal dynamics patterns in eDNA and mushroom communities.

**Conclusions:** Our results showed that, more than half of mushroom-forming fungi can be retrieved from water eDNA. Further studies are needed to reveal the reason for the difference in the observed mushroom and detected eDNA assemblage.
Effectors of melanin synthesis in *Auricularia polytricha* albino mutant type

Hao-yu Chen¹), Wen-Hsin Chung¹), Yun-Sheng Lu²)

¹)Department of Plant Pathology, National Chung Hsing University, Taiwan
²)Division of Plant Pathology, Taiwan Agriculture Research Institute, Taiwan

**Purpose:** Melanin synthesis is important for *Auricularia polytricha* (Ap) to be dark for marketing. Recently, albino mutant type of Ap was found in field and become a new product. The aim of this study were: 1) carrying out characteristics of wild type (Wt) and albino mutant type (Ab) of Ap, such as phenotype, nutritional metabolism, and biochemical synthesis; 2) finding effectors causing albino fruiting body of Ap.

**Methods:** Wt and Ab strains of Ap were from Taiwan Agricultural Research Institute. Series test were conducted, including growth rate, pH, exposed in ultraviolet light, different fungicide tolerance, effect of different carbon and nitrogen sources on the growth of Wt and Ab. Cultivation of Wt and Ab with sawdust bag was exposed under blue light LED, fluorescent lamp and dark room. Then, the fruiting body were soaked with different concentration of tyrosine.

**Results and conclusions:** Optimum temperature for Ap growth is 30 °C. Moreover, Ap could grow better in alkaline than acidic condition. Ap could not use lactose, sorbose and NH₄NO₃ well compared with control (sucrose, NaNO₃). Effects of temperature, pH, UV-light and fungicides tests revealed mycelia growth of Wt and Ab showed non-difference. However, Ab type could recover their color from white to black after soaking their fruiting body in tyrosine solution, which is a precursor of melanin synthesis. Thus, the reason of albino mutant type of ab strain in this study might associate with upstream tyrosine synthesis, and it’s downstream of melanin biosynthesis still work.
Yielding Production of *Agrocybe cylindracea* on Different Substrate Packing under Greenhouse with the Evaporative Cooling System

Thidaporn Theunpao¹, Todsaporn Thongthieng¹, Supawadee Punyadee¹, Sermsiri Mayteeworakoon²

¹King Mongkut’s University of Technology Thonburi, Thailand
²National Center for Genetic Engineering and Biotechnology, Klong Luang, Thailand

*Agrocybe cylindracea* is one type of widely edible mushroom in Thailand. High yield cultivation and quality improvement of *Agrocybe cylindracea* were rarely studies. The aim of this study was to investigate the suitable substrate packing for *Agrocybe cylindracea* production under greenhouse with the evaporative-cooling system. This study used two different substrates, compacted substrate and loose substrate for 1 kg packing bag. Mycelium growth presented the significantly highest with 1 kg of loose substrate for 3 weeks. The yielding results were not significantly differed between the compacted and the loose substrate. Both results of the number of fruiting bodies shown significantly decreased. Length of fruiting body stalks was not significantly differed between the compact and loose substrate. The results of mycelium growth, yielding, and fruiting body production of the compacted substrate was selected for used to large scale cultivation. It showed significantly the highest percentage of yielding in the second harvesting cycle.
Study on bio-synthesis and enzymatic properties of xylanase by fermentation of *Hypsizygus marmoreus*

Chunyan Xie\(^{1,2,3}\), Zhiyan Wu\(^{1,2,3}\), Hongzhen Guo\(^{1,2,3}\), Juan Du\(^{1,2,3}\)

\(^1\)Department of Life Science and Technology, Langfang normal university, China
\(^2\)Technical Innovation Center for Utilization of Edible and Medicinal Fungi in Hebei Province, China
\(^3\)Edible and Medicinal Fungi Research and Development Center of Hebei Universities, China

Effects of different additional nutrients on the activity of xylanase and the enzymatic properties of xylanase were studied in this paper. Xylanase was secreted by submerged fermentation of *Hypsizygus marmoreus* with corn bran medium. Results showed that carbon and nitrogen source significantly influenced activity of xylanase, and higher activity was obtained when D-galactose or beef paste added in the corn bran medium. And the optimal condition of xylanase was at temperature of 50 °C, pH of 5.5, and Ca\(^{2+}\) and Cu\(^{2+}\) were the activation and inhibitor of xylanase, respectively. This result could provide the theoretical basis for the utilization of *Hypsizygus marmoreus* and for the preparation of some functional oligosaccharides.
Co-infection of a Novel Mycovirus and its defective RNA Detrimental to Biocontrol Properties of *Trichoderma* spp.

Jiaqi You\(^1,2\), Kang Zhou\(^1,2\), Xiaolin Liu\(^1,2\), Mingde Wu\(^1,2\), Weidong Chen\(^3\), Guoqing Li\(^1,2\)

\(^1\)State Key Laboratory of Agricultural Microbiology and Key Laboratory of Plant Pathology of Hubei Province, Huazhong Agricultural University, China  
\(^2\)The Key Laboratory of Plant Pathology of Hubei Province, Huazhong Agricultural University, China  
\(^3\)U.S. Department of Agriculture, Agricultural Research Service, Washington State University, USA

**Purpose:** *Trichoderma* spp. are a group of fungi commonly used as biocontrol agents for many plant diseases. This study aimed to analyze the full-length sequences of *Trichoderma harzianum* hypovirus 1 (ThHV1) and its defective RNA ThHV1-S, and to evaluate the possible effects of ThHV1 and ThHV1-S on the application of *Trichoderma* species for control of plant disease.

**Methods:** The genomes of ThHV1 and ThHV1-S were sequenced by using conventional methods. The horizontal transmission of ThHV1 and ThHV1-S was conducted by using the pairing culture technique. The mycoparasitism assays were conducted through dual-cultural test with *Botrytis cinerea*.

**Results and Conclusions:** The ThHV1 genome contained two Open Reading Frames (ORFs), namely ORF1 and ORF2. The start codon of ORF2 overlapped with the stop codon of ORF1 in a 43 nt long region. The polypeptide encoded by ORF2 of ThHV1 shared sequence similarity with those of betahypoviruses. Isolate T-70D carrying both ThHV1 and ThHV1-S showed abnormal biological properties, especially decreased mycoparasitism ability. Both ThHV1 and ThHV1-S could be vertically transmitted to conidia and horizontally transmitted to *T. harzianum* isolate T-68 and *T. koningiopsis* T-51, resulting in the decreased mycoparasitism ability of the derivative strains carrying both ThHV1 and ThHV1-S. However, the strains carrying ThHV1 alone were normal. ThHV1 was widely detected in *Trichoderma* spp. of China. Therefore, viruses in fungal biocontrol agents may also be a factor associated with the stability of their application.
Determination of Mycotoxins Isolated from Exposed and Refrigerated Smoke-Cured *Sardinella albella* and *Decapterus macarellus*

Rina Isabel G. David¹, Patricia Mae C. De Guzman¹, Arcibel Bal Bautista²

¹Biology (Class of 2016) , Department of Biology, College of Science, Polytechnic University of the Philippines, Philippines
²Faculty, Department of Biology, College of Science, Polytechnic University of the Philippines

**Purpose:** Tinapa is a popular Filipino food preserved through smoking. This cannot significantly extend the fish shelf-life and the product may still be susceptible to fungal contamination. Toxigenic fungi, under optimum conditions, secrete mycotoxins that could cause health problems upon ingestion or exposure. In the Philippines, no study was conducted yet regarding mycotoxin contamination on smoke-cured fish products. This study determined the potential toxicity of the fungal mycoflora of smoke-cured *Sardinella albella* (tamban) and *Decapterus macarellus* (galunggong) stored in two different conditions: exposed and refrigerated.

**Materials and Methods:** Three fishes per species were placed in an exposed area while another three were stored inside the refrigerator. Filamentous fungi were isolated, identified and quantified. Aflatoxins and ochratoxin A were extracted from the fish samples using liquid-liquid partitioning and the presence of mycotoxins was detected using thin layer chromatography.

**Results** showed that exposed samples were contaminated with *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus* and *Mucor* spp. The refrigerated tamban was contaminated with *Mucor* sp., while the refrigerated galunggong was not contaminated with filamentous fungi. CFU/g determination revealed that the exposed tamban was more contaminated compared to the other samples. TLC revealed that refrigerated tamban was positive for aflatoxins B2 and G1, exposed tamban was positive for aflatoxins G1 and G2, exposed galunggong was positive for ochratoxin A and the refrigerated galunggong was negative for all mycotoxin tests.

**Conclusion** This study concluded that factors such as morphology of the fish, temperature and other environmental factors influence fungal growth and production of mycotoxins on the smoke-cured fish samples.
Rediscovery of *Rhizopodopsis javensis*, monotypic genus of Indonesian Mucorales with taxonomically ambiguous status

Gayuh Rahayu¹, Rudy Hermawan², Iman Hidayat³, Yusuke Takashima⁴,⁵, Kazuhiko Narisawa⁴, Yousuke Degawa⁵

¹Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agriculture University, Indonesia  
²IPBCC, Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agriculture University, Indonesia  
³Microbiology Division, Research Center for Biology, Indonesian Institute of Sciences (LIPI), Indonesia  
⁴Ibaraki University College of Agriculture, Japan  
⁵Sugadaira Research Station, Mountain Science Center, University of Tsukuba, Japan

**Purpose:** Southeast Asia including Indonesia is a biodiversity hotspot of fungi. In 1958, a Dutch mycologist KB Boedijn investigated Mucorales in Indonesia and he proposed four monotypic genera *Phascolomyces*, *Rhizopodopsis*, *Sporodiniella*, and *Utharomyces*. Although these monotypic genera were rather rarely recorded, three genera (*Phascolomyces*, *Sporodiniella*, and *Utharomyces*) have been rediscovered. Yet, *R. javensis* has not been rediscovered and the status of this species remained unclear. Therefore, reinventing the specimens and evaluation of taxonomic status of this species were done in this study.

