UNIVERSITY OF ALBERTA MICROFUNGUS COLLECTION AND HERBARIUM (UAMH)

A Unit of the Devonian Botanic Garden, Faculty of Agriculture, Life and Environmental Sciences
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SUMMARY OF ACTIVITIES FOR 2013

Supporting fungal research for over 50 years

Staff, Volunteers

Professor Emeritus (Curator to June 30) - **L. Sigler**
Devonian Botanic Garden/UAMH, Fac. Agriculture, Life & Environmental Sciences
Medical Microbiology & Immunology, Fac. of Medicine
Adjunct Professor in Biological Sciences
Consultant in Mycology, PLNA/UAH Microbiology & Public Health
Acting Curator from July 1, 2013 (.5 FTE Non-academic, .5 FTE trust) – **C. Gibas**
Technicians (trust, casual): **A. Anderson; V. Jajczay**
Volunteer- **M. Packer**

Cultures Received, Distributed and Accessioned

Cultures received for identification, deposit or in exchange (**Table 1**) **210**

Cultures distributed on request or in exchange (**Table 2**)..... **179**

Culture Collection and Herbarium Accessions

Accessions processed to Dec 31. ..... **147**

Total accessions..... **11819**

Information on Culture Accessions available through print and on-line CATALOGUES

Catalogue of the University of Alberta Microfungus Collection and Herbarium. [PDF]
https://secure.devonian.ualberta.ca/uamh/searchcatalogue.php

Collection Activities

- In 2013, 210 fungal isolates were received for deposit or identification and 179 isolates were distributed for various research purposes to scientists in universities, government and industry (**Tables 1 and 2**). Of the latter, 162 were distributed within Canada and the US, and 17 were sent internationally.
- The UAMH houses the largest North American repository of ecto-, orchid and ericoid mycorrhizal fungi as well as numerous collections of plant root associated fungi for which the ecological function is less clear. Significant collections of plant root associated fungi received in 2013 included 34 strains of dark septate endophytes collectively known as members of the *Phialocephala fortinii* s.l. – *Acephala applanata* species complex (PAC). These fungi are common colonizers of plant roots and their genetic diversity and ecosystem functions have been studied intensively by Drs TN Sieber of the Swiss Federal Institute of Technology, Zurich, Switzerland, C Grünig, Microsynth AG, Balgach, Switzerland and their associates (Refs **17, 19 – 21, 29 – 31**). Their analyses determined that the PAC comprises 7 species, *P. europaea, P. glacialis, P. helvetica, P. letzii, P. subalpina, P. turiciensis, P. uotolensis*, that are now represented by multiple well characterized isolates in the UAMH.

- Fifty one fungal isolates including many from rare or endangered orchid species were received from Dr. L. Zettler, Illinois College, Jacksonville, IL, whose research focuses on conservation of threatened orchids and the symbiotic germination of orchid seed with host -specific and locally adapted mycorrhizal fungi [**Ref 41**]. These add to the 250 isolates of orchid mycorrhizal fungi already accessioned. In nature, orchids depend on mycorrhizal fungi for seed germination and to provide a source of energy. Approximately 70 isolates held in UAMH have been demonstrated to initiate symbiotic seed germination. Many of the native orchid species in North America are under severe threat due to habitat destruction and many species are likely to become extirpated unless action is taken soon to conserve them and their fungal partners. Over 50 isolates held in UAMH are derived from the US federally threatened native species *Platanthera leucophaea*.
- We are collaborating with the North American Orchid Conservation Center (NAOCC) (http://northamericanorchidcenter.org/) housed at the Smithsonian Environmental Research Center (Edgewater, MD). The roles of the NAOCC are to establish collections of seeds and orchid mycorrhizal fungi and to develop protocols for propagation and restoration of native orchid species. A newly developed interactive website (http://goorchids.northamericanorchidcenter.org/) offers a mechanism to identify and learn about the features of North American orchids. A meeting of the NAOCC is planned for March 2014 to develop the seed and orchid banks. In September, we also met with directors of the local Orchid Species Preservation Foundation to discuss possible cooperation regarding development of a permanent orchid interpretive centre in Edmonton.
- The UAMH also houses large collections of beetle-associated wood-inhabiting fungi. Over 130 fungal isolates have been accessioned over several years through mountain pine beetle genomics projects. These isolates, representing six species of ophiostomatoid fungi (blue stain fungi), have been extensively genotyped [**Refs 32, 33**]. The genomes of a saprophyte, *Ophiostoma piceae* (UAMH 11346) [**Ref 8** http://www.ncbi.nlm.nih.gov/genome/?term=ophiostoma+piceae] and a pathogen *Grosmannia clavigera* (UAMH 11150 (=KW1407) http://www.ncbi.nlm.nih.gov/genome/?term=grosmannia+clavigera; completed in 2011) have now been fully sequenced by members of the UBC, U of A, TRIA research group (http://www.thetriaproject.ca). We continue to work with members of this research group to voucher newly obtained mountain pine beetle associated fungi and to supply cultures for further biologic and genomic studies.
- Additional information on the use of UAMH strains in diverse research applications is provided in section ***Publications citing UAMH cultures or assistance*** beginning on page 4 of this report. For example, the utilization of specific fungal species and isolates in producing decorative strains in wood to add commercial value and improvements in methods to enhance pigmentation have been studied extensively by SC Robinson, D Tudor and PA Cooper (Forestry, Univ Toronto) and their associates [**Refs 22 – 25, 35, 36**]. Characteristics of phytoalexins from the plant pathogen *Alternaria brassicicola* and of toxins and antigenic proteins produced by *Chaetomium globosum*, a common fungus in the indoor environment, have been evaluated by Canadian chemists MS Pedras (Univ Sask) (**Refs 14, 15**) and JD Miller (Carleton Univ) (**Refs 11, 16**) and their colleagues.

External Funding (Grants/Fees for Services)

NSERC Major Resources Support (ended Mar 31, 2013). The University of Alberta Microfungus Collection and Herbarium (UAMH). Sigler (PI), Currah, Hausner, Berbee (2008-2013) (Total $273,000). A moratorium on the MRS program in April 2012 left no option for renewal of this grant.	54,600
Shared / Core Research Facility (U of A). Microfungus Collection and Herbarium (UAMH). Sigler, L. (2013-2016) (Total $146,000)	48,660
Income from all services (cultures distributed, identifications, microbial assessments, consultation)	18,000

Other Activities (Sigler)

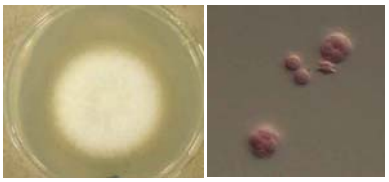
- May 31 – June 2: Invited participant in Alberta Microbiology Retreat, Banff, AB, sponsored by Medical Microbiology Residency Program, Dept of Medical Microbiology & Immunology, Fac Medicine & Dentistry
- June 12: University of Alberta Microfungus Collection and Herbarium (UAMH) – a Canadian Fungal Biodiversity Centre; invited talk to Alberta Health Services Medical Microbiology Journal Club

- Sept 24: visited collaborating veterinarian, Dr. Jean Paré at Bronx Zoo, Bronx NY for tour of animal health facilities; co-author of **Ref 2** cited below.
- Reviews: Antonie van Leeuwenhoek Journal of Microbiology (1); Journal of Clinical Microbiology (1)

In-house and Collaborative Research

Refereed Journal Articles Published

1. Malejczyk K, Sigler L, Gibas CFC, Smith SW. Invasive sino-orbital mycosis in an aplastic anemia patient caused by *Neosartorya laciniosa* . J Clin Microbiol 2013; 51 1316-1319. doi: 10.1128/JCM.02919-12. [reported as submitted in 2012 Annual Report]



We report the first case of *Neosartorya laciniosa* invasive sinusitis involving the orbit in an immunocompromised male with aplastic anemia. Treatment included surgical debridement with enucleation of the eye, combination voriconazole and micafungin therapy followed by voriconazole alone. The fungus was identified using sequencing of beta-tubulin and calmodulin gene regions. [UAMH 11627 isolated from patient at UA Hospitals]

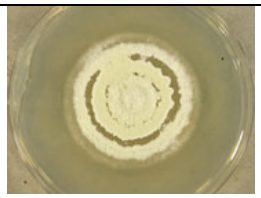
2. Sigler L, Hambleton S, Pare JA. Molecular characterization of reptile pathogens currently known as members of the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV) complex and relationship with some human-associated isolates. J Clin Microbiol 2013; 51(10):3338-3357. doi: 10.1128/JCM.01465-13.

