Western White Pine Endophyte Isolate Collection
Order: *Xylariales* and allies (potential blister rust inhibitors)

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Background and taxonomy. The *Xylariaceae* (*Sordariomycetidae, Xylariales*) contain 48 genera and 386 species[1]. *Xylariaceae spp.* occur worldwide, with the greatest number of species found in the tropics[2,12]. Morphological and genetic characteristics tend to align in phylogenetic analyses of *Sordariomycetes* taxa, and the *Xylariaceae* remains a monophyletic group of fungi with a reliable taxonomy[3].

Ecology. Endophytes in the *Xylariaceae* co-evolved with angiosperms (flowering plants)[12]; living and dead tropical woody plants remain their dominant niche. Notorious taxa in the *Sordariomycetes* include the rice blast fungus (*Magnaporthe grisea*)[4], the causal agent of chestnut blight (*Cryphonectria parasitica*)[17], and a typical model organism for genetics studies (*Neurospora crassula*)[64]. Most fungi in the *Xylarialeceae* decompose woody debris in late stages of decay, but phytopathogens and endophytic species also occur in this family[3]. Production of cellulolytic and ligninolytic enzymes allows *Xylariaceous* fungi to decompose various woody substrates[24], and they produce antimicrobial secondary metabolites to compete with bacteria and other fungi. These chemicals generate interest among bioprospectors, pharmaceutical researchers, and applied ecologists[22,40,47].

Image: *Xylaria digitata* stromata[26]. Rapid decomposition of dead wood returns carbon-rich compounds to the soil

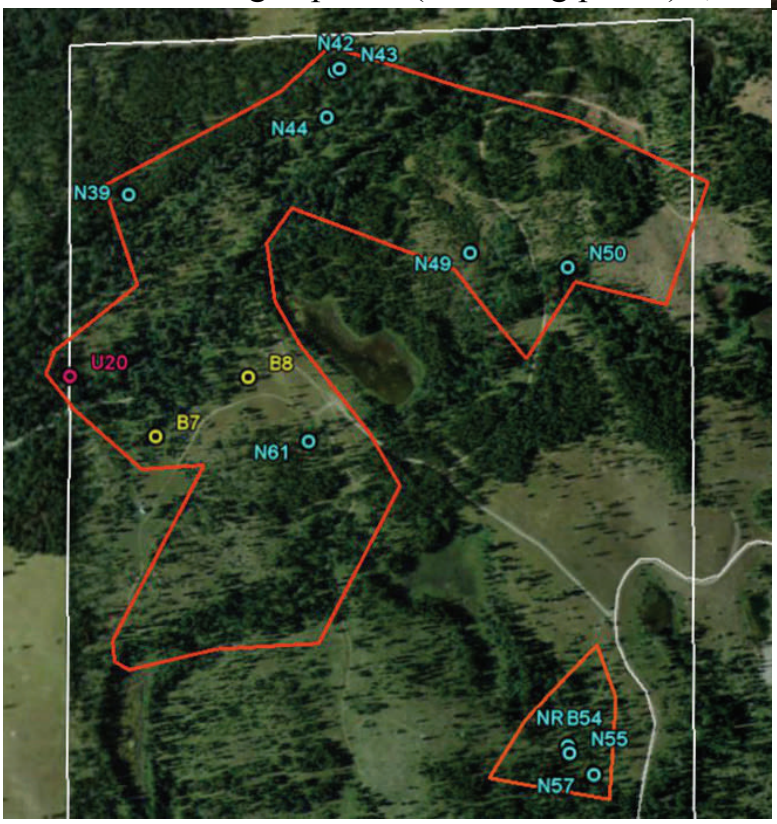


Figure 1: *Xylariales* and allies distribution on MPG North[5] The orange border surrounds the white pine sampling area. Circles represent trees that host xylariaceous fungi; text color indicates tree group. Blue=site native, mature trees; magenta=unbrowsed, planted seedlings; yellow=browsed, planted seedlings.

Table 1: Xylariales & allies distribution on MPG North

White pine sub-population	# of trees infested	# isolates obtained
Unbrowsed seedlings	1	1
Browsed seedlings	2	3
Natural regeneration	9	11
Blister-rust infected	1	1
Needle cast infected	0	0

Xylariaceous fungi on MPG North. Why do xylariaceous saprotrophs colonize living plant tissue rather than abundant woody debris? One explanation, the host jumping hypothesis, suggests that asymptomatic colonization of an alternate host by saprotrophs increases dispersal and subsequent colonization of their preferred substrate[12]. If this hypothesis is true, asymptomatic infestations in alternate hosts should occur intermittently over time and result in more frequent isolation of these fungi from older trees by chance. Fungi in the

Xylariales (and allies) occurred more often in the older, site-native white pines (table 1), consistent with the "host jumping" hypothesis. Widespread distribution of *Xylariaceous* fungi on MPG North indicates efficient dispersal (figure 1).

Xylariales and allies in restoration and pathogen defense. Xylariaceous metabolism of cellulose and lignin allows active transport of simpler carbon compounds into the soil profile, which results in improved soil physical and chemical properties. This encourages plant growth[27,28]. Soil restoration projects on MPG properties could harness this process by applying fungus-inoculated woody debris on roads and clearcuts ("mycoremediation"). Species in the genus *Xylaria* also form mutualisms with plant hosts that limit damage from fungal pathogens[22,46]. *Chaetomium globosum* liquid culture and purified culture filtrates inhibited rust fungus growth in laboratory and field trials[47]. Future research could explore the effect of these fungi against blister rust and other tree diseases on MPG properties.

Methods summary

We amplified isolates' nuclear internal transcribed spacer region (ITS) DNA and determined similarity to known fungal cultures based on homology to sequences stored in NCBI/Genbank. Microscopic analysis of isolates in culture provided further evidence to supplement the gene-based identification where possible.

Family: *Xylariaceae*

Genus: *Xylaria*

Background. The genus *Xylaria* contains at least 100 saprotrophic, pathogenic, and endophytic species that associate with most plant lineages worldwide[13,16,17]. Known for their large, club-shaped stromata (image 1), saprophytic *Xylaria spp.* accelerate the return of nutrients from woody debris to soil. Pathogenic *Xylaria spp.* cause lethal root rots and stem decay[17]. Endophytes in this genus inhabit woody plants worldwide, including *Artemesia spp.* across Asia[18], cupprecaeous trees in North America[19], and tropical woody plants like coffee shrubs[20], rubber[21], and cacao trees[22].

Xylaria spp. and endophyte research. The host jumping hypothesis proposes that endophytes infest alternative hosts to aid in dispersal, improve access to their preferred substrate, or survive through a decline in their preferred substrate. In contrast, the classic paradigm of endophyte function hypothesizes that asymptomatic endophytes persist in a latent state in their preferred host until tissue senescence triggers saprotrophic growth. The classic paradigm also suggests that latent saprobes have an incentive to invest in the fitness of their host because they rely on a continuous supply of senesced tissue for survival rather than the ability to escape to an alternate host. Host jumping endophytes stand to gain less from investment in their alternate hosts, as they simply leave when they can access their preferred substrate.