**Methods:** We conducted field surveys at Cibodas Botanical Garden which is the type locality of *R. javensis*. *Rhizopodopsis javensis* grown on fallen fruits were sampled, isolated and characterized on the basis of morphological observation. We also attempted to do a phylogenetic analysis for the first time using these cultures.

**Results and Conclusions:** During field surveys, we found *R. javensis* on fallen fruits of *Elaeagnus* which is the substrate for the original description, and fallen fruits of *Ficus* as an additional substrate. The diagnostic morphological characteristics of *R. javensis* such as umbellate sporangiophores and granulated sporangiospores were found on the living cultures from these substrates. These cultures were phylogenetically assigned to *Rhizopodaceae* (*Mucorales*) and related to *Rhizopus sexualis* and *R. stolonifer*. Furthermore, the results of additional investigations such as distributions, growth temperature, detailed morphologies and multi-locus phylogeny of *R. javensis* are discussed.
Role of citizen scientists and local natural history museums for mycology

Daisuke Sakuma
Osaka Museum of Natural History, Japan

There are numbers of local natural history museums in Japan, but rarely have mycological herbarium. Many of the Japanese local museums have only small numbers of scientists, so rarely have mycologists or experience to treat with fungal collection. Sometimes, citizen scientists who can collect good quality specimens served for local museums as guest scientists, and these amateur mycologists made basis of mycological collections in corporate with voluntary scientists. It is true to the Osaka Museum of Natural History, where had only one botanist, until 1974, then 2 botanists but no mycologist until1996. During that period, collection was cared by Toshiho UEDA, high school teacher and amateur mycologist and he made the basis of the collection. It is true to many local museums, collections depend on the local amateurs. So, it is important to educate local amateur scientists for gathering local mycological biodiversity information. Local museums have potentials to be a basis to invite young mycologists, empower local amateur, save and utilize lifelong personal mycological collections. Those will be valuable research materials for next centuries.

To promote these activities of local amateurs and museums, strong supports from academic mycologists and academic societies, science education authorities are needed. Such as repeated lectures for amateurs, useful textbooks, technical management supports for curators, and so on. Some of them have already started by Japanese Mycological society and National Museum of Nature and Science, Japan.
Phylogeny and morphology of micro-fungi associated with Pandanaceae

Saowaluck Tibpromma\textsuperscript{1,2}, Kevin D. Hyde\textsuperscript{1,2}, Peter E. Mortimer\textsuperscript{1}, Jianchu Xu\textsuperscript{1}

\textsuperscript{1}Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Science, People's Republic of China
\textsuperscript{2}Centre of Excellence in Fungal Research, Mae Fah Luang University, Thailand

**Purpose:** Collections of micro-fungi on Pandanaceae in China and Thailand resulted in the discovery of new/known species as the previous studies of micro-fungi on Pandanaceae have not included phylogenetic support.

**Methods:** This study, all data presented herein based on both morphological examination of specimens, coupled with phylogenetic sequence data to better integrate taxa into appropriate taxonomic ranks and infer their evolutionary relationships.

**Results and conclusions:** Inspiration for this study came from the book Fungi Associated with Pandanaceae by Whitton, McKenzie and Hyde in 2012. This study shown micro-fungi on Pandanaceae is particularly rich in hyphomycetes based on morphology and phylogeny support.
**Soil microfungal functional diversity in a Mexican cloud forest**

Cinthya Gabriela Leocadio¹, Patricia Velez¹, Yunuen Tapia²

¹Instituto de Biologia, Universidad Nacional Autonoma de Mexico, Mexico
²Escuela Nacional de Estudios Superiores, Campus Morelia, Mexico

**Purpose:** Montane cloud forest plays a key role in the regulation of hydrological and nutrient cycles. Even though, microfungi are important primary decomposers in soil communities, their taxonomic and functional diversity remains unknown in pristine montane cloud forests of the Neotropic. Herein, we evaluated the taxonomic diversity of soil microfungi in a pristine cloud forest of Mexico, and inferred their role in the regulation of carbon, nitrogen and phosphorus cycles using biogeochemical data.

**Methods:** Soil biogeochemical properties (enzymatic activity, C:N:P ratio, microbial C, N, and P, humidity and pH) and microfungal community composition in a pristine montane cloud forest of Mexico.

**Results:** Overall 101 microfungi were isolated, clustering into 37 OTUs based on the analysis of the ITS region. Our results on the total C:N:P ratio (256:18:1) agree with further published data for C-rich systems. Moreover, our data on the analysis of microbial enzymatic activity of laccase (6.18 ?mol h⁻¹ gSOM⁻¹), indicated significant lignin degradation processes. Additionally, the bioavailable C:N:P (85:5:1) and microbial C:N:P (75:8:1) ratios, suggest a rapid and efficient use of resources, attained by fungal organic matter mineralization and nutrient immobilization.

**Conclusions:** The comparison between microbial C:N:P and soil microbial mass data, reveals that phosphorus may represent a limiting factor for microfungi in this system. Decisively, our results suggest that microfungi are able to proficiently synthetize and use enzymes needed for the decomposition of C-rich organic matter, confirming their role as important players in the regulation of biogeochemical cycles through the organic matter decomposition in a Neotropical pristine montane cloud forest.
For the diversity analyses, endophytic fungi were isolated from 6 oak species in the spring and fall. Petioles, leaves, branches and stems were collected from Quercus acutissima, Q. mongolica, Q. dentata, Q. serrata, Q. variabilis, and Q. aliena, respectively. After surface disinfection with 0.5% NaOCl (3 min), 70% ethanol (1 min) and sterile water (15 sec.), segments were placed on PDA and kept at 25°C. A total of 463 isolates, i.e., 100, 148, 147 and 68 from the branches, leaves, petioles and stems, were identified by conventional and molecular methods based on rDNA ITS, LSU, TEF, TUB and RPB2 phylogenetic analyses. The genera, Stereum, Annulohypoxylon, and Daldinia, were dominant from the branches with 28, 17 and 16 isolates, respectively. From the leaves, Paraconiothyrium, Colletotrichum, Daldinia, Nigrospora, and Pestalotiopsis were dominant with 21, 19, 16, 12 and 10 isolates, respectively. The genera Paraconiothyrium, Daldinia, Diaporthe, and Colletotrichum were popular from the petioles with 23, 20, 15, and 11 isolates, respectively, while Daldinia, Biscogniauxia and Annulohypoxylon were common from the stems with 19, 19 and 8 isolates, respectively. Daldinia childiae was the most dominant species with 71 isolates, followed by Paraconiothyrium verruculosa with 45 isolates, Stereum hirsutum with 41 isolates and A. annulatum with 41 isolates. Q. serrata and Q. acutissima showed the highest similarity index of 0.68, while Q. dentata and Q. alien had the lowest of 0.23. The dominant species in the spring were D. childiae (53 isolates), P. verruculosa (46 isolates) and S. hirsutum (46 isolates), while D. childiae (62 isolates) and B. maritima (25 isolates) in the fall. Diversity, abundance, dominance and evenness indices from all parts of 6 oak species in the fall were higher than those in the spring.
Tree functional diversity regulates the diversity of soil fungal guilds in cool temperate montane forests

Nobuhiko Shigyo, Toshihide Hirao
The University of Tokyo Chichibu Forest, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan

Purpose: Soil fungal communities exhibit guild structures, composed primarily of plant saprotrophs, ectomycorrhizal fungi, arbuscular mycorrhizal fungi, and plant pathogens, which are related to forest ecosystem functioning. Although the evidence is mounting that plant diversity can promote soil fungal diversity, there is a lack of knowledge about how forest plant diversity affects fungal guild diversity. Here, we examine how climate conditions, soil properties, and plant diversity affect the diversity of soil fungal taxa and guilds in cool temperate forests.

Methods: Fungal communities were investigated in 60 survey plots across elevational gradients in the University of Tokyo Chichibu Forest in central Japan. Structural equation modeling (SEM) was used to consider the causal relationships between environmental factors (i.e., climate conditions, soil properties, and plant diversity) and soil fungal diversity.

Results: The observed diversity of fungal guilds was positively correlated with tree functional diversity and the taxonomic richness of saprophytic fungi. The SEM showed that the diversity of soil fungal taxa and guilds was mainly affected by both the direct effects of soil properties and the indirect effects of climate conditions. However, in the SEM, the significant path between tree functional diversity and fungal guild diversity improved the model fit and the coefficient of determination for fungal guild diversity.

Conclusions: Our study found a vital effect of tree functional diversity on the diversity of soil fungal guilds. These results imply that an increase in the variety of plant litter might facilitate the diversity of saprotrophic fungi, which shape guild structures of soil fungi in cool temperate forests.
The effect of thinning on the succession of deadwood inhabiting fungi and bacteria in a tropical forest of Taiwan

Yu-Ting Wu
Department of Forestry, National Pingtung University of Science and Technology, Taiwan

**Purpose:** Coarse woody debris (CWD) namely deadwood, the major organic carbon pool in forest ecosystem provides plentiful nutrient sources and shelter for organisms, primarily fungi and saproxylic insects. However, some studies currently revealed that deadwood inhabiting bacteria have emerged as an important group taking part in the decomposition, but the interaction between fungi and bacteria still remains unknown.

**Methods:** The study was conducted at a 1-ha experimental site, established in five tree thinning degrees in Tajen Forest Station, located in tropical area of Taiwan. Fresh logs of *Cyclobalanopsis pachyloma* and *Machilus thunbergii* had been randomly laid on the ground for three years. Bacterial and fungal composition of the initial phase of the deadwood decomposition were disentangled by Illumina MiSeq and PacBio Sequel, respectively.