In recent years, the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV), *Chrysosporium guarroi*, *Chrysosporium ophiodiicola* and *Chrysosporium* species have been reported as the cause of dermal or deep lesions in reptiles. Infections are contagious, often fatal and affect both captive and free ranging animals. Forty nine CANV isolates from reptiles and six isolates from human sources were compared with *Nannizziopsis vriesii* based on cultural characteristics and DNA sequence data. Analyses of sequences of the internal transcribed spacer and small subunit of the nuclear ribosomal gene revealed the reptile pathogens and human isolates to belong in well supported clades corresponding to three lineages distinct from all other taxa within the family Onygenaceae of the Onygenales. One lineage represents the genus *Nannizziopsis* and comprises *N. vriesii* and seven additional species encompassing isolates from chameleons and geckos, crocodiles, agamid and iguanid lizards and humans. Two other lineages comprise the genus *Ophidiomyces* with the species *Ophidiomyces ophiodiicola* occurring only on snakes, and *Paranannizziopsis* gen. nov. with three species from squamates and tuataras. The species newly described are *Nannizziopsis dermatitidis, Nannizziopsis crocodili, Nannizziopsis barbata , Nannizziopsis infrequens, Nannizziopsis hominis, Nannizziopsis obscura, Paranannizziopsis australasiensis, Paranannizziopsis californiensis* and *Paranannizziopsis crustacea. Chrysosporium guarroi* is transferred as *Nannizziopsis guarroi*. *N. guarroi* causes yellow fungus disease, a common infection in bearded dragons and green iguanas, and *O. ophiodiicola* is an emerging pathogen of captive and free-ranging snakes. Human-associated species were not recovered from reptiles and reptile-associated species were recovered only from reptiles, thereby mitigating zoonotic concerns.
[UAMH 3526, 3527 Type of *N. vriesii*, 6610, 7582, 7583 Type of *N. dermatitidis*, 7861, 11231, 11232, 9664-9666 Type of *N. crocodili*, 11185 Type of *N. barbata*, 10171, 10211, 10351 – 10353, 10409, 10918, 10928, 10938, 10944, 10960, 10417 Type of *N. infrequens*, 7859 Type of *N. hominis*, 7860, 7932, 5875 Type of *N. obscura*, 10439, 10440, 11644, 11645 Type of *P. australasiensis*, 11665, 11719, 10692, 10693 Type of *P. californiensis*, 10199 Type of *P. crustacea*, 10200, 10201, 6218 *O. opiodiicola*, 6642, 6688, 9832, 9985, 10079, 10768 – 10770, 10949, 11295]

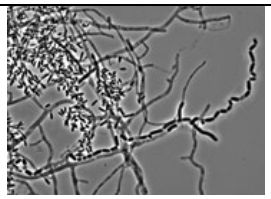
***Ophidiomyces ophiodiicola*, a fungus represented by 14 UAMH isolates, is associated with snake fungal disease, an emerging disease in some wild snake populations.**

Lesion on broad-headed snake (*Hoplocephalus bungaroides* courtesy D. McLelland)

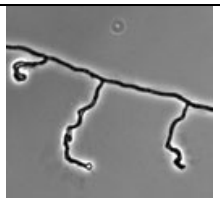
Fungus grown from biopsy of green anaconda snake



Isolate grown from Eastern diamondback rattlesnake



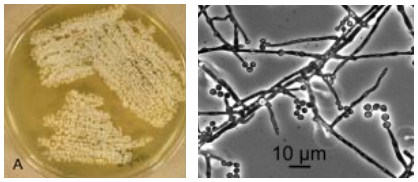
Microscopic characters of file snake isolate



Characteristic undulate hyphal branches

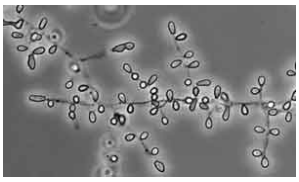
3. Sigler L, Hanselman B, Ruotsalo K, Tsui KG, Richardson S. Cytological, microbiological and therapeutic aspects of systemic infection in a dog caused by the fungus *Phialosimplex caninus.* Medical Mycology Case Reports 2013; 2:32–36. Doi 10.1016/j.mmcr.2012.12.007 [online 11 January 2013 http://dx.doi.org/10.1016/j.mmcr.2012.12.007]

A seven-year-old immunocompetent dog presenting with lymphadenopathy, mesenteric masses and splenic nodules was diagnosed with *Phialosimplex caninus* infection. Cytology of a mesenteric mass aspirate demonstrated few intact cells but numerous variably sized fungal cells and rare hyphal fragments. The identity of the cultured fungus was confirmed by DNA sequencing. Itraconazole therapy improved clinical signs, but the fungus was reisolated at follow-up. *P. caninus* systemic infection should be suspected in dogs presenting with lymphadenopathy and splenomegaly. [UAMH 11502, 11532]



4. Toplon DE, Terrell SP, Sigler L, Jacobson ER. Dermatitis and cellulitis in leopard geckos (*Eublepharis macularius*) caused by the *Chrysosporium* anamorph of *Nannizziopsis vriesii*. Vet Pathol. 2013; 50:585-9. doi: 10.1177/0300985812465324. [published online 16 November 2012]

An epizootic of ulcerative to nodular ventral dermatitis was observed in a large breeding colony of 8-month to 5-year-old leopard geckos (Eublepharis macularius) of both sexes. Two representative mature male geckos were euthanized for diagnostic necropsy. The Chrysosporium anamorph of Nannizziopsis vriesii (CANV) was isolated from the skin lesions, and identification was confirmed by sequencing of the internal transcribed spacer region of the rRNA gene. Histopathology revealed multifocal to coalescing dermal and subcutaneous heterophilic granulomas that contained septate fungal hyphae. There was also multifocal epidermal hyperplasia with hyperkeratosis, and similar hyphae were present within the stratum corneum, occasionally with terminal chains of arthroconidia consistent with the CANV. In one case, there was focal extension of granulomatous inflammation into the underlying masseter muscle. This is the first report of dermatitis and cellulitis due to the CANV in leopard geckos. [UAMH 11231, 11232] [Gecko image courtesy of ER Jacobson].



Publications Citing UAMH Cultures or Assistance

5. Cariello PF, Wickes BL, Sutton DA, Castlebury LA, Levitz SM, Finberg RW, Thompson EH, Daly JS. *Phomopsis bougainvilleicola* prepatellar bursitis in a renal transplant recipient. J Clin Microbiol. 2013; 51:692-5 doi: 10.1128/JCM.02674-12.

Prepatellar bursitis is typically a monomicrobial bacterial infection. A fungal cause is rarely identified. We describe a 61-year-old man who had received a renal transplant 21 months prior to presentation whose synovial fluid and surgical specimens grew *Phomopsis bougainvilleicola*, a pycnidial coelomycete. [UAMH 11634]

6. Diederich P, Ertz D, Lawrey JD, Sikaroodi M, Untereiner WA. Molecular data place the hyphomycetous lichenicolous genus *Sclerococcum* close to *Dactylospora* (*Eurotiomycetes*) and *S. parmeliae* in *Cladophialophora* (*Chaetothyriales*). Fungal Diversity. 2013; 58:61-72 doi: 10.1007/s13225-012-0179-4.

The lichenicolous anamorphic fungus *Sclerococcum parmeliae* was isolated in pure culture, and ITS, nuLSU and mtSSU sequences were obtained from these isolates. For comparison, sequences from S. sphaerale, the generic type, were obtained directly from freshly collected specimens. Phylogenetic analyses place *S. sphaerale* with species of *Dactylospora* and an unidentified lichen-inhabiting isolate in a strongly supported clade that is sister to a lineage comprising members of the Chaetothyriales and Pyrenulales. In contrast, *S. parmeliae* is inferred as a member of the Herpotrichiellaceae (Chaetothyriales) and belongs to a robustly supported clade that also includes species of *Cladophialophora*, *Capronia semiimmersa*, and *Phialophora verrucosa*. Within the Herpotrichiellaceae, *S. parmeliae* most closely resembles members of the anamorph genus *Cladophialophora*. Accordingly, we propose the transfer of *S. parmeliae* and the morphologically similar species *S. cladoniae*, *S. hawksworthii* and *S. normandinae* to *Cladophialophora*. A new lichenicolous species, *Clad. megalosporae*, collected twice on *Megalospora* in Florida and

Papua New Guinea, is also described. [UAMH 11090]

7. Doyon JB, Sutton DA, Theodore P, Dhillon G, Jones KD, Thompson EH, Fu J, Wickes BL, Koehler JE, Schwartz BS. *Rasamsonia argillacea* pulmonary and aortic graft infection in an immune-competent patient. J Clin Microbiol. 2013; 51:719-22 doi: 10.1128/JCM.02884-12.

Rasamsonia argillacea (formerly known as *Geosmithia argillacea*) is a fungus recently recognized as a pathogen of immunocompromised patients. Here we report the first case of *Rasamsonia* infection in an immunocompetent host, presenting as a pulmonary and aortic graft infection. Its morphological similarity to nonpathogenic *Penicillium* species delayed the diagnosis and initiation of appropriate treatment. [UAMH 11662, 11663]

8. Haridas S, Wang Y, Lim L, Alamouti SM, Jackman S, Docking R, Robertson G, Birol I, Bohlmann J, Breuil C. The genome and transcriptome of the pine saprophyte *Ophiostoma piceae*, and a comparison with the bark beetle-associated pine pathogen *Grosmannia clavigera*. BMC Genomics. 2013; 14:373 doi: 10.1186/1471-2164-14-373.