The presence of *Xylaria spp.* saprotrophs in asymptomatic plant tissues could support either the classic paradigm or host jumping scenarios. Evidence that saprotrophic endophytes also protected their hosts tends to support the classic paradigm. In *Theobroma cacao* (cocoa tree), *Xylaria spp.* inoculations reduced leaf damage by *Phytophthora spp.* pathogens and improved host survival[22]. Other research challenges latent saprotrophy as an explanation for endophyte presence. Phylogenetic analysis revealed that xylariaceous endophytes in liverworts clustered apart from known saprobes in the genus[14]; this suggests a cryptic lifestyle. Further, because liverworts produce no lignin and little cellulose[65], putative saprotrophs would gain little from persistence in that tissue. This scenario favors host jumping over the classic paradigm, although the evolution of a pure asymptomatic state in these endophytes also explains the observed cryptic lifestyle.

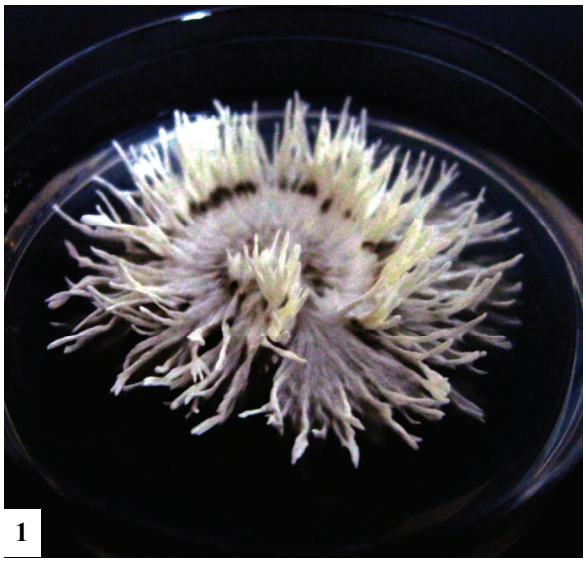
Xylaria and MPG Operations. On MPG properties, saprotrophic *Xylaria spp.* could help us meet restoration goals. Clear-cut or roaded landscapes suffer from increased soil density, poor water infiltration, and low available carbon. Placement of high-carbon substrates (straw, slash, etc.) over degraded soils improves soil physical properties, helps the microflora recover, and encourages plant growth[27], but migration of carbon compounds from woody debris into compacted soils relies on fungi already present on-site. In compacted soils, damage to fungal communities slows this

process. Fungal inoculation of added woody substrates ("mycoremediation") accelerates decomposition and translocation of carbon compounds into degraded soils, and speeds plant recovery[28].

Isolate 424

100% similarity to *Xylaria digitata*, accession GU322456.1[29]

The earliest description of *X. digitata* described its growth on a cut maple trunk in France[30]. Although the majority of *X. digitata* isolates originated in continental Europe and Great Britian[31], mid-twentieth century publications recorded the presence of this fungus in nine North American tree species[7]. Later, academic conflict over the methods used to describe *X. digitata* in North America led to the assignment of *X. acuta* to North American *Xylaria sp.* isolates with a similar morphology to *X. digitata*[32]. Molecular evidence could dispute this reassignment: according to the NCBI/GenBank database, ITS sequences in *X. acuta* and *X. digitata* differ by 4%, which is sufficient to differentiate between species according to typical protocol (3% difference between species with ITS sequences[66]). Isolate 424 showed 100% ITS similarity to *X. digitata,* but 96% similarity to *X. acuta*. Further, conidiophore and conidia morphology (images 2 and 3) match the descriptions of both *X. acuta* and *X. digitata*, but robust colony growth of isolate 424 (image 1) differed from *X. acuta*[32]. Since fungal databases lack any reference to *X. digitata* in North America based on genetic information[4,31], isolate 424 presents an opportunity to confirm the presence of this fungus in North America for the first time. Future research with isolate 424 could include a publication on the isolation of *X. digitata* in North America.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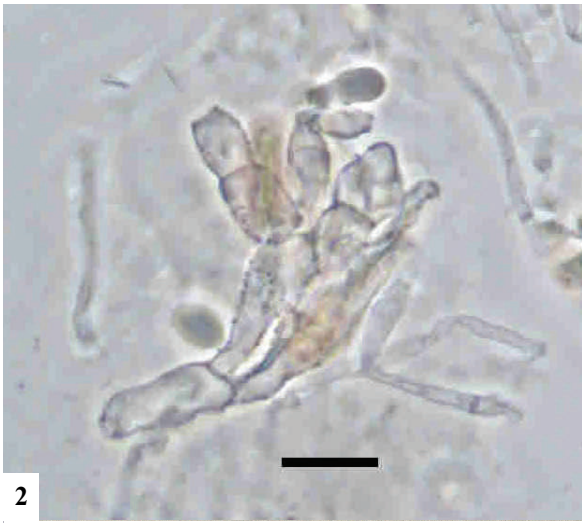
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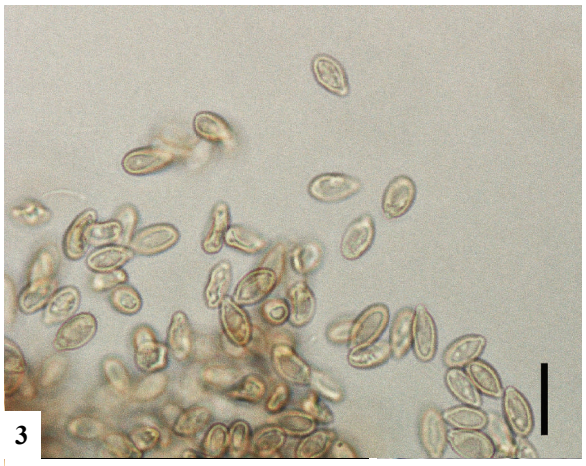
Isolate 234

97% similarity to *Xylaria cubensis*, accession AF163032.1[33]
Anamorph: *Xylocoremium flabelliforme*