**Results and conclusions:** A total of 7,333,022 qualified bacterial and 243,615 qualified fungal sequences were obtained from 40 logs. A total of 18 phyla 45 classes 136 families 221 genera 242 species were detected with *Enterobacter sp.*, *Pseudomonas viridiflava* and *Pantoea agglomerans* dominated the bacterial assemblage while 8 phyla 40 classes 289 families 637 genera 976 species were detected with *Pestalotiopsis sp.*, *Pestalotiopsis maculans* and *Cryptococcus sp.* dominated the fungal assemblage. NMDS based on deadwood inhabiting bacterial and fungal composition at initial decayed phase indicates that both assemblages differed between the two deadwood species across the five thinning treatments. Moreover, both assemblages did not significantly interact at initial decomposition based on the procrustes analysis ($m= 0.96, r= 0.25$). In conclusion, both microbiome and mycobiome of initial decomposition were affected by deadwood species, not tree thinning degrees.
Diversity of saprobic fungi on wood of *Magnolia garrettii* (Craib) V.S.Kumar

Rampai Kodsueb
Faculty of Science and Technology, Pibulsongkram Rajabhat University, Thailand

The purpose of this study was to evaluate fungal diversity of saprobic fungi found on woody litter of *Magnolia garrettii* (Craib) V.S.Kumar collected from Phu Hin Rongkla National Park in Phitsanulok Province, Thailand during late June 2008 to April 2009. One hundred wood samples have been collected and examined, the fungal diversity and community then were compared with the previous study of Kodsueb et al. (2008). Based on morphological characteristics, 141 taxa were obtained and classified as 40 ascomycetes, five basidiomycetes, 42 lichens, one unidentified taxa and 53 anamorphic fungi. The number of taxa recovered indicated that dry season samples support a more diverse fungal community than samples collected in the wet season although the common genera of fungi obtained during each season were similar. Distinct fungal communities of saprobic fungi collected from each site suggest that site characteristics affect the community composition. Samples from Phu Hin Rongkla National Park provided higher numbers of fungi (especially lichens) than were collected in a previous study in Doi Suthep-Pui National Park in Chiang Mai Province, with relatively few species overlapping in the two sites.
Determination of fungal indicator taxa of the tidal flats in South Korea by metagenomic analysis

Young Mok Heo, Hanbyul Lee, Sun-Lul Kwon, Jae-Jin Kim
Division of Environmental Science & Ecological Engineering, Korea University, Republic of Korea

**Purpose:** Tidal flats were widely distributed in South Korea, but greatly reduced due to the reclamation and the construction of salt ponds. Recently, the government has tried to restore it, as the ecological importance of the tidal flats were reevaluated. However, there was no reliable indicator for assessing the recovery, and it was necessary to find the indicator taxa that exist specifically in the tidal flats that are not interfered by human.

**Methods:** Environmental DNA was extracted from the sediments of the tidal flats and closed salt ponds in the west coast of South Korea. The internal transcribed spacer region sequences were amplified and sequenced using an Illumina MiSeq platform. Four kinds of statistical analyses were conducted using the fungal operational taxonomic units and their abundance data.

**Results and conclusions:** The indicator taxa selected for each analytical method were different from each other. Among them, *Glomeromycetes* and *Umbelopsidomycetes* were commonly selected from three different analyzes, and determined to be the indicator taxa of the tidal flats in the west coast of South Korea. Since *Glomeromycetes* are mycorrhizal fungi, it was suggested that they are strongly related to the indigenous halophytes like *Phragmites australis*. We demonstrate that these two indicator taxa can be used as reliable indicators for tidal flat restoration assessment.
Diversity and Distribution of Lichens in Balochistan, Pakistan: an Overview

Abdul Nasir Khalid
University of the Punjab, Pakistan

Purpose: In order to explore lichen diversity in Balochistan province, current study is carried out. Lichens are symbiotic phenotype of lichenized fungi and play important role in soil formation, nutrient entrapment and mineral recycling. Balochistan is largest province of Pakistan in terms of area but least explored for lichen diversity. Most part of Balochistan is semi arid and vegetation is scarce.

Methods: Recently, two sites, Ziarat and Mastung, Balochistan were selected to study lichen flora of this region. During visit of these areas, 40 specimens were collected from rocks and studied using morpho-anatomical and molecular analyses.

Results and Conclusions: From boulders near roadside to rocks in deserts, interesting and diverse lichen communities were observed indicating presence of rich diversity of this group of fungi present but hidden in Balochistan. Lichen communities present in this area are different from those observed in other parts of the country, especially Swat from where 26% of total lichen flora of Pakistan is reported. These lichens belong to families Acarosporaceae, caliciaceae, candellariaceae, lecanoraceae, Megasporaceae, Teloschisteacea and Verrucariaceae. Among these, 10 lichen species are new to science and 6 are new records for Pakistan. Genus Rinodinella is first time reported from Pakistan while all 40 lichens are new records for Balochistan as before this study, only 9 lichens are reported from this province. More extensive surveys are required to fully explore lichen flora and evaluate its ecological role.
Exploring the transcriptome-methylome dynamics in *Termitomyces eurhizus*

Yu-Chun Huang, Pao-Yang Chen
Institute of Plant and Microbial Biology, Academia Sinica, Taiwan

**Purpose:** *Termitomyces eurhizus* is an edible fungus which has fruiting body produced through symbiotic association with fungus-growing termites, black-winged subterranean termite (*Odontotermes formosanus*). Until now, the fruiting body can only be acquired from the wild, but not able to be cultivated in the lab condition, making it difficult for mushroom farming. While the mystery of fruiting in another basidiomycete *Coprinopsis cinerea* is well studied it is never clear with *T. eurhizus*, possibly due to its complicated symbiotic relationship with termite, and the precise environmental conditions implying epigenetic co-regulation. Our long-term goal is to understand the fruiting dynamics and life cycle of *T. eurhizus* through molecular profiling including both genetics and epigenetics.

**Methods:** In this project we performed whole genome bisulfite sequencing and RNA sequencing for 5 critical stages of *T. eurhizus* life cycle, and compare their molecular profiles.

**Results and Conclusions:** We have identified one specific tissue displaying a unique pattern of DNA methylation at CHG sites, that may associate with the fruiting dynamics. This pattern may be served as potential biomarkers for fruiting initiation. In addition, we also compared the findings in *T. eurhizus* with the fruiting in *C. cinerea* and *S. commune*. Our work reveals an important local fungus-termite system in Taiwan, with a great potential to decipher its fruiting mystery and to benefit mushroom farming in agriculture.
Dikaryotization seems essential for hypha formation and infection of coccid in the life-cycle of *Auriculoscypha anacardiicola*

Patinjareveettil Manimohan, Anjitha Thomas  
Department of Botany, University of Calicut, India

**Purpose:** *Auriculoscypha* (Basidiomycota, Pucciniomycetes, Septbasidiaceae) is a monotypic genus and *A. anacardiicola*, the only known species, is endemic to southwest India. This fungus is part of an interesting instance of plant-insect-fungus interaction. It is invariably associated with a coccid, *Neogreenia zeylanica*, belonging to the scale insect family Margarodidaeae. This fungus gets its nourishment from the coccid using its haustoria that enter the body cavity of the insect and submerge in the haemolymph, while the insect feeds on the sap of the sieve tubes of host trees using its stylet. The basidiocarps of the fungus emerge from a tubercle partially immersed on the bark of the host tree within which the enslaved coccids reside. In several phytopathogenic fungi such as the rusts and the smuts, only a dikaryon can infect the host tissues. The present study was an attempt to verify the hypothesis that dikaryotization is a prerequisite for hypha formation and coccid-infection in the life-cycle of *A. anacardiicola*.

**Methods:** Light-microscopic observations using Giemsa staining and fluorescent microscopic observations of DAPI-stained material were made to determine the number of nuclei in cells/hyphal compartments at various stages in the life-cycle of the fungus.

**Results:** Our studies revealed that while the basidiospores were consistently unicellular and uninucleate at the time of discharge, the hyphae of *A. anacardiicola* were consistently dikaryotic.

**Conclusions:** Coupled with the observed inability of a single basidiospore to establish a mycelium, our study indicates that dikaryotization is essential for hypha formation and the establishment of infection in the life cycle of *A. anacardiicola*. 
Comparison of fungal flora in female adults of an ambrosia beetle, *Euwallacea interjectus* (Scolytinae), among wild and rearing populations

Zi-Ru Jiang\(^1\), Hayato Masuya\(^2\), Hisashi Kajimura\(^1\)

\(^1\)Graduate School of Bioagricultural Sciences, Nagoya University, Japan
\(^2\)Forestry and Forest Products Research Institute, Japan

**Purpose:** The aim of this study was to identify and compare the fungal symbionts among wild and rearing *Euwallacea interjectus*, the fig tree-boring ambrosia beetle.

**Methods:** Dispersal female adults of *E. interjectus*, which were collected from logs of infested fig tree (51 individuals) caused by the plant pathogenic fungus, *Ceratocystis ficicola*, and from artificial diets (54 individuals), were used for fungal isolation. After surface sterilization, head, thorax and abdomen of the adults were placed on PDA plates at 25°C. Isolated fungi were identified based on morphological characteristics and DNA sequence data.

**Results:** In total of the tested female adults, 13 filamentous fungi were detected in the body of wild population. Specific fungus, *Fusarium* sp., was dominant in head, probably because of its oral mycangia (fungus-carrying organ). By contrast, 9 filamentous fungi and 1 yeast were found in rearing population, showing that *Fusarium* sp., *Fusarium* cf *solani* and *Meyerozyma guilliermondii* (yeast) were more frequently isolated from head than thorax and abdomen.

**Discussion:** Regardless of wild and rearing populations, *Fusarium* sp. is closely associated with female adults of *E. interjectus*. The present investigation also showed *C. ficicola* is not transmitted via mycangia of *E. interjectus*.  

---

Asian Mycological Congress 2019
Purpose: To demonstrate the plant-growth promoting potential of a wood-decay mushroom.