Background: *Ophiostoma piceae* is a wood-staining fungus that grows in the sapwood of conifer logs and lumber. We sequenced its genome and analyzed its transcriptomes under a range of growth conditions. A comparison with the genome and transcriptomes of the mountain pine beetle-associated pathogen *Grosmannia clavigera* highlights differences between a pathogen that colonizes and kills living pine trees and a saprophyte that colonizes wood and the inner bark of dead trees.
Results: We assembled a 33 Mbp genome in 45 scaffolds, and predicted approximately 8,884 genes. The genome size and gene content were similar to those of other ascomycetes. Despite having similar ecological niches, *O. piceae* and *G. clavigera* showed no large-scale synteny. We identified *O. piceae* genes involved in the biosynthesis of melanin, which causes wood discoloration and reduces the commercial value of wood products. We also identified genes and pathways involved in growth on simple carbon sources and in sapwood, *O. piceae's* natural substrate. Like the pathogen, the saprophyte is able to tolerate terpenes, which are a major class of pine tree defense compounds; unlike the pathogen, it cannot utilize monoterpenes as a carbon source.
Conclusions: This work makes available the second annotated genome of a softwood ophiostomatoid fungus, and suggests that *O. piceae's* tolerance to terpenes may be due in part to these chemicals being removed from the cells by an ABC transporter that is highly induced by terpenes. The data generated will provide the research community with resources for work on host-vector-fungus interactions for wood-inhabiting, beetle-associated saprophytes and pathogens. [UAMH 11346, 11672]

9. LeBlanc RE, Meriden Z, Sutton DA, Thompson EH, Neofytos D, Zhang SX. *Cunninghamella echinulata* causing fatally invasive fungal sinusitis. Diagn Microbiol Infect Dis. 2013; 76:506–9 doi: 10.1016/ j.diagmicrobio.2013.03.009.

We report a fatal case of invasive fungal sinusitis caused by *Cunninghamella echinulata* in a febrile, neutropenic 15-year-old male with relapsing acute leukemia. The isolate was recovered from a nasal biopsy from the right middle meatus, and microscopic examination of the tissue revealed angioinvasion and necrosis. Human infection caused by this organism has not been well documented; however, this report alerts us to its life-threatening potential. [UAMH 11661]

10. Ma A, Wong Q. Identification of esterase in *Aspergillus flavus* during degradation of polyester polyurethane. Canadian Young Scientist Journal. 2013; 2:24-31 doi 10.13034/cysj-2013-004.

Polyurethane not only has applications including tires and insulation, but also is an essential element of our lives. Based on previous reports, some strains of fungus including *Pestalotiopsis microspora* have been reported to degrade this plastic. The ultimate objective is to create a genetically engineered variant of *Pichia pastoris* which will digest polyester polyurethane (PUR) using the enzyme responsible for PUR degradation from *Aspergillus flavus*; this year we aim to identify the esterase, believed to be responsible for the biodegradation. The degradation of TPU (a granular form of thermoplastic PUR) and water-based PUR by *A. flavus*, was tested using incubation with shaking for one month. Although no significant weight loss was observed, the fungus was capable of growing on the water-based PUR as a food source. Esterase, the enzyme responsible for the degradation of the water-based PUR, was observed in the water-based PUR lane of the Native PAGE gel and has a molecular weight of approximately 20 kDa. Since the only difference between the experimental water-based lane and experimental TPU lane, was the esterase band, it can be confirmed that the degradation of the plastic and growth of fungus can be attributed to the esterase. [UAMH 7574]

11. McMullin DR, Sumarah MW, Miller JD. Chaetoglobosins and azaphilones produced by Canadian strains of *Chaetomium globosum* isolated from the indoor environment. Mycotoxin Research. 2013; 29 (1): 47-54. doi: 201210.1007/s12550-012-0144-9.

Chaetomium globosum is one of the most common species of fungi found growing on damp building materials in North America and Europe. At doses that could be experienced in a building with some mould damage, exposure to metabolites from other fungi results in inflammatory changes in vivo and in vitro. This research requires knowledge of the dominant toxins produced by fungal strains from the built environment and characterization of pure compounds for toxicity testing. We examined 25 strains of *C. globosum* isolated from the built environment in Canada. In varying amounts, these strains primarily produced chaetoglobosin A, C and F, chaetomugilin D, and chaetoviridin A. Spectroscopic data of the major isolated compounds are provided. Previous studies reported a number of metabolites from this species that we did not find. However, this appears to be due to misidentifications of the fungi they examined as well as problems with the analytical methods used. In addition, our data support the use of metabolite profiles for resolving the taxonomy of some economically important *Chaetomium* species. [UAMH 7142, 7773]

12. Neafsey DE, Barker BM, Sharpton TJ, Stajich JE, Park DJ, Whiston E, Hung CY, McMahan C, White J, Sykes S, Heiman D, Young S, Zeng Q, Abouelleil A, Aftuck L, Bessette D, Brown A, FitzGerald M, Lui A, Macdonald JP, Priest M, Orbach MJ, Galgiani JN, Kirkland TN, Cole GT, Birren BW, Henn MR, Taylor JW, Rounsley SD. Population genomic sequencing of *Coccidioides* fungi reveals recent hybridization and transposon control. Genome Res 2010; 20(7): 938-46. doi: 10.1101/gr.103911

We have sequenced the genomes of 18 isolates of the closely related human pathogenic fungi Coccidioides immitis and *Coccidioides posadasii* to more clearly elucidate population genomic structure, bringing the total number of sequenced genomes for each species to 10. Our data confirm earlier microsatellite-based findings that these species are genetically differentiated, but our population genomics approach reveals that hybridization and genetic introgression have recently occurred between the two species. The directionality of introgression is primarily from *C. posadasii* to *C. immitis*, and we find more than 800 genes exhibiting strong evidence of introgression in one or more sequenced isolates. We performed PCR-based sequencing of one region exhibiting introgression in 40 *C. immitis* isolates to confirm and better define the extent of gene flow between the species. We find more coding sequence than expected by chance in the introgressed regions, suggesting that natural selection may play a role in the observed genetic exchange. We find notable heterogeneity in repetitive sequence composition among the sequenced genomes and present the first detailed genome-wide profile of a repeat-induced point mutation (RIP) process distinctly different from what has been observed in *Neurospora*. We identify promiscuous HLA-I and HLA-II epitopes in both proteomes and discuss the possible implications of introgression and population genomic data for public health and vaccine candidate prioritization. This study highlights the importance of population genomic data for detecting subtle but potentially important phenomena such as introgression. [UAMH 1704 *Uncinocarpus reesii*]

13. Orós J, Hernández JD, Gallardo J, Lupiola P, Jensen HE. Dermatophytosis caused by *Trichophyton* spp. in a Tenerife Lizard (*Gallotia galloti*): an immunohistochemical study. J Comp Pathol. 2013; 149:372–375 doi: 10.1016/j.jcpa.2012.11.245.

Reports of dermatophytosis in reptiles are rare. This report describes the microscopical and immunohistochemical findings in a case of dermatophytosis caused by Trichophyton spp. in a 2-year-old Tenerife lizard (*Gallotia galloti*) with ulcerative and pustular skin lesions. Microscopically, the lesions were characterized by superficial epidermal pustules containing heterophils with numerous fungal hyphae that stained by periodic acid–Schiff and Grocott's stain. Fungal culture was not performed, but a panel of polyclonal antibodies specific for different fungal genera was applied to tissue sections. These immunohistochemical studies demonstrated reactivity of the hyphae only with antiserum specific for *Trichophyton spp*. [UAMH 7583]

14. Pedras MS, Abdoli A. Metabolism of the phytoalexins camalexins, their bioisosteres and analogues in the plant pathogenic fungus Alternaria brassicicola. Bioorg Med Chem 2013: 21(15) 4541-9. doi: 10.1016/j.bmc.2013.05.026. Epub 2013 May 30.

The metabolism of the phytoalexins camalexin (1), 1-methylcamalexin (10) and 6-methoxycamalexin (11) by Alternaria brassicicola and their antifungal activity is reported. This work establishes that camalexins are slowly biotransformed (ca. six days) to the corresponding indole-3-thiocarboxamides, which are further transformed to the indole-3-carboxylic acids. These metabolites are substantially less inhibitory to *A. brassicicola* than the parent camalexins, indicating that these enzyme-mediated transformations are detoxifications. In addition, analyses of the

metabolism of synthetic isomers and bioisosteres of camalexin (1) indicate that isomers of camalexin in the thiazole ring are not metabolized. Based on these results, the potential intermediates that lead to formation of indole-3-thiocarboxamides are proposed. [UAMH 7474]

15. Pedras MSC, Minic Z. The phytoalexins brassilexin and camalexin inhibit cyclobrassinin hydrolase, a unique enzyme from the fungal pathogen *Alternaria brassicicola*. Bioorg Med Chem. 2014; 22(1):459-67. doi: 10.1016/j.bmc.2013.11.005.Epub Nov 2013

Alternaria brassicicola is a fungal pathogen of many agriculturally important cruciferous crops. Cyclobrassinin hydrolase (CH) is an enzyme produced by *A. brassicicola* that catalyzes the transformation of the cruciferous phytoalexin cyclobrassinin into S-methyl[(2-sulfanyl-1H-indolyl-3)methyl]carbamothioate. The purification and characterization of CH was performed using a four-step chromatography method. SDS–PAGE and gel exclusion chromatography indicated that CH is a tetrameric protein with molecular mass of 330 kDa. Sequence analysis and chemical modification of CH with selective reagents suggested that the enzyme mediates hydrolysis of cyclobrassinin using a catalytic amino acid triad. Enzyme kinetic studies using cyclobrassinin and 1-methylcyclobrassinin as substrates revealed that CH displayed positive substrate cooperativity. Investigation of the effect of nine phytoalexins and two derivatives on the activity of CH indicated that six compounds displayed inhibitory activity: brassilexin, 1-methylbrassilexin, dioxibrassinin, camalexin, brassicanal A and sinalexin. The enzyme kinetics of CH strongly suggested that brassilexin and 1-methylbrassilexin are noncompetitive inhibitors of CH activity, and that camalexin is a competitive inhibitor while dioxibrassinin inhibits CH through a mixed mechanism. The phytoalexin brassilexin is the most effective inhibitor of CH ($K_i = 32 \pm 9$ μM). These results suggest that crops able to accumulate higher concentration of brassilexin would display higher resistance levels to the fungus. [UAMH 7474]

16. Provost NB, Shi C, She YM, Cyr TD, Miller JD. Characterization of an antigenic chitosanase from the cellulolytic fungus *Chaetomium globosum*. Med Mycol. 2013; 51(3): 290-299. doi:10.3109/13693786.2012.715246.