X. cubensis and its anamorph occur on woody substrates[6,7] in tropical, subtropical, and temperate regions throughout the world[34]. It produces large stroma characteristic of *Xylaria spp.* fungi (image 4[67]) The ITS sequence obtained from isolate 234 resolved to both of the allied families *Xylariaceae* and *Diatrypaceae.* Isolate 234 produced chlamydospores in culture (image 6), but the lack of conidial structures challenges identification by morphology. Questionable genetic evidence and atypical colony morphology (image 5) indicate that this isolate is an undescribed species in the *Xylariales*.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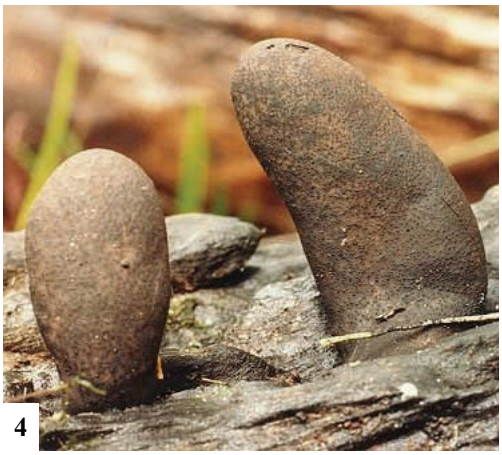
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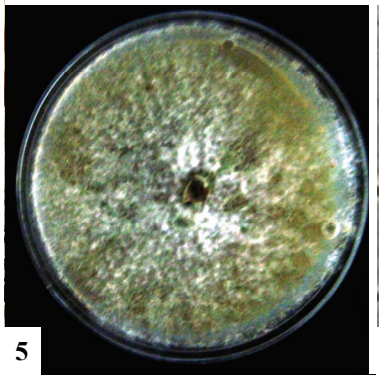
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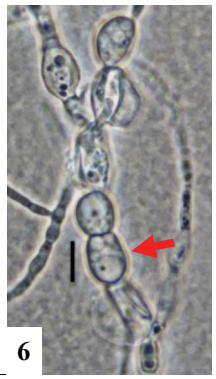
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Images 1-6: *Xylaria spp.* 1-3: isolate 424, in culture (1), with branched conidiophore (2), and conidia (3) consistent with *X. digitata*. 4: *X. cubensis* sporulating in a woody substrate in New Zealand[67]. 5-6: isolate 234, in culture (5) and with intercalary chlamydospores (6, arrow). Scale bar=10µm in 2, 3, 6. LH except 4[67], and 6: BL

Other Xylariaceae isolates

Isolate 242

>97% similarity to *Daldinia grandis*, accession AF176982.1[35]
Anamorph genus: *Nodulisporium*

Isolate 242 (colony, image 7) produced numerous truncate conidia (images 8 and 9), and conidiophores grew into brush-like appendages (*penicillate structure*, image 10). This morphology is consistent with the type description for *Nodulisporium spp.*[4] Unknown structures reminiscent of parasitic growth appeared in microscope images (image 11, arrows); darkened hyphae near these structures might indicate a defensive reaction by the host fungus.

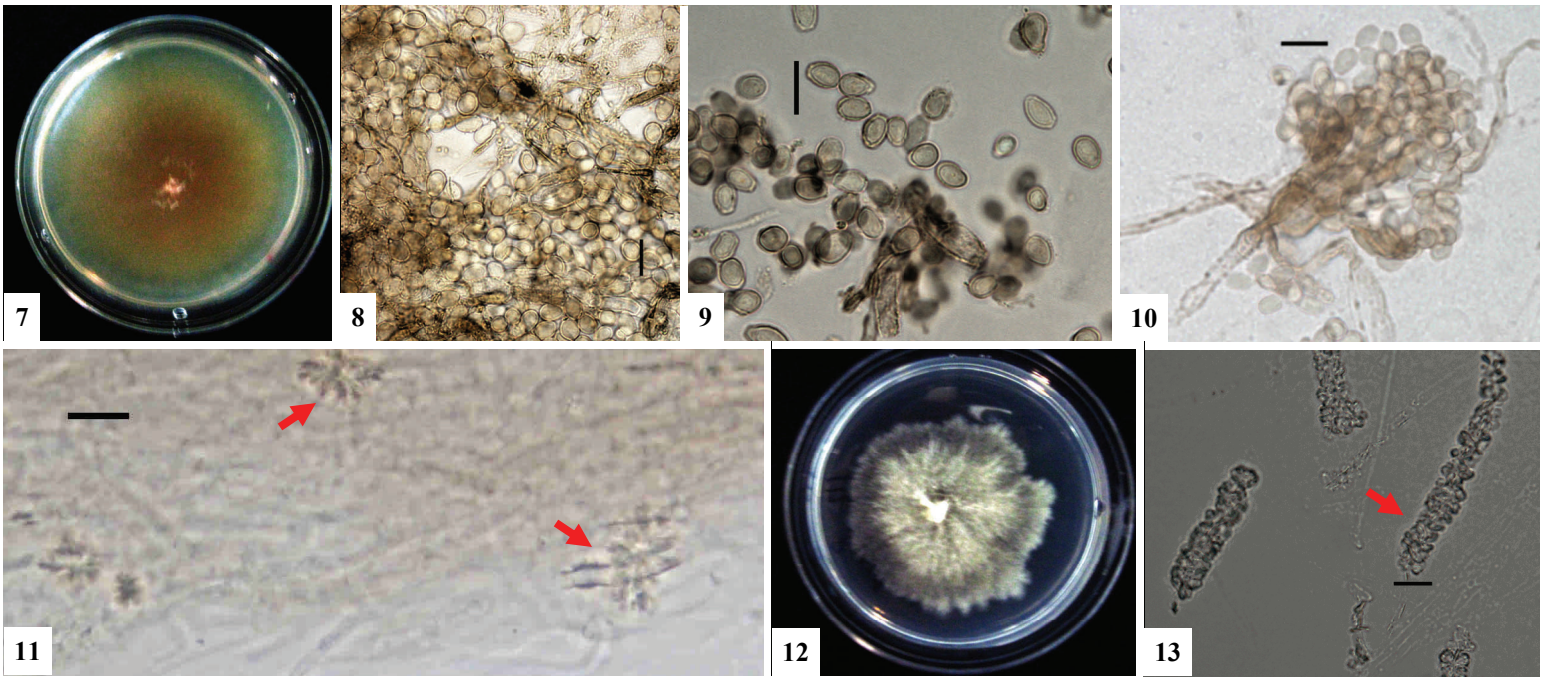
Daldinia spp. show weak to moderate host specificity on woody dicotyledonous plants, and parasitize them until host death triggers saprotrophic proliferation[13]. Medical research found that *Nodulisporium spp.* caused brain infections in humans[36] and produced cytotoxic[37], anti-cancer[38,39], and anti-plasmodium (malaria)[40] compounds. Because *Nodulisporium spp.* anamorphs belong to various saprotrophic or plant-pathogenic *Xylariaceae* genera[4,17], confirmation of *D. grandis* would require induction of sexual spore formation from isolate 242.

Isolate 112

99% similarity to *unknown foliar endophyte*, accession AY561198.1
98% similarity to *Rosellinia spp.*, accession AB017661.1, AY805591.1
Anamorph genera: *Dematophora, Nodulisporium, and Sporothrix*

Rosellinia spp. cause root rot diseases in at least 170 hardwood tree species worldwide[17]. They produce cytochalasin E[17], a potent phytotoxin that impairs actin polymerization in plant cells[41]. Plant cells affected by cytochalasin E show slow root growth, reduced defensive responses, and rapid invasion by pathogens[16].