Methods and results: A wild strain of a white rot fungus (*Pleurotus pulmonarius*) was found to convert 10 mM L-tryptophan (TRP) to indole-3-acetic acid (IAA) under the optimal growth conditions of 30 °C and pH 5. Results of gas chromatography-mass spectrometry indicated IAA synthesis through the IPA pathway when using cellulose as a sole carbon source. The mycelium as well as the culture filtrate promoted the growth and chlorophyll content of seedlings. In a monocotyledonous plant (rice), the number of lateral roots was increased experimentally, whereas in a dicotyledonous plant (tomato), the fungus led to an increased length of shoots and roots.

Conclusions: TRP-dependent IAA production was demonstrated for the first time for *Pleurotus pulmonarius* and may be responsible for enhancing plant growth in vitro.

Significance and impact: Synthesis of IAA as the most prevalent phytohormone in plants has been demonstrated for soil microfungi. *Pleurotus pulmonarius* is reported as IAA-producing wood-decay macrofungus. The higher temperature optimum of *Pleurotus pulmonarius* isolated from subtropical environment compared to other *Pleurotus* species from temperate regions makes it more suitable for application in subtropical/tropical regions.
Rapid nursery system for ectomycorrhizal seedlings of boreal forest tree species

Yutaka Tamai¹, Kei Kitamura¹, Seiki Gisusi²

¹Graduate School of Agriculture, Hokkaido University, Japan
²Forest Products Research Institute, Hokkaido Research Organization, Japan

Purpose: The Japanese white birch (Betula platyphylla var. japonica) is a fast growing tree that is native to Hokkaido, and is known to be symbiotic with various ectomycorrhizal fungi. Studies on mycorrhizal seedlings for edible mushroom cultivation have just begun, and there are various problems, such as enlargement of the seedlings and shortening of the nursery period. Therefore, in this study, we examined a method for the rapid growth of mycorrhizal seedlings using the fast growing birch.

Methods: After the pre-moist chilling treatment, the birch seeds were sown on sterilized borax, grown for about one month, and developed for one month using a hydroponic culture vessel. Then, they were grown for 46 weeks with mycorrhizal fungus inoculum, and the growth condition was observed every 4 weeks.

Results: The result shows that until the 12th week, root hairs were found throughout the root system and no mycorrhizae formation was confirmed, but at 16 to 20 weeks, the disappearance of root hairs and mycorrhizae formation gradually started, and the developed mycorrhizae was confirmed at the 24th week. At 46 weeks after inoculation of mycorrhizal fungi, the height of the seedlings reached approximately 65 cm.

Conclusions: From the above, it was thought that the combination of a fast-growing tree with hydroponic culture makes it possible to grow large mycorrhizal synthetic seedlings that can be planted in a short period.
Effects of different applications on enzymatic activity of ectomycorrhizas of Japanese black pine seedlings planted at a coastal area

Satoaki Yamaguchi¹, Toko Tanikawa², Keisuke Obase³, Yosuke Matsuda¹

¹Graduate School of Bioresources, Mie University, Japan
²Graduate School of Bioagricultural Sciences, Nagoya University, Japan
³Forestry and Forest Products Research Institute, Japan

Purpose: Along the coast in Japan, Pinus thunbergii has been planted to keep windbreak and tidewater controls. Ectomycorrhizal (ECM) fungi can decompose soil organic matter by secreting extracellular enzymes and supply nutrients to host trees. Since most P. thunbergii roots are colonized by ECM fungi, the fungi might play a pivotal role to maintain coastal forests under harsh environmental conditions. The aim of this study was to clarify the extracellular enzymatic activity of ECM fungi associated with P. thunbergii seedlings in a coastal forest.

Methods: Pine seedlings were treated with 3 different applications by adding with/without charcoal and bark compost at the time of transplanting. After 5 years, 6 soil blocks were arbitrarily sampled within each application plot. ECM roots were divided into black, white or brown based on its color under a stereoscopic microscope, and the formation rate of the roots was calculated. Representative ECM morphotypes were applied for measuring 8 enzymatic activities involved in the acquisition of nitrogen, phosphorus, and carbon.

Result and Conclusion: The formation rate of black ECM roots was significantly higher at the no application plot, and white and brown ones were significantly higher in application plots. The activity of acid phosphatase was significantly higher at the application plots than in the no application plot. The activities of N-acetyl-glucosaminidase, β-glucosidase and acid phosphatase were significantly higher in both white- and brown-ECM roots than black ones in all plots. These facts suggest that applications can alter associating ECM fungal assemblages which in turn affect enzyme activities.
Purpose: Japanese cedar (*Cryptomeria japonica*) is a common planting species in Japan and its fine roots have an association with arbuscular mycorrhizal (AM) fungi. AM fungi deemed to play a pivotal role for nutrient acquisitions such as phosphorus and nitrogen, and other microorganisms also supposed to be involved, e.g. nitrogen uptakes. Recent studies showed that archaea in acidic forest soils were associated with nitrification. In this study, we surveyed the taxonomic identity of archaea inhabiting at different root order of fine roots of *C. japonica* in order to elucidate archaea communities associated with trees.

Methods: Soil samples were taken at seven cedar forests in central Japan. In each forest, soil blocks (15 cm³) were collected at 3 points within a 100 x 100 m area. A total of 10 cm in length of fine roots were separately collected from 1st- to 3rd- ordered roots within root systems retrieved from the blocks. The infection rate of AM fungi in each root order was calculated as ((the number of observed cells colonized by AM fungi/ the number of all cells observed) x 100%). Furthermore, genomic DNAs were extracted from each root order and partial 16S sequences of archaea were determined by a cloning method. Successfully sequenced samples were executed for homology searches by using the BLAST search to infer taxonomic groups.

Results and conclusions: In total, the infection rate of AM fungi was ranged from 5.8 to 49.7%, and the rates in 1st ordered roots were significantly higher than those in 3rd order roots. Some of our sequences were matched the best with members of the phylum of Euryarchaeota and Thaumarchaeota belonging to a 1.1b group. Based on the obtained data, we will consider how archaea taxonomical characteristics are associated with AM fungal colonization in the fine roots of Japanese cedars.
Plasmodium behavior of the myxomycete *Physarum melleum* in response to its consumer *Vitronura pygmaea* (Collembola: Neanuridae)

Mayuko Kataoka, Taizo Nakamori
Graduate School of Environment and Information Sciences, Yokohama National University, Japan

**Purpose:** Studying interactions between soil organisms is important for a mechanistic understanding of soil ecosystems. Myxomycetes are unicellular organisms that live in soil and grow into large multinucleate plasmodia. They play a crucial role in nutrient cycling. Plasmodia are sometimes consumed by species of the collembolan family, Neanuridae. While plasmodia are known to move to avoid ultraviolet rays and to search for high-quality food, little is known about their behavioral response to consumers. Here, the object in this study was finding how plasmodium behave with consumer.

**Methods:** We examined the behavior of *Physarum melleum* plasmodia in the presence/absence of the neanurid collembolan *Vitronura pygmaea*.

**Results and conclusions:** In laboratory experiments, *P. melleum* plasmodia moved toward the area without *V. pygmaea* and sometimes dropped the attacked parts of their bodies. These results suggest that *P. melleum* can avoid the consumer *V. pygmaea* by its behavior.
Endophytic Fungi Isolated from leaves of various plants in 4 Islands in KOREA

JongChul Lee, Ahn-Heum Eom
Department of Biology Education, Korea National University of Education, Korea

‘Endophytic fungi’ are the common name for fungi that live in plants and do not damage host plants. And they also do not transform appearance of hosts. They are known to encourage growth of plants and protect plants from herbivore. Studies needed to be conducted about endophytic fungi isolated from lots of plants, because endophytic fungi are essential for plant’s life.

In this study, we isolated endophytic fungal strains from leaves of various plants in 4 Islands (Shinan, Ullengdo, Baengyeongdo, Hansando). The isolated strains were identified based on morphological characteristics and molecular analysis of ITS rDNA regions. As a result, we confirmed 135 fungal species from 57 genera from leaves. These results showed differences between endophytic fungi isolated from each island.
Fungal endophytes associated with roots of *Calanthe discolor*, an endangered native orchid in Korea

Sun-mi Lee, Ahn-Heum Eom  
Department of Biology Education, Korea National University of Education, Korea

Orchids, most diverse and widespread family of flowering plants, depend on specialized endophytic fungi from the Basidiomycota at some point in their lives. Orchid seeds are tiny and don't contain sufficient nutrients to support the growing embryonic plant, so they get what they need from the mycorrhizal association. Mycorrhizal and non-mycorrhizal endophytes directly or indirectly contribute to the growth and development of orchids as well as the production of valuable secondary metabolites. Many orchid species are threatened or even endangered all over the world, because of habitat destruction, excessive overcollection, and climate change. *Calanthe discolor* is a well-known native orchid species that is endangered in Korea. Since the majority of orchids are difficult to cultivate artificially, there is a need to find suitable fungal partners for the conservation and restoration of endangered orchids.

In this study, we investigated the species diversity of endophytic fungi, including mycorrhizal fungi, isolated and identified from roots of *Calanthe discolor* (terrestrial orchid) in the Jeju island, Korea. The taxa of strains were classified based on morphological characteristics and molecular analysis. Currently, 68 species of endophytic fungi, including species of orchid mycorrhizal fungi belonging to the genus *Tulasnella* and *Ceratobasidium* were identified in *C. discolor*. There are needed more studies about this diverse and valuable association to restoration of endangered species belonging to Orchidaceae.
Purpose: Various insects harbor the yeasts in their intestines, some of which have symbiotic relationships each other. Since the discovery of the yeasts in lacewing guts by Hagen et al. (1970), yeasts have been detected from 6 spp. (of 4 genera, fam. Chrysopidae, ord. Neuroptera) in North America. This study aims at surveying the intestinal yeast flora of lacewings in Asia for the first time.