We are interested in identifying human fungal allergens and antigens from species common on water-damaged or damp building materials for use as marker proteins and diagnostic tests. The cellulolytic fungus *Chaetomium globosum* is common on damp materials in the building environment worldwide. ELISA and immunoblotting tests identified two related proteins of molecular weights 45 and 47 kDa which were identified as fungal antigens found on spore surfaces and in culture filtrate. The sequences were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS), which indicated that the two proteins were chitosanases, confirmed by enzyme assay. The 47 kDa protein was not glycosylated and had an acidic pI of 4.5. These proteins have not been reported from other fungi and similar antigens were not seen in other fungi common in buildings. The production of polyclonal antibodies in rabbits showed the antigenicity of the target proteins and confirmed they were not artifacts of the isolation process. The proteins isolated are useful biomarkers for the detection of *C. globosum* in the building environment. [UAMH 7142, 7773]

17. Queloz V, Sieber TN, Holdenrieder O, McDonald BA, Grünig CR. No biogeographical pattern for a root-associated fungal species complex. Global Ecology and Biogeography, 2011; 20:160–169. doi: 10.1111/j.1466-8238.2010.00589.x.

Aim: The biogeography of microbes is poorly understood and there is an open debate regarding if and how microbial biodiversity is structured. At the beginning of the 20th century, Baas Becking laid the foundations for the biogeography of microbes by stating that 'Everything is everywhere, but the environment selects' (the EisE hypothesis). This hypothesis remained dogma for almost a century. However, the recognition that microbial 'species' are often assemblages of reproductively isolated lineages challenged the EisE hypothesis, leading to the now common assumption that microbial communities possess cryptic biogeographic structures. We tested the presence of a cryptic biogeographical structure for a well-characterized fungal species complex (the *Phialocephala fortinii s.l.–Acephala applanata* species complex, PAC) using precise molecular species resolution. In addition, we analysed factors that could govern PAC community assembling.
Locations: Forty-four study sites in temperate and boreal forests across the Northern Hemisphere were included.
Methods: (1) The distance–decay relationship among PAC communities was calculated and a resampling procedure was applied to analyse the effect of sampling intensity and geographic distances among PAC communities. (2) Factors shaping PAC communities (e.g. climatic factors and tree species composition) were studied. (3) We tested PAC communities for random composition.
Results: We found that the similarity of species assemblages did not decrease with increasing geographical distance.

Moreover, species diversity did not increase by expanding the area sampled. Instead, species diversity increased by increasing the sampling effort. Community composition correlated neither with tree species composition nor climate, and no association among species was observed.
Main conclusions: We could not discover any cryptic biogeographic structure even after applying refined species assignment but we demonstrate the importance of sampling effort for understanding the biogeography of microorganisms. Moreover, we show that primarily stochastic effects are responsible for the species composition of PAC communities. [UAMH 11679, 11680, 11681, 11682, 11683]

18. Réblová M, Untereiner WA, Réblová K. Novel evolutionary lineages revealed in the *Chaetothyriales* (fungi) based on multigene phylogenetic analyses and comparison of its secondary structure. PLOS. Epub May 2013; doi 10.1371/journal.pone.0063547.

Cyphellophora and *Phialophora* (Chaetothyriales, Pezizomycota) comprise species known from skin infections of humans and animals and from a variety of environmental sources. These fungi were studied based on the comparison of cultural and morphological features and phylogenetic analyses of five nuclear loci, i.e., internal transcribed spacer rDNA operon (ITS), large and small subunit nuclear ribosomal DNA (nuc28S rDNA, nuc18S rDNA), β-tubulin, DNA replication licensing factor (mcm7) and second largest subunit of RNA polymerase II (rpb2). Phylogenetic results were supported by comparative analysis of ITS1 and ITS2 secondary structure of representatives of the *Chaetothyriales* and the identification of substitutions among the taxa analyzed. Base pairs with non-conserved, co-evolving nucleotides that maintain base pairing in the RNA transcript and unique evolutionary motifs in the ITS2 that characterize whole clades or individual taxa were mapped on predicted secondary structure models. Morphological characteristics, structural data and phylogenetic analyses of three datasets, i.e., ITS, ITS-β-tubulin and 28S-18S-rpb2-mcm7, define a robust clade containing eight species of *Cyphellophora* (including the type) and six species of *Phialophora*. These taxa are now accommodated in the *Cyphellophoraceae*, a novel evolutionary lineage within the Chaetothyriales. *Cyphellophora* is emended and expanded to encompass species with both septate and nonseptate conidia formed on discrete, intercalary, terminal or lateral phialides. Six new combinations in *Cyphellophora* are proposed and a dichotomous key to species accepted in the genus is provided. *Cyphellophora eugeniae* and *C. hylomeconis,* which grouped in the *Chaetothyriaceae*, represent another novel lineage and are introduced as the type species of separate genera. [2981, 3632, 4344, 4411, 4935, 11090, 10396, 10872]

19. Reininger V, Grünig CR, Sieber TN. Microsatellite-based quantification method to estimate biomass of endophytic *Phialocephala* species in strain mixtures. Microb Ecol. 2011; 61:676-83. doi: 10.1007/s00248-010-9798-z.

Fungi of the *Phialocephala fortinii* sensu lato-*Acephala applanata* species complex (PAC) are ubiquitous endophytic colonizers of tree roots in which they form genotypically diverse communities. Measurement of the colonization density of each of the fungal colonizers is a prerequisite to study the ecology of these communities. Up to now, there is no method readily available for the quantification of PAC strains co-colonizing the same root. The new DNA quantification method presented here is based on the amplification of microsatellites by competitive polymerase chain reaction (PCR). The method proved to be suitable to detect and quantify at least two strains within one single sample by the addition of a known amount of mycelium of a reference strain before DNA extraction. The method exploits the correlation between the reference/target ratio of light emitted during microsatellite detection (peak ratio) and the reference/target ratio of mycelial weights to determine the biomass of the target strain. Hence, calibration curves were obtained by linear regression of the peak ratios on the weight ratios for different mixtures of reference and target strains. The slopes of the calibration curves and the coefficients of determination were close to 1, indicating that peak ratios are good predictors of weight ratios. Estimates of fungal biomass in mycelial test mixtures of known composition laid within the 95% prediction interval and deviated on average by 16% (maximally 50%) from the true biomass. On average, 3-6% of the root biomass of Norway spruce seedlings consisted of mycelial biomass of either one of two inoculated PAC strains. Biomass estimates obtained by real-time quantitative PCR were correlated with the estimates obtained by the microsatellite-based method, but variation between the two estimates from the same root was high in some samples. The microsatellite-based DNA quantification method described here is currently the best method for strainwise estimation of endophytic biomass of PAC fungi in small root samples. [UAMH 11676, 11677, 11695]

20. Reininger V, Grünig CR, Sieber TN. Host species and strain combination determine growth reduction of spruce and birch seedlings colonized by root-associated dark septate endophytes. Environ Microbiol. 2012; 14: 1064–1076. doi: 10.1111/j.1462-2920.2011.02686.x.

Interactions of *Betula pendula* and *Picea abies* with dark septate endophytes of the *Phialocephala fortinii-Acephala*

applanata species complex (PAC) were studied. PAC are ubiquitous fungal root symbionts of many woody plant species but their ecological role is largely unknown. Sterile birch and spruce seedlings in monoculture and mixed culture were exposed to four PAC strains, added either singularly or paired in all possible combinations at 18°C and 23°C. Plant and fungal biomass was determined after 4 months. The most significant factors were strain and host combination. One of the strains significantly reduced biomass gain of spruce but not of birch. Plant biomass was negatively correlated with total endophytic fungal biomass in half of the strain - plant combinations. Endophytic PAC biomass was four times higher in spruce (≈ 40 mg g(-1) drw) than in birch (≈ 10 mg g(-1) drw). Competition between strains was strain-dependent with some strains significantly reducing colonization density of other strains, and, thus, attenuating adverse effects of 'pathogenic' strains on plant growth in some strain - plant combinations. Biomass gain of spruce but not of birch was significantly reduced at higher temperature. In conclusion, host, fungal genotype, colonization density and presence of a competing PAC strain were the main determining factors for plant growth. [UAMH 11676, 11677, UAMH 11695]

21. Reininger V, Sieber TN. Mycorrhiza reduces adverse effects of dark septate endophytes (DSE) on growth of conifers. PLoS ONE. 2012; 7:e42865. doi:10.1371/journal.pone.0042865.