Isolate 112 (image 12) produced conidiophores in culture, but their high density and frilled, gelatinous appearance (image 13, arrow) resisted placement into any putative *Rosellinia* anamorph taxa. Future work could attempt to confirm the sequence-based identity of this isolate.



Images 7-13: *Xylariaceae spp.* 7-11: isolate 242, in culture (7), profuse conidiogenesis (8), individual truncate conidia typical of *Nodulisporium spp.* (9), penicillate conidiophore consistent with *Nodulisporium spp.* (10), unknown structures that could be parasites or extracellular nutrient-absorbtive tissues (11, arrows). 12-13: isolate 112, in culture (12), unusual conidiophore in isolate 112 (arrow) that is difficult to classify. Scale bar=10µm in 8-11, 13. LH except 13: BL

Genus: *Nemania*
Anamorphs: *Geniculosporium spp.*[15]

Nemania spp. exist in soil and living or dead plant material worldwide[4,6]; they also decompose other fungi (image 19[68]). Most reported plant diseases caused by *Nemania spp.* occur in European hardwoods[7], but these fungi also form endophytic relationships with *Pinus spp.* in China[8] and Idaho[9]. *Nemania serpens* decomposes maple wood, but persists as a quiescent endophyte in adjacent white spruce foliage[10]. This observation provides anecdotal evidence of host jumping: spruce needles may serve as an alternate host and allow the fungus to disperse across the landscape[11]. Further, persistence in white spruce may allow the fungi to survive as maples decline in forest succession. Then, when forest disturbance allows maples to re-colonize, spruce needles fall and deliver the fungus back to its preferred host.

BLAST analysis of ITS sequences showed poor resolution between *Nemania* and *Xylaria spp.* Although micromorphological analysis supports identification of these isolates as *Nemaina spp.* anamorphs (*Geniculosporium spp.*), morphological similarity of anamorphs in this clade[13] makes species confirmation difficult. Applications of *Nemania spp.* on MPG properties include mycoremediation treatments in hardwood substrates.

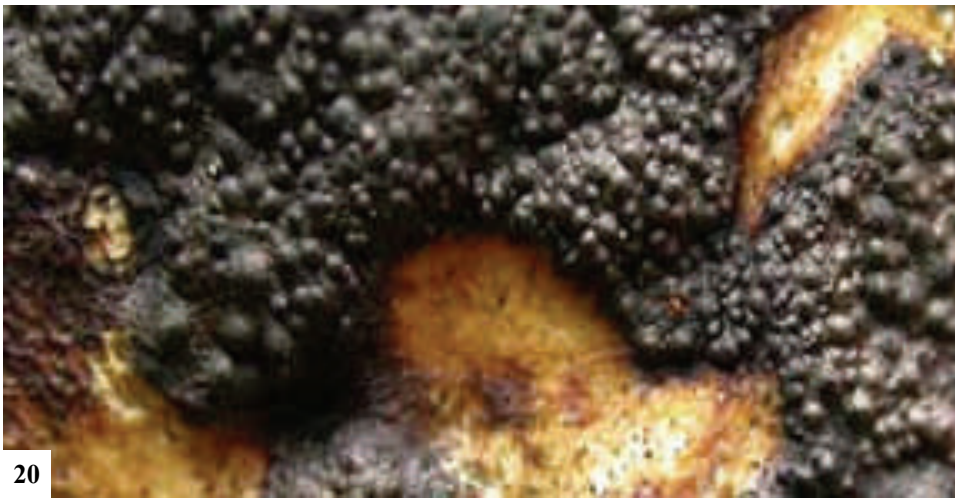
Isolates 34 (images 14 and 15), 366, 367, 412

97% similarity to *Nemania aenea*, accession AF201704.1
34: 98% to fungal endophyte, accession EU686035.1[14]
366, 367, 412: 97% to *Xylaria sp.*, accession GQ906941.1

Isolate 387

100% similarity to ARIZ AZ0792, accession HM123490.1
99% similarity to *Nemania serpens*, accession EF155504.1

The accepted description for *N. serpens's* anamorph identified conidia that measure 2.5-6 by 1.5-3µm[4], but conidia in isolate 397 measure 3.6 by 3.0µm (images 17-19). This slight deviation in shape challenges the otherwise strong genetic identification of this isolate as *N. serpens,* and little research on ARIZ AZ0792 illuminates any morphological similarity to isolate 387.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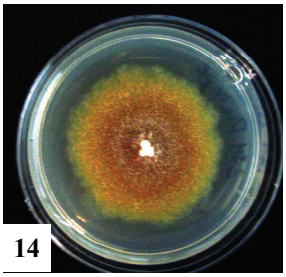
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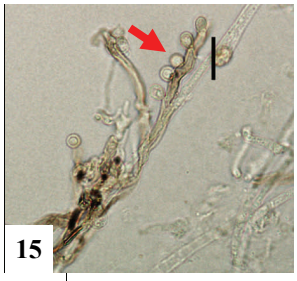
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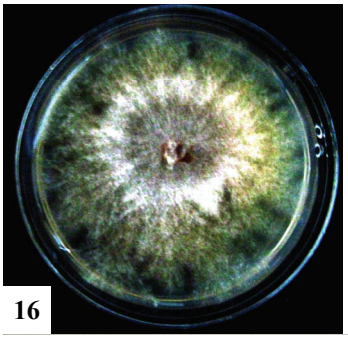
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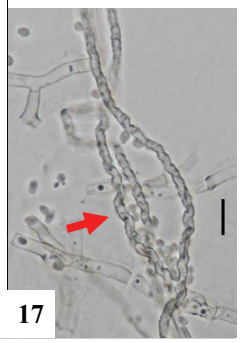
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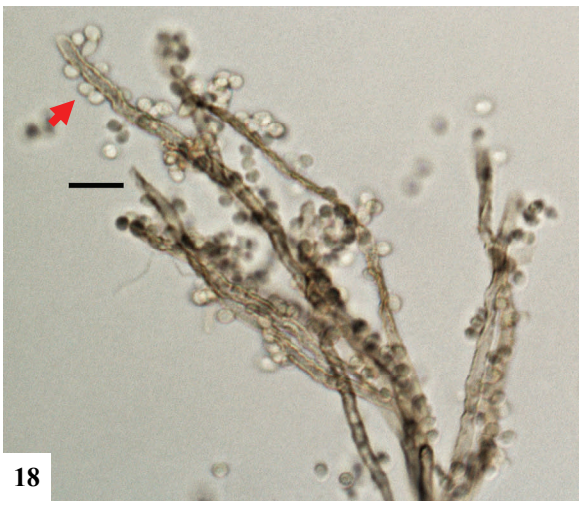
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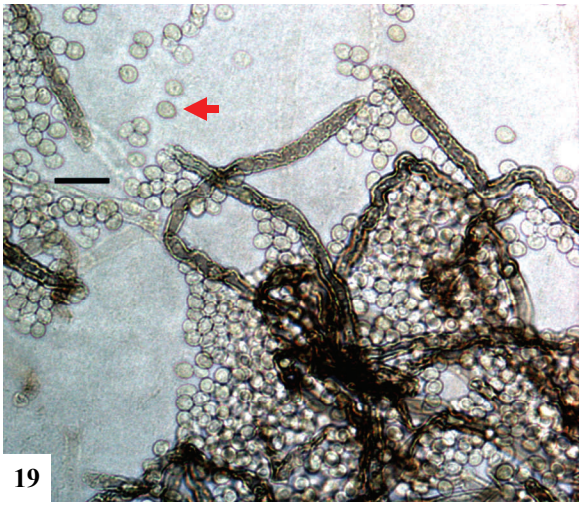
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Images 14-20: *Nemania spp.* related isolates. 14-15: isolate 34, in culture (14), conidiophore and discrete, solitary conidiogenesis (15, arrow). 16-19: isolate 387, in culture (16), geniculate (bent "like a knee") conidiophore (17, arrow), sympoidal (alternating) conidiogenesis (18, arrow), and detached conidia (19, arrow). 20: *Nemania serpens* sporulating on a decomposed fungal fruiting body, British Colombia, Canada[68]. Scale bar=10µm in 3, 5-7. LH except 20[68], and 5: BL