Methods: In 2018, we collected 38 individuals of 9 spp. (of 6 gen., fam. Chrysopidae) lacewings in Japan (Nagano, Tokyo, Ibaraki), and isolated yeasts from their intestines. To identify them, molecular phylogenetic analysis was carried out using rDNA LSU D1/D2 region by RAxML-NG(Kozlov et al. 2019).

Results and conclusions: We established 38 yeast isolates. As a result of preliminary BLAST analyses, all of isolates were identified as members of the genus Metschnikowia. Further phylogenetic analyses showed that they were divided into two major clades. Clade 1 contained M. picachoensis and M. pimensis known from the guts of lacewings (Chrysoperla sp.) in North America.

Clade 2 was a completely new one which is the sister clade to M. corniflorae known from the guts of Coleoptera and divided into five subclades.

Some lacewings feed on the nectar or honeydew. Considering the possibility that they obtain the yeasts from the nectar, it was checked whether the sequences of the present yeasts are detected or not in the environmental sequences of nectars in the collection site (Nagano). However, there was no matching of the sequences.
Impact of cover cropping with rotary- or no-tillage practice on the arbuscular mycorrhizal fungal communities colonizing maize roots

Yuya Tatewaki\textsuperscript{1)}, Masao Higo\textsuperscript{2)}, Yoshihiro Kawamura\textsuperscript{1)}, Koya Nakamura\textsuperscript{1)}, Katsunori Isobe\textsuperscript{2)}

\textsuperscript{1)}Graduate School of Bioresource Sciences, Nihon University, Japan
\textsuperscript{2)}College of Bioresource Sciences, Nihon University, Japan

**Introduction:** Community structure of arbuscular mycorrhizal fungi (AMF), may be a key component utilized in sustainable cover cropping systems. However, it still remains unclear whether introducing cover crops with or without tillage agricultural environments change the diversity of AMF communities in the subsequent maize roots. Thus, we investigated how combined agricultural practice with tillage and cover cropping affects the AMF community structure of subsequent maize roots.

**Methods:** A field trial was performed at Nihon University, in Kanagawa, Japan. Three cover crops treatments (hairy vetch, brown mustard and fallow) with rotary- or no-tillage practice were established to determine the diversity and structure of AMF communities in maize roots using amplicon sequencing.

**Results:** AMF colonization rate in no-tillage plot regardless of cover crop treatments was significantly higher than that in tillage plot. AMF communities in maize roots showed a significant difference between tillage and no-tillage. However, there were no significant differences in the diversity of AMF communities among the difference of cover crops regardless of tillage management.

**Conclusions:** In this study, the difference in the AMF communities of maize roots between tillage and no-tillage regardless of cover cropping was distinct. This suggests that the difference in tillage management may be more related to shaping the AMF community structure compared with cover cropping. However, we could not determine why cover cropping did not affect the AMF communities in the maize roots.

**Acknowledgments:** This study was financially supported by JSPS KAKENHI Grant Number JP19K06005.
Mycorrhizal fungi associating with germination of mycoheterotrophic Pyrola japonica

Yusuke Yamaguchi1), Shosei Kawai1), Takashi Uesugi1), Manami Mori2), Yousuke Matsuda3)
1)Graduate School of Bioresources, Mie University, Japan
2)Faculty of Bioresources, Mie University, Japan

Purpose: A green plant of Pyrola japonica is supposed to be a mycoheterotrophic obtaining carbon via not only photosynthesis but also mycorrhizal fungi. Such the plant is considered to be a pre-adaptation of the evolution to a fully mycoheterotrophic plant. Mycoheterotrophic plants generally produce minute seeds called as "dust seeds". Since P. japonica also produces such the seeds, the plant deemed to be mycoheterotrophic. However, no information is available which fungi are involved for the germination of P. japonica, although mycorrhizal fungal communities associated with adults and seedlings have been elucidated. The purpose of this study was to clarify mycorrhizal communities involved in the initial growth of P. japonica. For this purpose, genomic DNA was extracted from both germinated seeds of P. japonica, and associating fungi were examined based on DNA barcording.

Method: Seeds of P. japonica were put into seed packs and buried into three deciduous or evergreen forests in Tsu City, Mie, Japan in 2008 and 2014. The packs were collected in 2018 and the remaining seeds were examined under a stereomicroscope. When seeds were germinated, they were used for the extraction of DNA and applied for PCR amplification on the ITS region, TA-cloning and sequence. Successfully sequenced samples were executed for the BLAST search. The resulting sequences were compared with the previously obtained sequences of P. japonica seedlings and adult plants, and the mycorrhiza community was examined for changes.

Results and conclusions: Germinated seeds were confirmed and accounting for 15.2% (536/3522) in 2008 and for 0.1% (14/11021) in 2014. Based on DNA sequences obtained from germinated seeds, we discuss the succession of mycorrhizal communities with the initial stage of P. japonica.
Classification and functional significance of root endophytic fungi collected from Japanese cedar forests in the central Japan

Mei Fukatsu¹, Kanta Imaeda², Yosuke Matsuda²
¹Faculty of Bioresources, Mie University, Japan
²Graduate School of Bioresources, Mie University, Japan

Purpose: Japanese cedar, Cryptomeria japonica, is one of the major planted tree species in Japan. Fine roots of the cedar have an association with arbuscular mycorrhizal fungi. However, limited information is available for other fungal taxa involved. This study aimed to clarify the identity and functional significance of root endophytic fungi associated with the cedar.

Method: Root samples were collected from 7 cedar forests in the central Japan. First- and second-ordered root tips were surface sterilized and then separately incubated on PDA media. Fungi appeared from the tips were sub-cultured and used for DNA barcoding. Obtained DNA sequences were clustered as MOTUs (molecular operational taxonomic units) with a 97% homology and executed for the BLAST search to infer taxonomic identities. One representative strain in each MOTU that appeared from three or more different forests, i.e. 17 strains, were used for following in vitro tests. Each the strain was inoculated to cedar seedlings grown on modified MMN media excluded malt but containing 1%(w/w) glucose. After 3 months, the growth and health condition of the seedlings were measured based on their appearance, above- and belowground-biomass, and photosynthetic activity.

Results and conclusions: In a total of 1650 roots, fungi were endophytically appeared from 34.0% (561 roots). Among the successfully cultured 525 strains, 67.0% (352 strains) were determined DNA sequences which were divided into 79 MOTUs. The order Helotiales was the most dominant taxa. For some strains, fungal inoculation tended to show the increase in seedling biomass compared to the control. Based on these data, we discuss taxonomic traits and the extent of affinities with cedar trees of endophytic fungi.
Symbiotic culture revealed different mycorrhizal specificity among coexisting three epiphytic orchids

Kento Rammitsu$^{1,2}$, Masaru Goto$^3$, Yumi Yamashita$^4$, Tomohisa Yukawa$^4$, Yuki Ogura-Tsujita$^{1,2}$

$^1$United Graduate School of Agricultural Sciences, Kagoshima University, Japan
$^2$Faculty of Agriculture, Saga University, Japan
$^3$Shizuoka Calanthe Society, Japan
$^4$Tsukuba Botanical Garden, National Museum of Nature and Science, Japan

**Purpose**: Do coexisting orchids in natural habitats associate with distinct mycorrhizal fungi or share the same fungal partners? The purpose of this study is to reveal mycorrhizal association of coexisting three epiphytic orchids, *Oberonia japonica* (Oj), *Taeniophyllum glandulosum* (Tg) and *Thrixspermum japonicum* (Tj), by molecular identification and symbiotic culture experiments.

**Methods**: In total, 108 root samples were collected from two Japanese sites, where three orchid species coexisted, and used for molecular identification of mycorrhizal fungi. The ITS sequences obtained from mycorrhizal fungi were classified into operational taxonomic units (OTUs) based on 97% sequence similarity. The main fungal OTUs associated with three orchid species were isolated from their roots, and cultured with the seeds of each orchid species.

**Results**: In total, 11 OTUs were identified, and Two OTUs, CE1 and TU2 belonging to the Ceratobasidiaceae and Tulasnellaceae, respectively, were dominant fungi. The frequencies of CE1 and TU2 were 4, 84, and 50% and 74, 0, and 10%, in Oj, Tg and Tj, respectively. Symbiotic culture revealed that the developments of Tg and Oj were promoted only by CE1 or TU2, respectively, whereas that of Tj was stimulated by both fungi.

**Conclusions**: This study clearly showed the different mycorrhizal specificity among the coexisting three orchid species. Two orchids, Tg and Oj, displayed marked high fungal specificity toward particular mycorrhizal fungi and used different mycorrhizal partners, while Tj exhibited low mycorrhizal specificity, sharing the mycorrhizal partners with coexisting other two orchids.
Diversity of ectomycorrhizal fungi: from a root tip to trees

Ren-Cheng Liu\(^1\), Ying-Hsuan Chen\(^1\), Wan-Rou Lin\(^2\), Pi-Han Wang\(^1\)
\(^1\)Department of Life Science, Tunghai University, Taiwan
\(^2\)Bioresource Collection and Research Center (BCRC), Food Industry Research and Development Institute (FIRDI), Taiwan

Ectomycorrhizal symbiosis is important and complex in the forest ecosystem. In this study, the diversity of ectomycorrhizal fungi (EMF) in a sub-alpine cold temperate forest dominated by *Abies kawakamii* and *Tsuga chinensis* var. *formosana* was investigated by morphology and DNA sequencing identification. Six morphotypes of mycorrhizae were described from the roots of two conifer species and 19 EMF species were detected by Sanger sequencing of rDNA ITS. Results show that four and six EMF species were detected from two morphotypes of ectomycorrhizae, one to two EMF species were detected from the other four morphotypes. *Laccaria* sp. were found in two *A. kawakamii* ectomycorrhizal morphotypes. *Russula peckii* formed more than two morphotypes of mycorrhizae with both tree species. These indicated that a single mycorrhizal root tip of plant may associate with multiple EMF species and a fungus existed in different ectomycorrhizal morphotypes with the same host. In total, 80,552 reads were obtained by next-generation sequencing and of 73,374 reads were identified as 8 families 35 EMF species. Russulaceae, Sebacinaceae were dominant in both tree species. *A. kawakamii* and *T. chinensis* were associated with 18 and 29 EMF species, respectively. Different abundances of 12 EMF species exist between two host trees. It indicated that EMF associated with multiple hosts with host preference. Both plants sharing EMF and EMF associated with hosts benefit the stability of EMF community.
Effect of Biochar on the Growth and Ectomycorrhizal Fungal Community of Japanese Black Pine Seedlings

Sonoka Nanya, Yosuke matsuda
Mie University, Japan

Purpose: Japanese black pine (Pinus thunbergii) is widely planted along coastal areas in Japan and plays an important role for keeping landscape and for preventing Tunami disaster. Since most fine roots of the pine trees were colonized by ectomycorrhizal (ECM) fungi, ECM associations would be necessarily for water and nutrients absorption, especially in seedling stage. In recent studies, biochar, which is a charcoal derivative from various plant materials, is expected to be a soil ameliorant improving soil moisture as well as nutrient condition. Thus, the application of biochar might affect ECM fungal communities. In this study, we aimed to clarify the role of soil biotic and abiotic conditions for the growth of pine seedlings. For this purpose, we monitored the growth of the seedlings and evaluated the enzyme activity of ECM fungi under different substrate conditions.