Mycorrhizal roots are frequently colonized by fungi of the *Phialocephala fortinii* s.l. – *Acephala applanata* species complex (PAC). These ascomycetes are common and widespread colonizers of tree roots. Some PAC strains reduce growth increments of their hosts but are beneficial in protecting roots against pathogens. Nothing is known about the effects of PAC on mycorrhizal fungi and the PAC-mycorrhiza association on plant growth, even though these two fungal groups occur closely together in natural habitats. We expect reduced colonization rates and reduced negative effects of PAC on host plants if roots are co-colonized by an ectomycorrhizal fungus (ECM). Depending on the temperature regime interactions among the partners in this tripartite ECM-PAC-plant system might also change. To test our hypotheses, effects of four PAC genotypes (two pathogenic and two non-pathogenic on the Norway spruce), mycorrhization by *Laccaria bicolor* (strain S238N) and two temperature regimes (19°C and 25°C) on the biomass of the Douglas-fir (*Pseudotsuga menziesii*) and Norway spruce (*Picea abies*) seedlings were studied. Mycorrhization compensated the adverse effects of PAC on the growth of the Norway spruce at both temperatures. The growth of the Douglas-fir was not influenced either by PAC or mycorrhization at 19°C, but at 25°C mycorrhization had a similar protective effect as in the Norway spruce. The compensatory effects probably rely on the reduction of the PAC-colonization density by mycorrhizae. Temperature and the PAC strain only had a differential effect on the biomass of the Norway spruce but not on the Douglas-fir. Higher temperature reduced mycorrhization of both hosts. We conclude that ectomycorrhizae form physical and/or physiological barriers against PAC leading to reduced PAC-colonization of the roots. Additionally, our results indicate that global warming could cause a general decrease of mycorrhization making primary roots more accessible to other symbionts and pathogens. [UAMH 11676, 11677, 11695]

22. Robinson SC. Developing fungal pigments for "painting" vascular plants. Appl Microbiol Biotechnol 2012; 93(4): 1389–94. doi: 10.1007/s00253-011-3858-2

The use of fungal pigments as color additives to wood as a method to increase forest revenue is a relatively new, but quickly developing field. Sugar maple (*Acer saccharum*) is currently the primary utilized hardwood for spalting and appears to be the best suited North American hardwood for such purposes. The combination of *Trametes versicolor* and *Bjerkandera adusta* has been identified in several instances as a strong fungal pairing for zone line production; however, *Xylaria polymorpha* is capable of creating zone lines without the antagonism of a secondary fungus. Few fungal pigments have been developed for reliable use; *Scytalidium cuboideum* is capable of producing a penetrating pink/red stain, as well as a blue pigment after extended incubation, and *Chlorociboria* sp. produces a blue/green pigment if grown on aspen (*Populus tremuloides*). Several opportunities exist for stimulation of fungal pigments including the use of copper sulfate and changes in wood pH. [4802, 11517 –-1521, 11655 –11657]

23. Robinson SC, Tudor D, Cooper PA. Utilizing pigment-producing fungi to add commercial value to American beech (*Fagus grandifolia*). Appl Microbiol Biotechnol 2012; 93(3):1041-8. doi: 10.1007/s00253-011-3576-9.

American beech (*Fagus grandifolia*) is an abundant, underutilized tree in certain areas of North America, and methods to increase its market value are of considerable interest. This research utilized pigment-producing fungi to induce color in American beech to potentially establish its use as a decorative wood. Wood samples were inoculated with *Trametes versicolor, Xylaria polymorpha, Inonotus hispidus*, and *Arthrographis cuboidea* to induce fungal pigmentation. Black pigmentation (*T. versicolor, X. polymorpha, I. hispidus*) was sporadic, occurred primarily on the surfaces of the heartwood, but not internally. Pink pigmentation (*A. cuboidea*) occurred throughout all of the tested beech samples, but was difficult to see in the heartwood due to the darker color of the wood. To increase

the visibility of the pink stain, beech blocks were pretreated with *T. versicolor* for 4 weeks before being inoculated with *A. cuboidea*. This method significantly increased the saturation of the pink stain on both beech heartwood and sapwood, creating coloration similar to that found on sugar maple. This value-adding process should be particularly effective for small-scale wood pigmentation, and should help establish a market for this currently underutilized wood species. [UAMH 11517 (as ELS-1), 11520, 11521]

24. Robinson SC, Tudor D, Hipson S, Snider H, Sheena Ng S, Korshikov E, Cooper PA. Methods of inoculating *Acer* spp., *Populus tremuloides*, and *Fagus grandifolia* logs for commercial spalting applications. J Wood Sci 59 (4): 351-357, 2013. DOI 10.1007/s10086-013-1335-5

One of the most promising wood value-added processes currently under development is spalting, where pigment is added to wood via fungal colonization. Previous studies have shown laboratory level spalting to be achievable and highly predictable. However, large-scale spalting for potential commercial applications introduces a substantial number of additional variables which impact the spalting process. To test the potential of commercial-scale spalting, *Acer saccharum*, *Fagus grandifolia*, and *Populus tremuloides* logs were inoculated with multiple known spalting fungi utilizing both liquid spray cultures and live dowel pin cultures. Many of the fungi that successfully produce spalting in small, sterile cultures also produced significant amounts in large logs, with many spalting patterns identical to those found in small-scale testing. Pairings of *Trametes versicolor*/*Scytalidium cuboideum* and *Xylaria polymorpha*/*Xylaria polymorpha* (different isolates) produced significant amounts of zone lines. In addition, the method of inoculation impacted the amount of spalting: more zone lines were produced when fungi were introduced via plugs, while more stain was produced when liquid cultures were sprayed onto the logs. These results indicate that many of the standard spalting fungi are suitable for large-scale applications; however, the inoculation method appears to be a vital component for successful spalting under a restricted time schedule. [UAMH 1502, 11517, 11518]

25. Robinson SC, Tudor D, Zhang WR, Ng S, Cooper PA. Ability of three yellow pigment producing fungi to colour wood under controlled conditions. International Wood Products Journal. Epub 2013 ahead of print. doi: 10.1179/2042645313Y.0000000060.

Inonotus hispidus, Scytalidium ganodermophthorum and two strains of *Scytalidium lignicola* were tested for their ability to produce yellow extracellular pigment on media plates, sterile wood blocks and non-sterile logs to determine their suitability for use as spalting fungi. All three fungi produced a penetrating yellow pigment in the non-sterile logs after 12 weeks of incubation; however, results from the sterile block tests indicated that the incubation time necessary for *I. hispidus* to produce sufficient yellow pigment may be as low as 4 weeks of incubation. An incubation period of 4 weeks is the shortest recorded for controlled spalting and will allow for the currently utilised production time for yellow spalted wood of 12 weeks to be substantially decreased using an isolate of *I. hispidus* as the inoculum. [UAMH 1502, 5122, 10320]

26. Selbmann L, Grube M, Onofri S, Isola D, Zucconi L. Antarctic epilithic lichens as niches for black meristematic fungi. Biology. 2013; 2:784-97 doi: 10.3390/biology2020784.

Sixteen epilithic lichen samples (13 species), collected from seven locations in Northern and Southern Victoria Land in Antarctica, were investigated for the presence of black fungi. Thirteen fungal strains isolated were studied by both morphological and molecular methods. Nuclear ribosomal 18S gene sequences were used together with the most similar published and unpublished sequences of fungi from other sources, to reconstruct an ML tree. Most of the studied fungi could be grouped together with described or still unnamed rock-inhabiting species in lichen dominated Antarctic cryptoendolithic communities. At the edge of life, epilithic lichens withdraw inside the airspaces of rocks to find conditions still compatible with life; this study provides evidence, for the first time, that the same microbes associated to epilithic thalli also have the same fate and chose endolithic life. These results support the concept of lichens being complex symbiotic systems, which offer attractive and sheltered habitats for other microbes. [UAMH 9870]

27. Sethuraman J, Rudski SM, Wosnitza K, Hafez M, Guppy B, Hausner G. Evolutionary dynamics of introns and their open reading frames in the U7 region of the mitochondrial rnl gene in species of *Ceratocystis*. Fungal Biology. 2013; 117:791–806 doi: 10.1016/j.funbio.2013.10.002.

The mtDNA rnl-U7 region has been examined for the presence of introns in selected species of the genus *Ceratocystis*. Comparative sequence analysis identified group I and group II introns encoding single and double motif LAGLIDADG open reading frames (ORFs) at the following positions L1671, L1787, and L1923. In addition

downstream of the rnl-U7 region group I introns were detected at positions L1971 and L2231, and a group II intron at L2059. A GIY-YIG type ORF was located within one mL1923 LAGLIDADG type ORF and a degenerated GIY-YIG ORF fused to a nad2 gene fragment was found in association with the mL1971 group I intron. The diversity of composite elements that appear to be sporadically distributed among closely related species of *Ceratocystis* illustrates the potential for homing endonucleases and their associated introns to invade new sites. Phylogenetic analysis showed that single motif LADGLIDADG ORFs related to the mL1923 ORFs have invaded the L1787 group II intron and the L1671 group I intron. Phylogenetic analysis of intron encoded single and double motif LAGLIDADG ORFs also showed that these ORFs transferred four times from group I into group II B1 type introns. [UAMH 9550, 9783, 9644, 4906, 9396, 8395, 963, 8399, 9259, 9968, 8839, 11187, 11188]

28. Sutton DA, Marín Y, Thompson EH, Wickes BL, Fu J, García D, Swinford A, de Maar T, Guarro J. Isolation and characterization of a new fungal genus and species, *Aphanoascella galapagosensis*, from carapace keratitis of a Galapagos tortoise (*Chelonoidis nigra microphyes*). Med Mycol. 2013; 51:113-20. doi: 10.3109/13693786.2012.701767.