Other *Sordariomycetes:* allies of *Xylariales* fungi

Isolate 428

99% similarity to *Chaetomium globosum*, accession FN868476.1

The saprobe *C. globosum* (*Sordariomycetidae, Sordariales, Chaetomiaceae*)[4] occurs in soils, dung, wood, human environments[6], and oceanic algae[42]. *C. globosum* produces cellulolytic enzymes[43] that cause soft rots (i.e., cellulose degradation that leaves lignin intact) in plant material, lumber, and paper products worldwide[4]. *C. globosum* also produces antibiotic and antifungal compounds that antagonize fungal pathogens in sugar beet[44], apple[45], rice, and wheat[46,47]. One of the wheat pathogens inhibited by *C. globosum* (*Puccinia recondita*) belongs to the same order (*Puccinales*) as white pine blister rust[4]. Live mycelia, cultural filtrates, and purified metabolites produced by *C. globosum* reduced *P. recondita* growth in laboratory and field trials[47]. Purified alkaloids from *C. globosum* cultures also slowed human cancer cell growth[42,48].

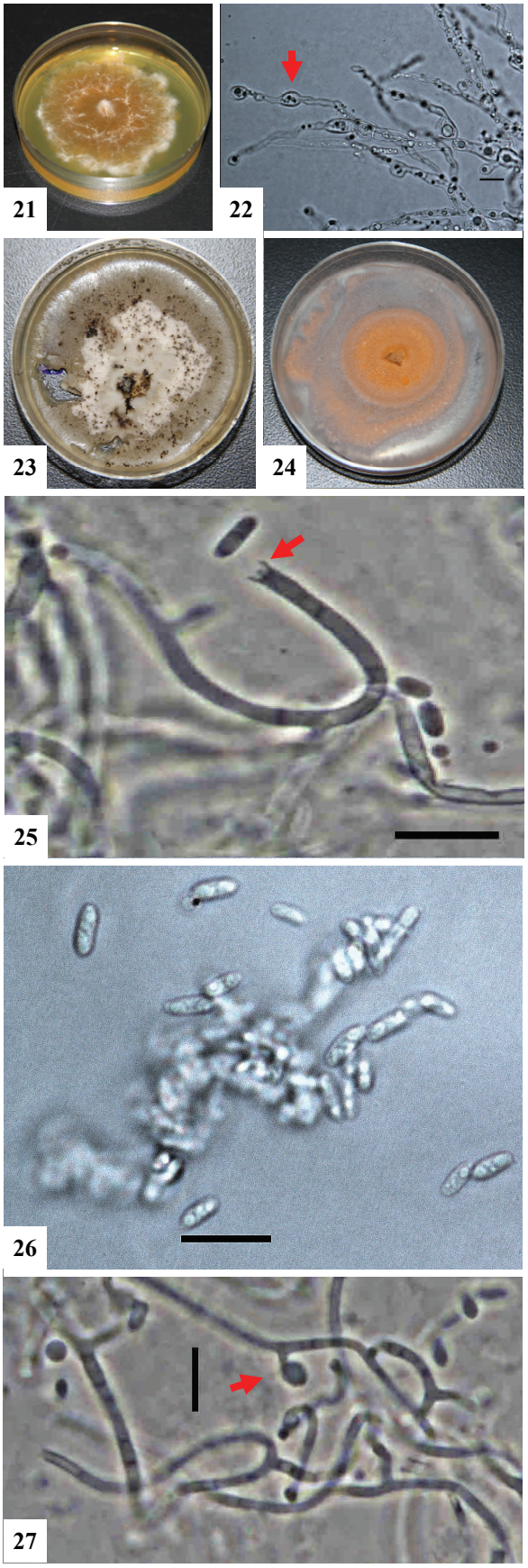
The orange hue created in laboratory culture by isolate 428 provides evidence of secondary metabolite production (image 21). Microscopic analysis revealed chlamydospores (image 22, arrow). In the absence of asexual or sexual spore production, future work with this isolate should attempt induction of sexual spore formation and confirm the sequence-based identity. The potential for isolate 428 to antagonize rust species underscores the need for positive identification and continued research. The purchase of verified *C. globosum* isolates constitutes an alternative option for further work.

Isolate 251

100% similarity to ARIZ AZ1006, accession HM123665.1[49]
98% similarity to *Fimetariella rabenhorstii*, accession AM921717.1
Anamorph: *Cladorrhinum spp.*

F. rabenhorstii (*Sordariomycetidae, Sordariales, Lasiosphaeriaceae*)[4] occurs as an endophyte in Scots Pine (*Pinus sylvestrus*)[50] and *Aquilaria sinensis* (an evergreen angiosperm endemic to China)[51] leaves. In soils, *F. rabensorstii* associates with ectomycorrhizal symbionts of *P. sylvestrus*[52], but the nature of this association remains unclear. Other *Fimetariella spp.* inhabit and metabolize dung[53]. Coprophilous fungi occur in a wide variety of substrates due to deposition by animals (on soil, plants, aqueous environments, etc.) and sporulation. Some dung fungi require passage through the gut of animals, and these species must exist in plants as part of their life cycle so herbivores will eat them[54].