Method: Soils collected from a coastal pine forest were sieved with a 2mm mesh, and were divided into two groups, i.e. sterilized or non-sterilized. Both the groups were further divided into with/without ECM fungal inoculation and with/without biochar application. In total, 8 treatments were prepared. After pine seeds were sown in multi-cavity containers, 94 seedlings were monitored and 34 of them were finally examined for biomass. Moreover, a part of the root system was used for mycorrhization, enzyme activity assay and DNA analysis.

Results and conclusions: In biochar added treatments, the biomass of seedlings tend to increase compared with control. Biochar applications increased ECM formation rates and the occurrence of whitish ECM roots tended to increased. Based on obtaining results, we discuss how the application of biochar as well as ECM fungi affect the growth of pine seedlings.
The genus *Beauveria* in Taiwan

Sung-Yuan Hsieh, Han-Yun Li
Food Industry Research and Development Institute, Taiwan

Six species of the entomopathogenic fungal genus *Beauveria* were collected and isolated from diverse insect cadavers in natural habitats. Morphological observations and DNA sequences from ITS, TEF and RPB1 genes were used to identify taxa and examine phylogenetic relationships. All species described in this study owned dense of mycelia, granular-pulverulent conidial mass on the host and formed a kind of sympodial and globose to flask-shaped short conidiogenous cells. Six species, including *B. australis*, *B. bassiana*, *B. lii*, *B. malawiensis*, *B. pseudobassiana*, and *B. scarabaeicola* (=*B. sungii*), were distinguished by morphological characteristics and DNA sequences. Detailed descriptions and illustrations of *Beauveria* species were provided in this study. Among of these species, four species including *B. australis*, *B. lii*, *B. malawiensis* and *B. pseudobassiana* were described as new records for Taiwan.
Potential contribution of a defective RNA segment of *Fusarium boothii* large flexivirus 1 on hypovirulence of the host Fusarium Head Blight fungus

Sotaro Chiba¹, Yukiyoshi Mizutani¹, Abraham Adane², Kazuma Uesaka³, Hideki Kondo², Haruhisa Suga⁴, Nobuhiro Suzuki²

¹Graduate School of Bioagricultural Sciences, Nagoya University, Japan
²Institute of Plant Science and Resources, Okayama University, Japan
³Center for Gene Research, Nagoya University, Japan
⁴Life Science Research Center, Gifu University, Japan

**Purpose:** Mycoviruses have a potential to be a biocontrol agent of pathogenic fungi. In the past 20 years, extensive mycovirus screenings identified diverse RNA and DNA mycoviruses, some of which were shown to affect host fungal growth and/or pathogenicity. This study was aimed at screening of virulence-attenuating mycoviruses that could control Fusarium Head Blight (FHB) disease of cereal crops.

**Methods:** Screening for virus-infected Fusarium species was conducted with conventional double-stranded RNA (dsRNA) detection. The sequence of dsRNAs was determined by construction of cDNA-library and subsequent sanger sequencing, and high-throughput RNAseq analysis. Biological properties of mycoviruses were analyzed by evaluation of growth and pathogenicity of host *Fusarium* fungi on a synthetic media and wheat plants. Virus curing was performed by single spore isolation and regeneration in the presence of an antiviral drug, ribavirin.

**Results and conclusions:** Growth-impaired, hypovirulent *Fusarium boothii* strain, BL13, was isolated and found to be virus-infected. A novel tymovirus-like virus namely *Fusarium boothii* large flexivirus 1 (FbLFV1) and a new mitochondrial virus, *Fusarium boothii* mitovirus 1 (FbMV1), each encoded single ORFs, were identified in BL13. The fungal strain additionally carried defective RNA form of FbLFV1 (D-RNA) that lacked most middle part of the FbLFV1 genome but retained the N- and C-terminal coding domains in-frame. The FbMV1 infection alone unlikely contributed to hypovirulence of the host fungus. Moreover, a possible contribution of the D-RNA to fungal growth inhibition was observed. Taken together, the D-RNA of FbLFV1 might produce a cytopathic protein that potentially control the FHB on wheat.
Identification of internal ribosomal entry sites in the genome of a fungal virus conferring hypovirulence to the white root rot fungus

Kanoko Murata1), Atif Jamal2), Hironori Kubo1), Nobuhiro Suzuki2), Sotaro Chiba1)
1)Graduate School of Bioagricultural Sciences, Nagoya University, Japan
2)Institute of Plant Science and Resources, Okayama University, Japan

Purpose: Fungal viruses have a potential to control fungal diseases. The prototypic megabirnavirus, Rosellinia necatrix megabirnavirus 1 (RnMBV1), confers hypovirulence to its natural host, the white root rot fungus Rosellinia necatrix that causes destructive diseases on fruit trees. The RnMBV1 genome consists of two dsRNA segments of 9 and 7 kbp in length (dsRNA1 and dsRNA2), and each encodes two ORFs. Unique features of RnMBV1 include extremely long 5' untranslated region (5' UTR) spanning 1.6 kbp that share significant sequence identity between the segments. Therefore, the 5' UTRs are suspected to carry essential roles in replication and translation. This study explored internal ribosomal entry site (IRES)-mediated translation of the RnMBV1 genes.

Methods: IRES activities were evaluated by transgenic expression of a bicistronic luciferase cassette where a codon-optimized Renilla luciferase (ORluc) gene was cap-dependently translated while a codon-optimized firefly luciferase (OFluc) coding domain by IRES-dependently. Viral sequences were inserted in between the ORluc and OFluc genes and subjected to dual luciferase assay.

Results and conclusions: The 5' UTR of two dsRNA segments of RnMBV1 showed almost equal IRES activities. A series of deletion mutation analyses on dsRNA1 5' UTR revealed that fully functional IRES required a region positioned at 434-1401 but not other non-coding or coding sequences. Interestingly, there are no potential AUG start codons from nucleotide 1402 to 1678 (the first AUG), although 23 AUGs are found in the region 1-1401. The 1 kb RnMBV1-IRES is one of the largest viral IRES elements reported so far.
RNA virus diversity in *Aspergillus* species revealed by FLDS, a comprehensive non-retro RNA virus surveillance method

Yuto Chiba1), Takashi Yaguchi2), Syun-ichi Urayama3,4), Daisuke Hagiwara2,3,4)
1)Graduate school of Life and Environmental Sciences, University of Tsukuba, Japan  
2)Medical Mycology Research Center, Chiba University, Japan  
3)Faculty of Life and Environmental Sciences, University of Tsukuba, Japan  
4)Microbiology Research Center for Sustainability, University of Tsukuba, Japan

**Purpose:** RNA virus diversity in filamentous fungi have been intensively investigated for these two decades. To find RNA viruses in fungi, detection of double-stranded RNA (dsRNA), a hallmark of RNA virus infection, and RNA sequencing (RNA-seq) were used. However, the sensitivity of dsRNA detection by electrophoresis was relatively low, and there were difficulties in capturing terminal sequences of the viral genome in RNA-seq analysis. Our knowledge of RNA virus diversity in filamentous fungi can be restricted by those methodological limitations. To overcome these limitations, we performed newly developed comprehensive virus detection method, fragmented and primer ligated dsRNA sequencing (FLDS).

**Methods:** We used clinical and environmental isolates of *Aspergillus fumigatus* and its related species for RNA virus identification. For high-sensitive detection of RNA virus and retrieval of its complete genome sequences, FLDS was used.

**Result:** We identified at least one RNA virus in 17 of 156 isolates. Among them, 8 isolates were infected by ssRNA viruses although they did not represent dsRNA bands by gel electrophoresis. This result suggested that ssRNA virus diversity had been underestimated by dsRNA electrophoresis. We also reconstructed complete RNA viral genomes based on terminal sequences of genome segments which are shared among segments in a single virus genome. Some sequences predicted to be one of the RNA viral segments did not show significant similarity to known RNA viral proteins, suggesting that these segments may have been missed by RNA-seq analysis.