A new fungal genus and species, *Aphanoascella galapagosensis*, recovered from carapace keratitis in a Galapagos tortoise residing in a south Texas zoological collection, is characterized and described. The presence of a pale peridium composed of textura epidermoidea surrounded by scarce Hülle cell-like chlamydospores, and the characteristic reticulate ascospores with an equatorial rim separates it from other genera within the Onygenales. The phylogenetic tree inferred from the analysis of D1/D2 sequences demonstrates that this fungus represents a new lineage within that order. As D1/D2 and ITS sequence data also shows a further separation of *Aphanoascus* spp. into two monophyletic groups, we propose to retain the generic name *Keratinophyton* for species whose ascospores are pitted and display a conspicuous equatorial rim, and thereby propose new combinations in this genus for four *Aphanoascus* species. [UAMH 11703]

29. Tellenbach C, Grünig CR, Sieber TN. Suitability of quantitative real-time PCR to estimate the biomass of fungal root endophytes. Appl Environ Microbiol. 2010; 76(17): 5764-5772.

A nested single-copy locus-based quantitative PCR (qPCR) assay and a multicopy locus-based qPCR assay were developed to estimate endophytic biomass of fungal root symbionts belonging to the *Phialocephala fortinii* sensu lato-*Acephala applanata* species complex (PAC). Both assays were suitable for estimation of endophytic biomass, but the nested assay was more sensitive and specific for PAC. For mycelia grown in liquid cultures, the correlation between dry weight and DNA amount was strong and statistically significant for all three examined strains, allowing accurate prediction of fungal biomass by qPCR. For mycelia colonizing cellophane or Norway spruce roots, correlation between biomass estimated by qPCR and microscopy was strain dependent and was affected by the abundance of microsclerotia. Fungal biomass estimated by qPCR and microscopy correlated well for one strain with poor microsclerotia formation but not for two strains with high microsclerotia formation. The accuracy of qPCR measurement is constrained by the variability of cell volumes, while the accuracy of microscopy can be hampered by overlapping fungal structures and lack of specificity for PAC. Nevertheless, qPCR is preferable because it is highly specific for PAC and less time-consuming than quantification by microscopy. There is currently no better method than qPCR-based quantification using calibration curves obtained from pure mycelia to predict PAC biomass in substrates. In this study, the DNA amount of *A. applanata* extracted from 15 mm of Norway spruce fine root segments (mean diameter, 610 microm) varied between 0.3 and 45.5 ng, which corresponds to a PAC biomass of 5.1 +/- 4.5 microg (estimate +/- 95% prediction interval) and 418 +/- 264 microg. [UAMH 11675, 11715]

30. Tellenbach C, Grünig CR, Sieber TN. Negative effects on survival and performance of Norway spruce seedlings colonized by dark septate root endophytes are primarily isolate-dependent. Environ Microbiol. 2011; 13:2508 – 17. doi: 10.1111/j.1462-2920.2011.02523.x.

Root endophytes are common and genetically highly diverse suggesting important ecological roles. Yet, relative to above-ground endophytes, little is known about them. Dark septate endophytic fungi of the *Phialocephala fortinii* s.l.-*Acephala applanata* species complex (PAC) are ubiquitous root colonizers of conifers and Ericaceae, but their ecological function is largely unknown. Responses of Norway spruce seedlings of two seed provenances to inoculations with isolates of four PAC species were studied in vitro. In addition, isolates of *Phialocephala subalpina* from two populations within and one outside the natural range of Norway spruce were also included to study the effect of the geographic origin of *P. subalpina* on host response. The interaction of PAC with Norway spruce ranged from neutral to highly virulent and was primarily isolate-dependent. Variation in virulence was much higher within than among species, nonetheless only isolates of P. *subalpina* were highly virulent. Disease caused by *P. subalpina* genotypes from the native range of Norway spruce was more severe than that induced by genotypes from outside

the natural distribution of Norway spruce. Virulence was not correlated with the phylogenetic relatedness of the isolates but was positively correlated with the extent of fungal colonization as measured by quantitative real-time PCR. [UAMH 11675 – 11695, 11704 – 11716]

31. Tellenbach C,. Sieber TN. Do colonization by dark septate endophytes and elevated temperature affect pathogenicity of oomycetes? FEMS Microbiology Ecology 2012; 82: 157–168.

Phialocephala subalpina is one of the most frequent dark septate root endophytes in tree roots but its function in forest ecosystems is largely unknown. A full-factorial infection experiment was performed, using six *P. subalpina* isolates, two pathogenic oomycetes (*Phytophthora plurivora* [syn. *Phytophthora citricola* s.l.] and *Elongisporangium undulatum* [syn. *Pythium undulatum*]) and two temperature regimes (17.9 and 21.6 °C) to examine the ability of *P. subalpina* to protect Norway spruce seedlings against root pathogens. Seedling survival, disease intensity and seedling growth were affected by *P. subalpina* genotype, temperature and pathogen species. Some *P. subalpina* isolates effectively reduced mortality and disease intensity caused by the two pathogens. Elevated temperature adversely affected seedling growth but did not aggravate the effect of the pathogens. *Elongisporangium undulatum* but not *P. plurivora* significantly reduced plant growth. Colonization density of *P. subalpina* measured by quantitative PCR was not affected by temperature or the presence of the pathogens. In conclusion, *P. subalpina* confers an indirect benefit to its host and might therefore be tolerated in natural ecosystems, despite negative effects on plant health and plant growth. [UAMH 11675, 11676, 11677, 11678, 11692, 11695]

32. Tsui CKM, DiGuistini S., Wang Y, Feau N, Dhillon B, Bohlmann J, Hamelin RC. Unequal recombination and evolution of the mating-type (MAT) loci in the pathogenic fungus *Grosmannia clavigera* and relatives. Genes, Genomes, Genetics. 2013; 3:465-80. doi: 10.1534/g3.112.004986.

Sexual reproduction in fungi is regulated by the mating-type (MAT) locus where recombination is suppressed. We investigated the evolution of MAT loci in eight fungal species belonging to *Grosmannia* and *Ophiostoma* (Sordariomycetes, Ascomycota) that include conifer pathogens and beetle symbionts. The MAT1-2 idiomorph/allele was identified from the assembled and annotated *Grosmannia clavigera* genome, and the MAT locus is flanked by genes coding for cytoskeleton protein (SLA) and DNA lyase. The synteny of these genes is conserved and consistent with other members in Ascomycota. Using sequences from SLA and flanking regions, we characterized the MAT1-1 idiomorph from other isolates of *G. clavigera* and performed dotplot analysis between the two idiomorphs. Unexpectedly, the MAT1-2 idiomorph contains a truncated MAT1-1-1 gene upstream of the MAT1-2-1 gene that bears the high-mobility-group domain. The nucleotide and amino acid sequence of the truncated MAT1-1-1 gene is similar to its homologous copy in the MAT1-1 idiomorph in the opposite mating-type isolate, except that positive selection is acting on the truncated gene and the alpha(α)-box that encodes the transcription factor has been deleted. The MAT idiomorphs sharing identical gene organization were present in seven additional species in the Ophiostomatales, suggesting that the presence of truncated MAT1-1-1 gene is a general pattern in this order. We propose that an ancient unequal recombination event resulted in the ancestral MAT1-1-1 gene integrated into the MAT1-2 idiomorph and surviving as the truncated MAT1-1-1 genes. The α-box domain of MAT1-1-1 gene, located at the same MAT locus adjacent to the MAT1-2-1 gene, could have been removed by deletion after recombination due to mating signal interference. Our data confirmed a 1:1 MAT/sex ratio in two pathogen populations, and showed that all members of the Ophiostomatales studied here including those that were previously deemed asexual have the potential to reproduce sexually. This ability can potentially increase genetic variability and can enhance fitness in new, ecological niches. [UAMH 11150 (=KW1407), UAMH 1363, UAMH 4875, UAMH 4838, UAMH 9584, UAMH 11095, UAMH 10623 [SL-KW1436] type, UAMH 10629 (B13)]

33. Tsui CKM, Roe AD, El-Kassaby YA, Rice AV, Alamouti SM, Sperling FAH, Cooke JEK, Bohlmann J, Hamelin RC. Population structure and migration pattern of a conifer pathogen, *Grosmannia clavigera*, as influenced by its symbiont, the mountain pine beetle. Mol Ecol 2012; 21(1) 71-86. doi: 10.1111/j.1365-294X.2011.05366.x.

We investigated the population structure of *Grosmannia clavigera* (Gc), a fungal symbiont of the mountain pine beetle (MPB) that plays a crucial role in the establishment and reproductive success of this pathogen. This insect–fungal complex has destroyed over 16 million ha of lodgepole pine forests in Canada, the largest MPB epidemic in recorded history. During this current epidemic, MPB has expanded its range beyond historically recorded boundaries, both northward and eastward, and has now reached the jack pine of Alberta, potentially threatening the Canadian boreal forest. To better understand the dynamics between the beetle and its fungal symbiont, we sampled 19 populations in western North America and genotyped individuals from these populations with eight microsatellite markers. The fungus displayed high haplotype diversity, with over 250 unique haplotypes observed in 335 single spore isolates. Linkage equilibria in 13 of the 19 populations suggested that the fungus reproduces

sexually. Bayesian clustering and distance analyses identified four genetic clusters that corresponded to four major geographical regions, which suggested that the epidemic arose from multiple geographical sources. A genetic cluster north of the Rocky Mountains, where the MPB has recently become established, experienced a population bottleneck, probably as a result of the recent range expansion. The two genetic clusters located north and west of the Rocky Mountains contained many fungal isolates admixed from all populations, possibly due to the massive movement of MPB during the epidemic. The general agreement in north–south differentiation of MPB and *G. clavigera* populations points to the fungal pathogen's dependence on the movement of its insect vector. In addition, the patterns of diversity and the individual assignment tests of the fungal associate suggest that migration across the Rocky Mountains occurred via a northeastern corridor, in accordance with meteorological patterns and observation of MPB movement data. Our results highlight the potential of this pathogen for both expansion and sexual reproduction, and also identify some possible barriers to gene flow. Understanding the ecological and evolutionary dynamics of this fungus–beetle association is important for the modelling and prediction of MPB epidemics. [UAMH 11150 (=KW 1407) *Grosmannia clavigera* control organism]

34. Troy GC, Panciera DL, Pickett JP, Sutton DA, Gene J, Cano JF, Guarro J, Thompson EH, Wickes BL. Mixed infection caused by *Lecythophora canina* sp. *nov*. and *Plectosphaerella cucumerina* in a German shepherd dog. Med Mycol. 2013; 51:455-60 doi: 10.3109/ 13693786.2012.754998.