The original description of this fungus occurred in 1964[4], and few studies describe its function in the environment or the diversity of its associated anamorphs. That 40 unidentified sequences retrieved from GenBank return *Fimetariella spp.* as a best match[56] suggests a widespread distribution and frequent isolation rate but little research interest or successful identification in culture. Isolate 251 produced numerous conidia in culture (image 26) under both UVB and normal lighting conditions, but colony pigmentation changed from tan to pink as a result of UVB exposure



Images 21-27: *Sordariomycetes.* 21-22: isolate 428 in culture with agar turned orange by a secondary metabolite (21), and with interhyphal chlamydospores (22, arrow). 23-27: isolate 251 in culture exposed to normal light (23) and UVB (24), conidiophore collar (25), detached conidia (26), and phialide-like chlamydospore (27, arrow). Scale bar=10µm in 21, 24-26. LH except 25-26: BL

(images 23 and 24). Conidiophore structure remained ambiguous after repeated observation attempts. A single conidiophore collar (image 25, arrow) and a lateral chlamydospore (image 27, arrow) fail to explain the genesis of multitudinous ovate conidia. Probable endoconidiogenesis (i.e., hyphal contents condense into conidia and disperse from ruptured ends), the lack of lateral conidiophore branches (phialides) in large number, and 1-3 septate conidia contraindicate inclusion in the *Cladorrhinum* genus[55]. As such, isolate 251 remains an unidentified hyphomycete related to *Fimetariella spp.*

Isolate 272

99% similarity to ARIZ AZ1004, accession HM123663.1
98% similarity to *Coniochaeta ligniaria*, accession AY198390.1
Anamorph: *Lecythophora* (= *Phialophora*)

C. ligniaria (*Sordariomycetidae, Sordariales, Coniochaetaceae*) produces cellulose, xylanase, and two lignin peroxidases in liquid batch cultivation[57]. It also metabolizes phenolic compounds that inhibit fermentation of cellulosic materials[58]. This combination of traits makes *C. ligniaria* a suitable organism for use in biofuels production, and a United States patent protects its use for this purpose[59].

Coniochaeta spp. and their anamorphs occur as asymptomatic endophytes[60]; they also cause plant diseases in woody hosts like stone fruit trees[61]. *C. ligniaria* causes disease in *Larix occidentalis*, *Pinus spp.*, and various shrubs (e.g., *Ceanothus velutinus, Alnus incana*) in the northern Rocky Mountains[7]. Production of potent anti-fungal compounds[62] helps this fungus compete against other pathogens for host tissue.

In culture, *Coniochaeta* and its anamorphs produce masses of slimy conidia via phialides (short, lateral outgrowths from hyphae) and endoconidiogenesis[63]. Microscopic investigation of isolate 272 is consistent with *Coniochaeta* anamorphs due to the presence of phialides (images 30 and 32), conidial morphology (images 29-31), and colony growth characteristics. Future research with isolate 272 could explore its potential as an antifungal producer and woody-debris decomposer.

Unidentified *Sordariomycetes*

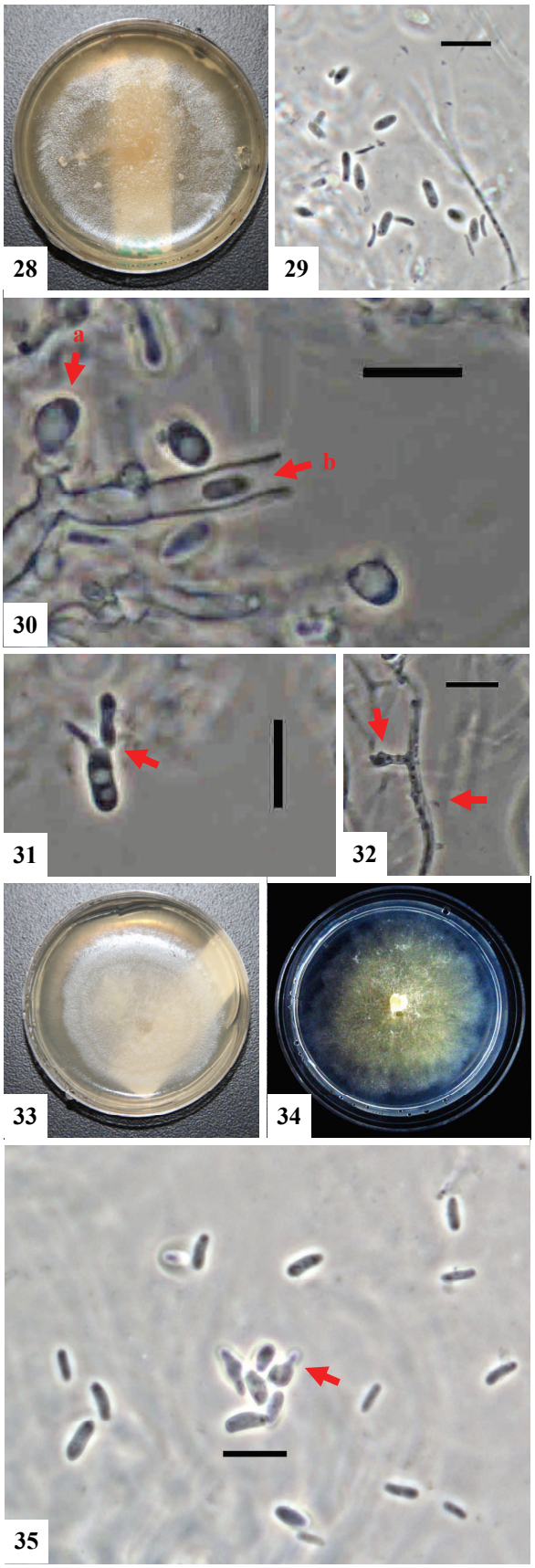
These isolates produced conidia and hyphal growth reminiscent of anamorphs in the *Soradriomycetidae*, but without conclusive genetic evidence or sexual sporulation, they remain unidentified. In the NCBI database, the closest named fungi to isolate 403 is in the *Lecythophora*; continued research may identify this isolate as another *Conichaeta sp.* anamorph.

Isolate 403

98% similarity to *Sordariomycetes sp.*, accession GQ152999.1

Isolate 191

99% similarity to unknown foliar endophyte, accession AY561215.1



Images 28-35: *Sordariomycetes*. 28-32: isolate 272, in culture (28), detached and endoconidia (29, 30b, arrows), phialidic conidiogenesis (30a,) a budding conidium (31, arrow), and phialides with conidia detached (32, arrows). 33-35: unknown *Sordariomycetes*; isolates 403 and 191 in culture (33, 34), and conidia with possible budding or microcyclic conidiogenesis (conidia sprouting, 35, arrow). Scale bar=10µm in 28-31, 33, 35. LH except 29-31: BL