**Conclusion:** We identified RNA viruses or RNA viral genes which could be overlooked by the limitations of conventional RNA virus detection methods. The RNA virus diversity in *Aspergillus* species was higher than previously expected. Our findings and the method will provide a deeper insight into RNA virus diversity in filamentous fungi.
Characterization of a botybirnavirus in the phytopathogenic fungus *Leptosphaeria biglobosa*

Yue Deng¹², Kang Zhou¹², Mingde Wu¹², Guoqing Li¹²

¹State Key Laboratory of Agricultural Microbiology and Key Laboratory of Plant Pathology of Hubei Province, Huazhong Agricultural University, China
²The Key Laboratory of Plant Pathology of Hubei Province, Huazhong Agricultural University, China

Leptosphaeria biglobosa is a worldwide pathogenic fungus that can cause black leg on many cruciferous crops, especially oilseed rape (Brassica napus L.). Here we characterize a double-strand RNA (dsRNA) virus, namely Leptosphaeria biglobosa botybirnavirus 1 (LbBV1), isolated from L. biglobosa strain GZJS-19 in China. The LbBV1 genome was sequenced by using conventional methods. The radial mycelial growth of L. biglobosa strains was determined on potato dextrose agar (PDA), and their pathogenicity assays were conducted on cotyledons of 14-day-old oilseed rape seedlings. LbBV1 has two dsRNA segments, namely dsRNA 1 and dsRNA 2, with the sizes of 6,190 and 5,900 bp, respectively. Each dsRNA segment contains one large open reading frames (ORF), putatively encoding a polypeptide of 202 and 192 kDa in size for dsRNA 1 and dsRNA 2, respectively. The polypeptide encoded by ORF2 (dsRNA 2) possesses several conserved domains including a protein-rich region and an RNA-dependent RNA polymerase (RdRp) domain. The cDNA sequences of dsRNA 1 and dsRNA 2 show high sequence identity of 78% and 81% to those of Alternaria botybirnavirus 1 (ABV1) at nucleotide level, respectively. The genomic organization and phylogenetic analysis supported that LbBV1 belongs to the genus Botybirnavirus. Purified spherical viral particles of LbBV1 are ~44 nm in diameter encompassing dsRNA 1 and dsRNA 2 and two structural proteins of 90 and 100 kDa, respectively. Strain GZJS-19 shows no significant difference in colony morphology, radial mycelial growth and pathogenicity compared with other LbBV1-free strains. However, introduction of LbBV1 virions into virulent strain HBtom-459 of Botrytis cinerea resulted in the reduced virulence and sclerotium formation of the derivative B. cinerea strains.
Quinine Production by Endophytic *Cercospora* spp. (Fungi, Mycosphaerellaceae) from *Cinchona calisaya* (Rubiaceae)

Iman Hidayat¹, Nani Radiastuti², Izumi Okane³, Suminar Setiati Achmadi⁴, Gayuh Rahayu⁵

¹Research Center for Biology, Indonesian Institute of Sciences (LIPI), Indonesia
²Faculty of Science and Technology, State Islamic University Syarif Hidayatullah, Indonesia
³Faculty of Life and Environmental Sciences, University of Tsukuba, Japan
⁴Chemistry Department, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University (IPB), Indonesia
⁵Biology Department, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University (IPB), Indonesia

**Aims:** Genus *Cercospora* has been recognized as a common fungal pathogen causing leaf spot on various plants. During the study of fungal endophytes from *C. calisaya* in Indonesia, two unsporulated fungal isolates (*Cercospora* sp. strain IPBCC 14.1189 and strain IPBCC 14.1190) resemble the colony characteristics of *Cercospora* were determined. The current study aimed at identifying the species name of both strains using combination of morphology data and phylogenetic analysis, and analyzing their cinchona alkaloids profile.

**Methodology and Results:** The phylogenetic analysis was carried out based on combination of the internal transcribed spacer (ITS), part of the elongation factor (EF) 1-α, actin (ACT), calmodulin (CAL) and histon (HIS) sequences. While cinchona alkaloids profile was analyzed using HPLC method. The phylogenetic tree generated from multilocus analysis confirmed that both isolates belong to the genus *Cercospora*, and formed an independent clade separated from other *Cercospora* sequences. This indicates that both strains belonging to a new taxon, however, further study involving sporulation induction assay is necessary to verify the identity of both isolates. Analysis of cinchona alkaloids using HPLC method showed that quinine was detected from *Cercospora* sp. strain IPBCC 14.1189 and strain IPBCC 14.1190 at concentration 1.2 mg/L and 0.7 mg/L, respectively.

**Conclusion:** Significance and Impact of study: Although *Cercospora* sp. strain IPBCC 14.1189 and strain IPBCC 14.1190 are capable in producing quinine, further fermentation study in optimizing quinine production is necessary for scaling up.

Key words: alkaloids, Cinchona, Fungi, Mycosphaerellaceae, phylogeny
Bioethanol production from *Arundo donax* as a raw material using wood rotting fungus *Schizophyllum commune* NBRC 4928

Yuka Yamanaka, Akie Inoue, Sakae Horisawa
Kochi University of Technology, Japan

**Purpose:** Pretreatment methods of cellulosic biomass such as delignification and saccharification are mainly performed physically/chemically, but there are problems such as high equipment cost and generation of fermentation inhibitors. Therefore, we examined a method to execute all the steps consistently by using a wood rot fungus which decomposes lignin, cellulose and hemicellulose which are main components of plant cell wall. In this study, we investigated ethanol production from soft biomass giant leads (*Arundo donax*) and water hyacinth (*Eichhornia crassipes*) using the highly fermentable wood rot fungus *Schizophyllum commune* NBRC 4928.

**Methods:** Sixty milliliter of liquid medium was placed in a 200-ml flask. Mycelial discs of *S. commune*, 12 mm in diameter, were punched out from colonies subcultured on potato dextrose agar. Four discs were put in a flask and then incubated on a rotary shaker (90 rpm) at 30 °C. Semi-anaerobic conditions were generated by an *N*₂ gas purge and a silicon rubber cap with a fermentation airlock. The concentration of ethanol in the supernatant of liquid medium was measured by using HPLC.

**Results and Conclusions:** Although *S. commune* NBRC 4928 did not produce ethanol from water hyacinth, ethanol production was observed when a giant reed was used as a raw material. The result that combination of *S. commune* and cellulase enhanced ethanol production from the giant reed suggested that improved saccharification efficiency might increase the yield of ethanol. We concluded that the CBP using the white rot fungus is available in the bioethanol production from cellulosic materials.
Efficiency of cytotoxicity against cancer cell lines and antioxidant activity from ethyl acetate extracts of *Xylaria* spp.

Niwana Wangsawat¹, Cherdchai Phosri², Rungpetch Khaengraeng³, Anthony JS Whalley⁴, Nuttika Suwannasai⁵

¹Department of Biology, Faculty of Science, Srinakharinwirot University, Thailand
²Department of Biology, Faculty of Science, Nakhon Phanom University, Thailand
³Environmental Science Programme, Faculty of Science and Technology, Pibulsongkram Rajabhat University, Thailand
⁴School of Pharmacy and Biomolecular Science, Liverpool John Moores University, UK
⁵Department of Microbiology, Faculty of Science, Srinakharinwirot University, Thailand

**Purpose:** *Xylaria* is one of the largest genera in family the Xylariaceae. Many of them are capable of synthesizing bioactive compounds including antimicrobial, antioxidant and anticancer activities. Cancer is a major health issue worldwide due to the high rate of its morbidity and mortality. Then, new sources of anticancer drugs with low side-effects remain a major focus in several researchs. In the present study, ten crude extracts of *Xylaria* spp. obtained from cultural broths were screened cytotoxicity activity against different cancer cell lines and antioxidant activity.

**Methods:** The crude extracts of *Xylaria* spp. were tested against four different cancer cell lines which were lung (A549), liver (HepG2), cervical (Hela) and kidney (Vero) by using the MTT assay. Moreover, the DPPH scavenging assay was also examined for antioxidant activity.

**Results and conclusions:** The results indicated that all *Xylaria* extracts exhibited cytotoxicity against at least one kind of the cancer cell lines. The highest cytotoxicity against Hela, A549, HepG2 and Vero cell lines belonged to *Xylaria* sp. PK16-11.1. The percentages of cell inhibition were 96.56, 96.10, 91.82 and 95.46, respectively. The antioxidant activities (IC₅₀ value) of *Xylaria* extracts varied from 0.93 to 4.6 mg/mL. However, the results from this study provide valuable information of potential fungal sources for anticancer and antioxidant drug discovery in future studies.
Effect of butyl cyanoacrylate nanoparticles on manganese-dependent peroxidase production by *Bjerkandera adusta*

Mizuki Kamio¹), Sakae Horisawa¹), Chikage Komatsu²)

¹)Kochi University of Technology, Japan
²)Chikami MIL-TEC.inc., Japan

**Purpose:** The isobutyl cyanoacrylate nanoparticles (IBCA-NPs) are made of isobutyl cyanoacrylate polymer and exhibit antibacterial activity against gram-positive bacteria. These nanoparticles exhibit antibacterial activity against Gram-positive bacteria and inhibit mycelial growth of filamentous fungi and Gram-negative bacteria. Ligninolytic enzyme production by white rot fungi were thought to increase due to starvation stress caused by the lack of nutrients (C and N). Therefore, we thought that ligninolytic enzyme production by white rot fungi increased by antimicrobial stress.

**Methods:** In this study, we investigated the effect of IBCA-NPs on the production of manganese-dependent ligninolytic enzymes by basidiomycete *Bjerkandera adusta*. We evaluated the production of Mn-dependent enzymes when nanoparticles with different particle sizes 200 nm and 30 nm. Activity of Mn-dependent ligninolytic enzyme from *B. adusta* in the liquid culture added IBCA-NPs 30 nm and 200 nm in diameter were measured, respectively.