We describe an opportunistic, disseminated infection in a German shepherd dog associated with two fungal organisms not previously reported to cause disease. *Lecythophora canina*, a new species here described, was isolated from an osteolytic bone lesion. A fine needle aspirate of the lesion demonstrated septate hyphae. *Plectospharella cucumerina* (anamorph *Plectosporium tabacinum*) was isolated from a urine sample. Clinical manifestations were blindness, altered mentation, and osteomyelitis. Treatment with itraconazole and terbinafine for greater than one year resulted in stable clinical disease. [UAMH 11633, 11702]

35. Tudor D, Robinson SC, Cooper PA. The influence of moisture content variation on fungal pigment formation in spalted wood. AMB Express. 2012; 17:69. doi: 10.1186/2191-0855-2-69.

Eight fungal species known to produce wood pigmentation were tested for reaction to various moisture contents in two hardwood species. Fungal pigmentation by *Trametes versicolor* and Xylaria polymorpha was stimulated at low water concentrations in both Acer saccharum (sugar maple) and Fagus grandifolia (American beech), while *Inonotus hispidus* and *Polyporus squamosus* were stimulated above 22-28% and 34-38% moisture content in beech and in sugar maple respectively. *Fomes fomentarius* and *Polyporus brumalis* produced maximum pigmentation in beech at 26 - 41% and in sugar maple at 59 - 96% moisture content. The pink staining *Scytalidium cuboideum* pigmented both wood species at above 35% moisture content. This research indicates that controlling the moisture content values of wood substrates can stimulate the intensity of pigmentation of specific fungi when spalting wood for decorative and commercial purpose. [UAMH 4802, 11518 – 11521, 11651 – 11654]

36. Tudor D, Robinson SC, Cooper PA. The influence of pH on pigment formation by lignicolous fungi. Int Biodeterior Biodegrad. 2013; 80:22e28. doi: http://dx.doi.org/10.1016/j.ibiod.2012.09.013.

Wood-decay patterns are strongly influenced by the conditions of the wood substrate, and the pH of the substrate is one of the most important factors. As a reaction to a stressed environment, some lignicolous fungi respond with pigment formation that helps to isolate and protect their mycelia; other fungi, with high specificity, produce pigmentation regardless of the changes in the conditions in which the fungus grows. These changes result in only minor variation in the color intensity of the pigment. The occasional dark-colored reaction pigment, also known as melanin, is the most common pigment formed by wood-decay fungi. To investigate pigment formation under the influence of pH variation, sugar maple and beech samples adjusted with buffer solutions to different pH values were inoculated with various basidiomycetes and ascomycetes fungi, known to produce pigmentation. Based on the range and increments of the pH treatments tested on the wood substrate, maximum pigmentation and minimum mass loss occur at adjacent values of pH treatments for all wood–fungus combinations, and never coincide. Maximum pigment production occurred at treatment with pH 4.5 for beech and sugar maple inoculated with *Trametes versicolor*, while *Xylaria polymorpha* produced external pigmentation in beech treated with buffer at pH 5 and sugar maple at pH 4.5. Fungi tested in agar substrate produced maximum pigmentation at the pH range 4–5.5, except for *Scytalidium cuboideum*, which produce maximum intensity of red pigment at pH 6 and blue pigment at pH 8. [UAMH 4802, 11518 – 11521, 11652 – 11655]

37. Untereiner WA, Bogale M, Carter A,Platt HW, Hanson SÅ, Læssøe T, Stepánek V, Réblová M. Molecular phylogeny of *Boliniales* (Sordariomycetes) with an assessment of the systematics of *Apiorhynchostoma*, *Endoxyla* and *Pseudovalsaria*. Mycologia. 2013; 105:564-88 doi: 10.3852/12-326.

The systematics of the ascomycete genera *Apiorhynchostoma*, *Endoxyla* and *Pseudovalsaria* are reevaluated based on the comparison of cultural characteristics, teleomorph morphology and DNA sequence data. Analyses of sequences of the internal transcribed spacer (ITS) of the ribosomal DNA operon and the large subunit (LSU) of the nuclear ribosomal DNA gene resolve Boliniales as a robustly supported lineage comprising *Apiorhynchostoma*, *Camarops*, *Camaropella*, *Cornipulvina*, *Endoxyla* and *Pseudovalsaria*. Within Boliniales, species of *Endoxyla* form a strongly supported lineage. *Apiorhynchostoma curreyi* and *Pseudovalsaria ferruginea* group with *Cornipulvina ellipsoides*. Species of Camarops are paraphyletic and comprise two clades, one of which includes Camaropella. Boliniaceae is emended, *Endoxyla mallochii* is described as new and *Apiorhynchostoma trabicola* is considered a synonym of *Apiorhynchostoma altipetum*. We also propose the combinations *Endoxyla occulta*, *Endoxylina luteobasis* and *Jobellisia peckii*. Keys to genera included in the Boliniaceae and to species of *Apiocamarops*, *Apiorhynchostoma* and *Endoxyla* are provided. [UAMH 11085, 11086, 11087 Type, 11088, 11490, 11491]

38. Vanderwolf KJ, McAlpine DF, Malloch D, Forbes GJ. Ectomycota associated with hibernating bats in eastern Canadian caves prior to the emergence of white-nose syndrome. Northeastern Naturalist. 2013; 20:115-30 doi: http://dx.doi.org/10.1656/045.020.0109.

The emergence of the fungal disease white-nose syndrome (WNS) among hibernating bats in North America and its causative pathogen, *Geomyces destructans*, underscores how little is known about fungi associated with bats and their subterranean environments. Investigating 8 caves and mines in New Brunswick, Canada, we cultured a diverse array of fungi from the fur and skin of apparently healthy, hibernating *Myotis lucifugus* (Little Brown Bat) and *M. septentrionalis* (Northern Long-eared Bat) in the year prior to the emergence of WNS in the province. Among the 117 isolated fungal taxa, we found an array of psychrophilic, psychrotrophic, keratinolytic, coprophilous, and saprobic fungi. The most common taxa were *Geomyces pannorum sensu lato*, *Penicillium spp*., *Mortierella spp*., *Mucor spp*., *Cephalotrichum stemonitis*, *Leuconeurospora spp*., *Penicillium solitum*, *Cladosporium spp*., and *Trichosporon dulcitum*. Each bat hosted 6.9 ± 3 (SD) fungal taxa, and 30.8 ± 5 taxa were isolated per hibernaculum. Number of taxa isolated per bat was positively correlated with mean and minimum winter temperatures in the dark zones of hibernacula. Forty-seven of the taxa have never been reported in caves, and an additional 31 taxa are new records for North American caves. The presence of *Geomyces pannorum sensu lato* on 70% of hibernating bats may complicate results of diagnostic techniques used for identifying *G. destructans*. Bats hibernating in eastern Canada harbor a rich reservoir of fungal species and probably play a role in moving fungal spores into and between hibernacula, as well as onto the landscape. [UAMH 11121, 11159–11164, 11182–11184, 11236–11251, 11296–11329, 11334–11345, 11379–11400, 11408–11436, 11438–11478, 11492–11496, 11499–11500, 11504–11516, 11528, 11529, 11594–11614, 11618, 11619, 11621–11626, 11639, 11642]

39. Váradi, G., Tóth, G. K., Kele, Z., Galgóczy, L., Fizil, Á. and Batta, G. Synthesis of PAF, an antifungal protein from *P. chrysogenum*, by native chemical ligation: native disulfide pattern and fold obtained upon oxidative refolding. Chem Eur J. 2013; 19:12684–92 doi: 10.1002/chem.201301098.

The folding of disulfide proteins is of considerable interest because knowledge of this may influence our present understanding of protein folding. However, sometimes even the disulfide pattern cannot be unequivocally determined by the available experimental techniques. For example, the structures of a few small antifungal proteins (PAF, AFP) have been disclosed recently using NMR spectroscopy but with some ambiguity in the actual disulfide pattern. For this reason, we carried out the chemical synthesis of PAF. Probing different approaches, the oxidative folding of the synthetic linear PAF yielded a folded protein that has identical structure and antifungal activity as the native PAF. In contrast, unfolded linear PAF was inactive, a result that may have implications concerning its redox state in the mode of action. [UAMH 7955]

40. Wang B, Wang L. *Penicillium kongii*, a new terverticillate species isolated from plant leaves in China. Mycologia. 2013; 105:1547-54.