References

1. Kirk PM, Cannon PF, David JC, Stalpers JA. Dictionary of the fungi. 9th ed. CAB International, UK. 2001
2. Davis EC, Franklin JB, Shaw AJ, Vilgalys R. Endophytic *Xylaria* (*Xylariaceae*) among liverworts and angiosperms: phylogenetics, distribution, and symbiosis. American Journal of Botany 2003;90(11):1661-1667
3. Zhang N, Castlebury LA, Miller An, Huhndorf SM, Schoch CL, Siefert KA, Rossman AY, Rogers JD, Kohlmeyer J, Volkmann-Kohlmeyer B, Sung GH. An overview of the systematic of the *Sordariomycetes* based on a four-gene phylogeny. Mycologia 2006;98 (6):1076-1087
4. Mycobank fungal databases nomenclature and species banks; online taxonomic novelties submission. International Mycological Association. Accessed 9 November 2010 from http://www.mycobank.com/MycoTaxo.aspx.
5. MPG North. 47°31'16.23" N and 113°40'13.54" W. Google Earth. Updated 23 June 2009, accessed 9 November 2010.
6. Centraalbureau voor Schimmelcultures (CBS) fungal database, Ulrecht, The Netherlands. Accessed 9 November 2010 from http://www.cbs.knaw.nl/databases/.
7. Farr DF, Rossman AY. Fungal databases, systematic mycology and microbiology laboratory, ARS, USDA. Accessed 9 November 2010 from http://nt.ars-grin.gov/fungaldatabases/
8. Wang Y, Guo LD, Hyde KD. Taxonomic placement of sterile morphotypes of endophytic fungi from *Pinus tabulaeformis* (*Pinaceae*) in northwest China based on rDNA sequences. Fungal Diversity 2005;20:235-260
9. Ganley RJ, Newcombe G. fungal endophytes in seeds and needles of *Pinus monticola.* Mycological Research 2006;110:318-327
10. Stefani FOP, Berube JA. Biodiversity of foliar fungal endophytes in white spruce (*Picea glauca*) from souther Quebec. Canadian Journal of Botany 2006;84:777-790
11. Carroll GC. The foraging ascomycete. 16th International Botanical Congress, Abstracts. International Botanical Congress, St. Louis, MO 1999;309
12. Rogers JD. Thoughts and musings on tropical *Xylariaceae*. Mycological Research 2000;104(12):1412-1420
13. Rogers JD, Ju YM, Adams MJ. Home of the *Xylariaceae.* 2002. Accessed from http://mycology.sinica.edu.tw/Xylariaceae/on 9 November 2010
14. Davis CE, Shaw JA. Biogeographic and phylogenetic patterns in diversity of liverwort-associated endophytes. American Journal of Botany 2008;95(8):914-924
15. Chesters CGC, Greenhalgh GN. *Geniculosporium serpens* gen. et sp.nov, the imperfect state of *Hypoxylon serpens.* Transactions of the British Mycological Society 1964;47(3):393-401
16. Whalley AJS, Edwards RL. The *Xylariaceae:* a case study in biological and chemical diversity. International Conference on Biodiversity and Bioresources: Conservation and Utilization. 1997. *In* Pure Applied Chemistry 1998;70(11)
17. Sinclair WA, Lyon HH. Diseases of Trees and Shrubs. 2nd ed. Cornell University Press, Ithaca, NY. 2005
18. Huang WY, Cai YZ, Surveswaran S, Hyde KD, Corke H, Sun M. Molecular phylogenetic identification of endophytic fungi isolated from three *Artemesia* species. Fungal Diversity 2009;36:69-88
19. Hoffman MT, Arnold AE. Geographic locality and host identity shape fungal endophyte communities in *Cupressaceous* trees. Mycological Research 2008;112:331-344
20. Santamaria J, Bayman P. Fungal epiphytes and endophytes of coffee leaves (*Coffea arabica*). Microbial Ecology 2005;50(1):1-8
21. Gazis R, Chaverri P. Diversity of fungal endophytes in leaves and stems of wild rubber trees (*Hevea brasiliensis*) in Peru. Fungal Ecology 2010;3:240-254
22. Arnold AE, Mejia LC, Kyllo D, Rojas EI, Maynard Z, Robbins N, Herre EA. Fungal endophytes limit pathogen damage in a tropical tree. PNAS, The National Academy of Sciences of the USA 2003;100(26):15649-15654
23. Sun G, Dilcher DL, Zheng S, Zhou Z. In search for the first flower; a Jurassic angiosperm, *Archaefructus,* from northeast China. Science 1998;282:1692-1695
24. Urairuj C, Khanongnuch C, Lumyong S. Ligninolytic enzymes from tropical *Xylariaceae.* Fungal Diversity 2003;13:209-219
25. Stadler M, Fouriner J, Laessoe T, Lechat C, Tichy HV, Piepenbring M. Recognition of hypoxyloid and xylarioid *Entonaema* species and allied *Xylaria* species from a comparison of holomorphic morphology, HPLC profiles, and ribosomal DNA sequences. Mycological Progress 2008;7(1):53-73
26. Maric V. *Xylaria digitata.* Mushroom Observer. Accessed 9 November 2010 from http://mushroomobserver.org/name/show_name?_js=on&_new=true&id=5763
27. Vander Meer, M. Personal communication. 15 September 2010
28. Staments P, Sumerlin D. Mycofiltration: a novel approach for the bio-transformation of abandoned logging roads. Fungi Perfecti LLC. Accessed 10 November 2010 from http://www.fungi.com/mycotech/roadrestoration.html
29. Hsieh HM, Lin CR, Fang MG, Rogers JD, Fournier J, Lechat C, Ju YM. Phylogenetic status of *Xylaria* subgenus *Pseudoxylaria* among taxa of the subfamily *Xylarioideae* (*Xylariaceae*) and phylogeny of the taxa involved in the subfamily. Molecular Phylogenetics and Evolution 2010;54(3):957-969
30. Pfister DH. Early illustrations of *Xylaria* species. North American Fungi 2008;3(7):161-166
31. Global Biodiversity Information Facility. Accessed on 10 November 2010 from http://www.gbif.org
32. Rogers JD. *Xylaria acuta, Xylaria cornu-damae,* and *Xylaria mali* in continental Unite States. Mycologia 1984;76(1):23-33
33. Lee JS, Ko KS, Jung HS. Phylogenetic analysis of *Xylaria* based on nuclear ribosomal ITS1-5.8S-ITS2 sequences. FEMS Microbiology Letters 20000;187(1):89-93
34. Rogers JD. *Xylaria cubensis* and its anamorph *Xylocoremium flabelliforme, Xylaria allantoidea,* and *Xylaria poitei* in continental United States. Mycologia 1984;76(5):912-923
35. Johannesson H, Laessoe T, Stenlid J. Molecular and morphological investigation of *Daldinia* in northern Europe. Mycological Research 2000;104(3):275-280
36. Umabala P, Lakshmi V, Mruthy AR, Prasad VSSV, Sundaram C, Beguin H. Isolation of a *Nodulisporium* species from a case of cerebral phaeohyphomycosis. Journal of Clinical Microbiology 2001;39(11):4213-4218