**Results and conclusions:** As a result, the 30 nm of IBCA-NPs exhibit the effect of increasing the production amount of Mn-dependent ligninolytic enzyme on *Bjerkandera adusta* Iwa5b.
New Illudane-type Sesquiterpenes from the Fruiting Body of Omphalotus japonicus

Satoki Aoki¹, Takako Aboshi¹,², Yoshihito Shiono¹,², Ken-ichi Kimura¹,³, Daisuke Arai⁴, Yoshiaki Iizuka⁴, Tetsuya Murayama¹,²

¹The United Graduate School of Agricultural Science, Iwate University, Japan
²Faculty of Agriculture, Yamagata University, Japan
³Graduate School of Arts and Sciences, Iwate University, Japan
⁴Field Science Center, Faculty of Agriculture, Yamagata University, Japan

Purpose: Wild mushrooms are important food ingredients in Japan. However, food poisoning due to misidentification of poisonous as edible mushrooms are occurring every year. In the most of cases were caused by Omphalotus japonicus (Tsukiyo-take in Japanese). In the previous study, illudin S, an illudane-type sesquiterpene was founded as the toxic substance from O. japonicus. Furthermore, several analogous, dihydroilludin S, neoilludin A and B, were also isolated. However, there were few studies on the detail food poisoning caused with O. japonicus. Furthermore, Omphalotaceae mushrooms were well known to produce a variety of sesquiterpenes. Therefore, we investigate constituents from the O. japonicus.

Methods: O. japonicus was collected in Yamagata University Forest, Yamagata prefecture. The fruiting body was extracted with methanol and then the solvent was removed under vacuum. The residue was suspended with distilled water and partitioned with hexane, ethyl acetate, and butanol, respectively. Ethyl acetate and butanol fractions were subjected to several steps chromatography to afforded compounds 1-6.

Results and conclusion: Based on NMR, HRMS, IR, and CD analyses, compounds 1-3 were identified as known compound, illudin S, neoilludins A and B, respectively. Compound 4 was found as a new epimer of neoilludin B at C-4. Compounds 5 and 6 were also new analogue compounds similar to those of neoilludins A and B, except 4-OH in neoilludins A and B were replaced to 4-OMe in 5 and 6, respectively. In addition, illudin S, 5 and 6 were exhibited Ca²⁺ inhibition activity.
Cytotoxicity activity of some small molecular weight peptides from tropical lichen forming fungi

Ek Sangvichien¹, Theerapat Luangsuphabool², Janthima Jaresitthikunchai³, Sittiruk Roytrakul³

¹Department of Biology, Ramkhamhaeng University, Thailand
²Biotechnology Research and Development Office, Department of Agriculture, Thailand
³Proteomics Research Laboratory, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency, Thailand

Trypetheliaceae is a family of pyrenocarpus crustose lichens which are widely found in tropical and some sub-tropical regions. The main genera of this fungal family found in Thailand belong to Astrothelium, Bathelium, Marcelaria, Polymeridium, Pseudopyrenula and Trypethelium. The fungal partner or lichen forming fungi from this representative genus Marcelaria and Trypethelium were isolated by the ascospores discharge method and cultivated on Malt Yeast Extract medium for 9 weeks at ambient temperature. Their major secondary metabolites from the fungal cultures were anthraquinones, lichexanthone naphthoquinones, parietin, phenalenones, and xanthones and these have had been investigated for antimicrobial and antioxidant activities. Compounds from a Trypethelium expressed strong activity in antioxidant reactions. There have been no reports of small molecular weight proteins or peptides from lichen forming fungi previously. In this study, the lichen forming fungi, Marcelaria cumingii (K11) and Trypethelium sp. (KRB 172) were selected as representatives. Natural peptides from fungal cells were extracted, partially purified following digestion by pepsin. Cytotoxicity testing revealed that peptides from M. cumingii (K11) exhibited selective inhibition against HepG2, MCF-7 and MDA-MB-231 cell lines at 58.0, 60.7 and 69.9 % inhibition respectively. Peptides from Trypethelium sp. (KRB172) also inhibited HepG2, MCF-7 and MDA-MB-231 cell lines at 58.1, 76.8 and 71.0 % inhibition respectively. This data represents important implications of lichen forming fungi for pharmaceutical applications.
Antimicrobial Activity of Fungi Isolated from Freshwater Environments against Methicillin-Resistant *Staphylococcus aureus*

Hye Jin Hwang¹, Buyng Su Hwang², Hye Yeon Mun¹, Jaeduk Goh¹, Eu Jin Chung³

¹Fungi Research Team, Microbial Research Department, Nakdonggang National Institute of Biological Resources, Republic of Korea
²Animal & Plant Utilization Team, Animal & Plant Research Department, Nakdonggang National Institute of Biological Resources, Republic of Korea
³Environmental Microbiology Research Team, Microbial Research Department, Nakdonggang National Institute of Biological Resources, Republic of Korea

**Purpose:** The antibiotics resistant microorganism has increased in past three decades. Methicillin resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen that causes severe morbidity and mortality worldwide. The aim of the present study was to evaluate the antimicrobial activity against MRSA in fungi isolated from freshwater environments.

**Methods:** Purely isolated fungi were cultivated using potato dextrose broth medium at 25°C and 150 rpm 10 days shaking-flask fermentation. Fermentation broths from 50 isolated fungi were tested for antimicrobial activity against MRSA by the disc diffusion method. The secondary metabolite produced by fungi were extracted using n-hexane, ethyl acetate, n-butanol as a solvent.

**Results:** *Penicillium rubefaciens*(NNIBRFG5039) and *Filosporella exilis*(NNIBRFG1552) were pre-screened for potential antimicrobial activity against MRSA. The antimicrobial compounds from ethyl acetate fraction of these two fungi were identified as 4-hydroxybenzoic acid and 6-hydroxymellein, respectively.

**Conclusions:** We conclude that *P. rubefaciens*(NNIBRFG5039) and *F. exilis*(NNIBRFG1552) can be a source of antimicrobial compounds. However, continuous research is needed to prove its bioactive action.
Search for an “unlocker” compounds from microbial secondary metabolites, which release the ability of secondary metabolites production in fungi

Kazunari Sakai1), Mutsumi Nagoya2), Emi Arakawa3), Yoshihiro Watanabe1), Masato Iwatuki1,3), Kenichi Nonaka1,3)
1) Kitasato Institute for Life Science, Kitasato University, Japan
2) School of Science, Kitasato University, Japan
3) Graduate School of Infection Control Sciences, Kitasato University, Japan

Purpose: Natural products are very attractive because they have a backbone that can’t be easily obtained by synthesis and play an important role as a lead compound for drug development. Recent advances in genome analysis technology had clear that there are more than 30 biosynthetic gene clusters for secondary metabolites in fungus but most of them are silent. Accordingly, we are searching for natural compounds, which release the ability of secondary metabolite production in fungi, and we named the compound as an unlocker.

Methods: The compounds from the Omura natural products library were used for this screening. Talaromyces siamensis FKA-61 used as a test fungal strain, whose secondary metabolic profile was already analyzed. In the 1st screening, seed-cultured FKA-61 was inoculated to an agar plate which a non-conidiation medium (PDB1), and then the paper disc method was used to evaluate the production of pigment and conidiation. In the 2nd screening, FKA-61 was incubated in the production media with and without the screening compound. The cultured broths were extracted with EtOH, and then analyzed the secondary metabolic profiles by LC/ESI-MS.

Results and conclusions: As a result of evaluating all 794 compounds in the 1st screening, 74 compounds passed. Twelve compounds were evaluated in the 2nd screening, and the result, one compound changed the secondary metabolic profile in a dose-dependent.
Identification of a sulfur amino acid biosynthetic gene in *Cryptococcus neoformans*

Phuong Thao Nguyen, Kiminori Shimizu  
Graduate School of Industrial Science and Technology, Tokyo University of Science, Japan

*Cryptococcus neoformans* is an environmental microorganism and causes meningitis in immunocompromised patients by its infection into lung. Since amino acid biosynthetic pathways have been reported as a factor for *C. neoformans* survival in the host, these pathways are proposed as targets for potential antimicrobial drugs. By using *Agrobacterium tumefaciens* mediated mutagenesis to *C. neoformans*, 10000 transformants were obtained and screened for auxotrophy. Among these transformants, we found a mutant which requires cysteine an amino acid. This transformant, T-DNA was inserted into a gene which encodes a putative sulfite reductase which is involved in sulfur amino acid biosynthesis.
Antiamebic compounds produced by Indonesian microbial strains

Mihoko Mori1), Arif Nurkanto2), Dyah Noor Hidayati3), Diana Dewi3), Kenichi Nonaka1), Atsuko Matsumoto1), Danang Waluyo3), Agung Eru Wibowo3), Kazuro Shiomi1), Tomoyoshi Nozaki2)

1) Kitasato Institute for Life Sciences, Kitasato University, Japan
2) University of Tokyo, Japan
3) Biotechnology Center, BPPT, PUSPIPTEK Area, Indonesia

Purpose: We have conducted a project titled "Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources" in SATREPS (Science and Technology Research Partnership for Sustainable Development) program between Indonesia and Japan. In this study, our purpose is finding anti-amebic compounds from Indonesian actinomycetes/fungal broths.

Methods: Indonesian actinomycetes and fungi were isolated from soils, litters, leaves, and insects collected at various parts of Indonesia. In Indonesia and Japan, these isolates were cultured in at least 2 kinds of media and an antiamebic activity of cultured broths against an intestinal protozoan parasite, Entamoeba histolytica, was measured by cell-based assay. Cytotoxicity against mammalian cells was measured using human fibroblast cell line MRC-5.

Results and conclusions: We screened more than 6,000 broths. Fumagillin, a secondary metabolite of Aspergillus, has potent antiamebic activity. This compound was frequently found in active fungal broths. The broths which did not contain fumagillin were selected and antiamebic compounds were obtained by antiamebic activity-guided purification. 5 liter-culture broth sample F.0932 was purified by ODS and LH-20 column chromatography and preparative HPLC, thus we obtained citrinin, a famous mycotoxin, as an antiamebic compound. Citrinin showed cytotoxicity against MRC-5 with the same concentration of ED$_{50}$ value against E. histolytica. The other Indonesian fungal strain Aspergillus nomius BioMCC-f.MO.018 was cultured at Kitasato University in Japan. The broth extract was purified by HP20 and ODS column chromatography and preparative HPLC. We obtained tenuazonic acid as an antiamebic compound from BioMCC-f.MO.018 broth extract. The possibility of tenuazonic acid to become a potent antiamebic agent is under evaluation.