A new *Penicillium* species isolated from plant leaves, characterized by restricted growth, terverticillate penicilli, ovoid to ellipsoidal conidia and a red soluble pigment on yeast extract sucrose agar is reported here. *Penicillium kongii* sp. nov. belongs to subgenus *Penicillium* section *Brevicompacta* and is morphologically similar to *P. bialowiezense* and *P. brevicompactum*. Phylogenetic analyses based on sequence data from calmodulin gene, β-tubulin gene and rDNA ITS1-5.8S-ITS2 show that *P. kongii* forms a distinctive clade. [UAMH 11674 Type strain =

NRRL 62674]

41. Zettler LW, Corey LL, Jacks AL, Gruender LT, Lopez AM. *Tulasnella irregularis* (Basidiomycota: Tulasnellaceae) from roots of *Encyclia tampensis* in south Florida, and confirmation of its mycorrhizal significance through symbiotic seed germination. Lankesteriana. 2013; 13:119—128.

Epiphytic orchids remain understudied with respect to their obligate mycorrhizal relationships – a key component of the integrated conservation model. Existing studies have revealed that these plants, like their terrestrial counterparts, commonly associate with ubiquitous basidiomycetes (e.g., Tulasnellaceae); however, few studies have verified their physiological role(s). Two strains of mycorrhizal fungi (UAMH 11541, UAMH 11543) were isolated from roots of an epiphytic orchid in south Florida, *Encyclia tampensis*; one was acquired from a seedling and one from a mature specimen. Seeds of four epiphytic taxa were subsequently inoculated (separately) with both fungal strains in vitro: *E. tampensis, Epidendrum amphistomum, Epidendrum nocturnum*, and *Prosthechea cochleata*. More than one-third of inoculated *E. tampensis* and *E. nocturnum* seeds developed leaves in total darkness after 100 days. No significant differences were detected between the two strains on germination, nor any interaction between fungus and seed source (ANOVA, $\alpha = 0.05$). Using ITS amplification and sequencing, both strains were identified as the teleomorph, *Tulasnella irregularis* (Basidiomycota: Tulasnellaceae), and both were genetically identical with a high (98%) degree of certainty. Thus, symbiotic germination and ITS sequencing results are in agreement that both strains are indeed the same fungus. This paper is meant to shed additional light into epiphytic orchid-fungal interactions and highlights the need to identify, test (through symbiotic germination) and safeguard mycorrhizal fungi necessary for conservation. [UAMH 11541 – 11543]

Table 1. Cultures Received in 2013

	Person or industry or culture collection and address	Purpose	Total
1.	Bemis DA, Biomedical and Diagnostic Sciences, Univ of Tennessee College of Veterinary Medicine, Knoxville, TN	D	1
2.	Breuil C (Wang Y), Forest Sciences Centre, Univ of British Columbia, Vancouver, BC	D	8
3.	Clough R, Investigation & Diagnostic Centre and Response, MAF Biosecurity NZ, Upper Hutt, New Zealand	D	1
4.	Fuller J (Jansen B), Mycology, Microbiology and Public Health, Univ of Alberta Hospitals, Edmonton, AB	ID	1
5.	Hausner G, Dept of Microbiology, Univ of Manitoba, Winnipeg, MB	D	6
6.	Iwen P, Univ of Nebraska Medical Center, Omaha, NE	D	1
7.	Seifert K (Tanney J), Biodiversity (Mycology and Botany), Agriculture & Agri-Food Canada, Ottawa, ON	D	1
8.	Sieber T, Forest Pathology and Dendrology, Swiss Federal Institute of Technology, Zurich, Switzerland	D	35
9.	Sutton DA, Fungus Testing Lab, Dept of Pathology, Univ of Texas Health Science Center, San Antonio, TX	ID/D	5
10.	Untereiner W (Vanderwolf K), Dept of Biology, Brandon Univ, Brandon, MB	D	7
11.	Vanderwolf K, Zoology, New Brunswick Museum, St. John, NB	D	85
12.	Wang L, State Key Lab of Mycology, Chinese Academy of Sciences, Institute of Microbiology, Beijing, China	D	1
13.	Zelazny A, National Institutes of Health Clinical Center, Bethesda, MD	D	1
14.	Zettler L, Biology Dept, Illinois College, Jacksonville, IL	D	55
15.	Zhang S (Lee R), Medical Microbiology, Johns Hopkins Hospital, Baltimore, MD	D	2

Cultures received from:

Internal (Univ Alberta/UA Hospitals)	1
North America	172
International	37

Total cultures received **210**

Codes: **D**= Deposit; **ID**= Identification ; **R**=Replacement

Table 2. Cultures Distributed in 2013

	Person or industry or culture collection and address	Purpose	Total
1.	Ammirati JF (Lindsley D, Sabiniano E), Dept of Biology, Univ of Washington, Seattle, WA	MR	6
2.	Assured Bio (Pope L, Sobek E), USA	IAQ	4
3.	Babcock C, Canadian Collection of Fungal Cultures, Agriculture & Agri-Food Canada, Ottawa, ON	EX	6
4.	Bell T, Centre sur la Biodiversité, Univ de Montréal, Montreal, QC	BR	1
5.	Bidochka M (Padilla I), Biological Sciences, Brock Univ, St. Catharines, ON	RD	10
6.	Blehert D, National Wildlife Health Center, US Geological Survey, Madison, WI	RD	16
7.	CABI Europe – UK (Centre for Agricultural Bioscience International) (Lawrence S, Godwin-Keene G), Egham, Surrey, UK	MS	13
8.	Danisco USA Inc./ Genencor (Bower B, Garland A), Palo Alta, CA	RD	12
9.	Davis S, Forest Control Products Lab, US Dept of Agriculture, Madison, WI	MS	8
10.	de la Bastide P (Hintz W), Biology, Univ of Victoria, Victoria, BC	BR	8
11.	Dietrich D (Clardy J), Dept of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA	B	2
12.	Dycor Technologies Ltd. (Vogrinetz J), Edmonton, AB	BD	4
13.	Gene J (Guarro J), Unitat de Microbiologia, Facultat de Medicina I Ciencies de la Salut, Universitat Rovira I Virgili, Reus, Spain	MS	3
14.	Hambleton S, Eastern cereal and oilseed research centre, Agriculture & Agri-Food Canada, Ottawa, ON	CR/MS	14
15.	Harrington T, Plant Pathology and Microbiology, Iowa State University, Ames, IA	MS	3
16.	Hausner G (Berg S), Dept of Microbiology, Univ of Manitoba, Winnipeg, MB	MS	5
17.	Hyde K (Chomnunti P), MFLU Herbarium, School of Science, Mae Fah Luang Univ, Changrai, Thailand	T	1
18.	Inderbitzin P, Dept of Plant Pathology, Univ of California Davis, Davis, CA	FG	1
19.	IRRST - Institut de recherche Robert-Sauve En sante et en securite du travail, Montreal, QC (Pepin C)	IAQ	6
20.	Jaspers C (O'Neil K), Biorefining Research Institute, Lakehead Univ, Oak Ridge, TN	RG	1
21.	Klironomos J (Gorzelak M), Biology, Univ of British Columbia-Okanagan, Kelowna, BC	MT	6
22.	Laboratoires d'analyses S. M. Inc. (S.M. Laboratory Services Inc.) (Laganiere G), Microbiology Dept, Longueuil, QC	TE	10
23.	Luminex Molecular Diagnostics (McKay J, Valcu M, Fernandes S), Design Transfer and Process Engineering, Toronto, ON	RD	11
24.	Pedras S (Surtees C), Bioorganic and Agricultural Chemistry, Dept of Chemistry, Univ of Saskatchewan, Saskatoon, SK	M	1
25.	Rossman A (Rehner S), Systematic Mycology & Microbiology Lab, US Dept of Agriculture – Agricultural Research Service, Beltsville, MD	MS	2
26.	Roy S, Dept of Biology, Univ of Sherbrooke, Sherbrooke, QC	MR	1
27.	Sain M (Ferhan M), Center of Biocomposites & Biomaterials Processing, Faculty of Forestry, Univ of Toronto, Toronto, ON	BD	2
28.	Seifert K (Tanney J), Biodiversity (Mycology and Botany), Agriculture & Agri-Food Canada, Ottawa, ON	EX	1

Table 2. Cultures Distributed in 2013

	Person or industry or culture collection and address	Purpose	Total
29.	Smith B, Western Ecology Division, US Environmental Protection Agency, Corvallis, OR	RD	3
30.	Sporometrics Inc. (Guardiola Y, Hollis E), Toronto, ON	QC/PT	7
31.	Vanderwolf K, Botany and Mycology, New Brunswick Museum, St. John, NB	T	9
32.	Warriner K (Varga L), Dept of Food Science, Univ of Guelph, Guelph, ON	RG	1
33.	Zhang N, Dept of Plant Biology and Pathology, Dept of Biochemistry and Microbiology, Rutgers, The State Univ of New Jersey, New Brunswick, NJ	MR/T	1

Cultures distributed to:

North America	162
International	17
Total cultures distributed	**179**

Codes: **B** – Biocontrol; **BD** – Biodegradation; **BR** – Bioremediation; **CR** – Collaborative Research; **EX** – Exchange; **FG** – Fungal Genetics; **IAQ** – Indoor Air Quality; **M** – Metabolites; **MR** – Mycorrhizae; **MS** - Molecular Systematics; **P** – Pathogenicity; **QC**/**PT** – Quality Control / Proficiency Testing; **RD** – Research Diagnostics; **RG** – Research General; **T** – Taxonomy; **TE** - Teaching.