37. Kamisuki S, Ishimaru C, Onoda K, Kuriama I, Ida N, Sugawara F, Yoshida H, Mizushina Y. Nodulisporol and Nodulisporone, novel specific inhibitors of human DNA polymerase λ from a fungus, *Nodulisporium sp.* Bioorganic and Medicinal Chemistry 2007;15(9):3109-3114
38. Zhao K, Zhou D, Ping W, Jinping G. Study on the preparation and regeneration of protoplast from Taxol-producing fungus *Nodulisporium sylviforme*. Nature and Science 2004;2(2):52-59
39. Pontius A, Krick A, Kehraus S, Foegan SE, Muller M, Klimo K, Gerhauser C, Konig GM. Noduliprevenone: a novel heterodimeric chromanone with cancer chemopreventive potential. Chemistry—A European Journal 2008;14(32):9860-9863
40. Kasettrathat C, Ngamrojanavanich N, Wiyakrutta S, Mahidol C, Ruchirawat S, Kittakoop P. Cytotoxic and antiplasmodial substances from marine-derived fungi, *Nodulisporium sp.* and CRI247-01
41. Mellersh DG, Foulds IV, Higgins VJ, Heath MC. H2O2 plays different roles in determining penetration failure in three diverse plant-fungal interactions. The Plant Journal 2002;29(3):257-268
42. Wang S, Li XM, Teuscher F, Li DL, Diesel A, Ebel R, Proksch P, Wang BG. Chaetopyranin, a benzaldehyde derivative, and other related metabolites from *Chaetomium globosum,* and endophytic fungus derived from the marine red alga *Polysiphonia urceolata.* Journal of Natural Products 2006;69(11):1622-1625
43. Umikalsom MS, Ariff AB, Shamsuddin ZH, Tong CC, Hassan MA, Karim MIA. Production of cellulose by a wild strain of *Chaetomium globosum* using delignified oil palm empty-fruit-bunch fibre as a substrate. Applied Microbiology and Biotechnology 1996;47(5):590-595
44. Di Pietro A, Gut-Rella M, Pachlatko JP, Schwinn FJ. Role of antibiotics produced by *Chaetomium globosum* in biocontrol of *Pythium ultimum*, a causal agent of damping-off. Phytopathology 1992;82:131-135
45. Davis RF, Backman PA, Rodriguez-Kabana R, Kokalis-Burelle N. Biological control of apple fruit diseases by *Chaetomium globosum* formulations containing cellulose. Biological Control 1992;2(2):118-123
46. Istifadah N, McGee PA. Endophytic *Chaetomium globosum* reduces development of tan spot in wheat caused by *Pyrenophora tritici-repentis*. Australasian Plant Pathology 2006;35:411-418
47. Park JH, Choi GJ, Jang KS, Lim HK, Kim HT, Cho KY, Kim JC. Antifungal activity against plant pathogenic fungi of chaetoviridins isolated from *Chaetomium globosum.* FEMS Microbiology Letters 2005;252(2):309-313
48. Ding G, Song Y, Chen JR, Xu C, Ge HM, Wang XT, Tan RX. Chaetoglobosin U, a cytochalasin alkaloid from endophytic *Chaetomium globosum* IFB-E019. Journal of Natural Products 2006;69(2):302-304
49. U"Ren JM, Lutzoni F, Miadlikowska J, Arnold AE. Connumity analysis reveals close affinities between endophytic and endolichenic fungi in mosses and lichens. Microbial Ecology 2010;60(2):340-353
50. Persoh D, Melcher M, Flessa F, Rambold G. First fungal community analyses of endophytic ascomycetes associated with *Viscum album* ssp. *Austracum* and its host *Pinus sylvestris*. Fungal Biology 2010;114(7):585-596
51. Wang L, Zhang WM, Pan QL, Li HH, Tao MH, Gao XX. Isolation and molecular identification of endophytic fungi from *Aquilaria sinensis*. Journal of Fungal Research 2009;1. Accessed 11 November 2010 from http://en.cnki.com.cn/Article_en/CJFDTotal-YJJW200901009.htm
52. Menkis A, Vasaitis R. Fungi in roots of nursery grown *Pinus sylvestris:* ectomycorrhizal colonization, genetic diversity and spatial distribution. Microbial Ecology 2010;5(2). Accessed 11 November 2010 from http://www.springerlink.com
53. Krug JC. *Periamphispora,* a new genus of the *Sordariaceae.* Mycologia 1989;81(3):475-479
54. Kruys AN. Phylogenetic relationships and species richness of coprophilous ascomycetes. Dissertation, Department of Ecology and Environmental Science, Umea University 2005.
55. Mouchacca J, Gams W. The hyphomycete genus *Cladorrhinum* and its teleomorph connections. Mycotaxon 1993;(48):415-440
56. Nilsson RH, Kristiansson E, Ryberg M, Larsson KH. Approaching the taxonomic affiliation of unidentified sequences in public databases—an example from the mycorrhizal fungi. BMC Bioinformatics 2005;6:178. Accessed 11 November 2010 from http://www.biomedcentral.com/1471-2105/6/178
57. Lopez MJ, Vargas-Garcia MC, Suarez-Estrella F, Nichols NN, Dien BS, Moreno J. Lignocellulose-degrading enzymes produced by the ascomycete *Coniochaeta ligniaria* and related species: application for a lignocellulosic substrate treatment. Enzyme and Microbial Technology 2007;40(4):794-800
58. Nichols NN, Dien BS, Lopez MJ, Moreno J. Use of *coniochaeta ligniaria* to detoxify fermentation inhibitors present in cellulosic sugar streams. *In*: Hou TC, Shaw JF (eds). Biocatalysis and Molecular Engineering. John Wiley and Sons, New York. 2010:253-263
59. Nichols NN, Lopez MJ, Dien BS, Bothast RJ. United States patent number 7067303. Accessed 11 November 2010 from http://www.freepatentsonline.com/7067303.html
60. Hoffman M, Gunatilaka M, Ong J, Shimabukuro M, Arnold AE. Molecular analysis reveals a distinctive fungal endophyte community associated with foliage of montane oaks in southeastern Arizona. Journal of the Arizona-Nevada Academy of Science 2008;40(1):91-100
61. Damm U, Fourie PH, Crous PW. *Coniochaeta* (*Lecythophora*), *Collophora* gen. nov. and *Phaeomoniella* species associated with wood necroses of *Prunus* trees. Persoonia 2010;24:60-80
62. Kim HY, Choi GJ, Lee HB, Lee Sw, Lim HK, Jang KS, Son SW, Lee SO, Cho KY, Sung ND, Kim JC. Some fungal endophytes from vegetable crops and their anti—oomycete activities against tomato late blight. Letters in Applied Microbiology 2007;44(3):332-337
63. Rogers JD. The conidial stage of *Coniochaeta lignaria*: morphology and cytology. Mycologia 1965;57(3):368-378
64. Taylor JW, Bowman BH, Berbee ML, White TJ. Fungal model organisms: phylogenetics of *Saccharomyces, Aspergillus,* and *Neurospora.* Systematic Biology 1993;42(4):440-457
65. Campbell NA, Reece JB. Biology, 6th ed. Benjamin Cummings, San Francisco, CA 2002
66. Newcombe G. Personal communication. University of Idaho College of Forestry and Conservation 2009.
67. Web image. The hidden forest. Hidden Forest Designs 2000. Accessed 18 November 2010 from http://hiddenforest.co.nz/
68. Callan BE. First report of *Nemania serpens* var. *hydnicola* in Canada, and production of the teleomorph in culture. North American Fungi 2008;3(7):187-192