

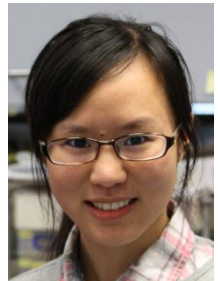


Fungal Community Associated with the Urban Trees and Prevention of Tree Disease

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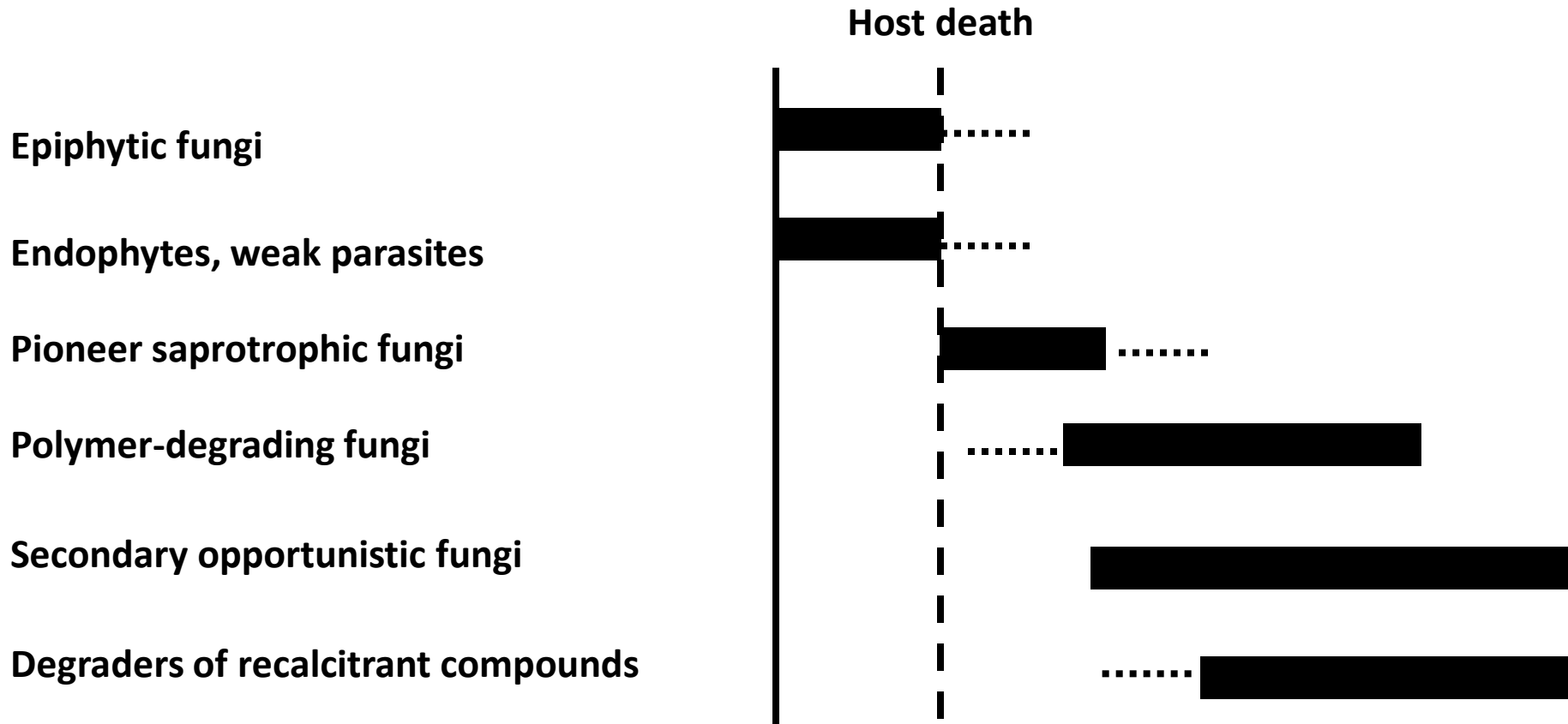
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Outline

- Wood-decaying in subtropical region
- *Phellinus noxius* and the Brown Root Rot Disease
- Current laboratory investigations on *P. noxius*
- Future work

Fungi as Agents of Decomposition



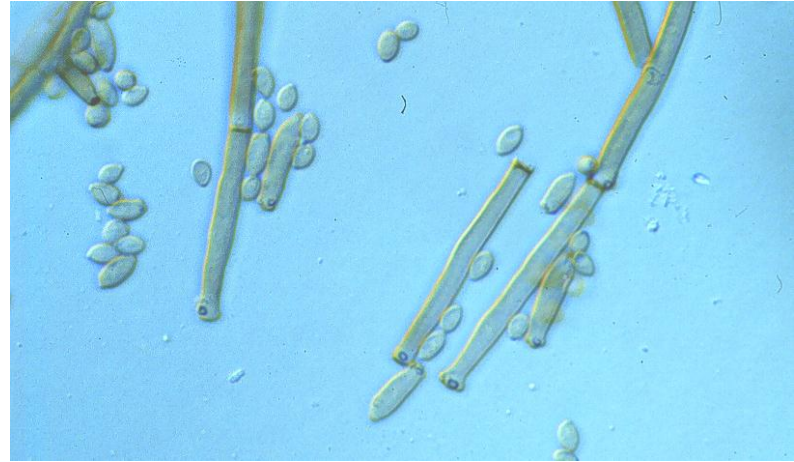
Epiphytes

- Phyllosphere: Bacteria and yeasts growing on soluble nutrients “leaking” from host tissue
 - Many have carotenoid pigments, capsules or slime for protection and adhesion



Endophytes and Weak Parasites

- Endophytes grow in living plant tissue
 - Many taxa
- Weak parasites colonize living tissue, or tissue just starting to senesce
 - Common parasites include *Cladosporium*, *Alternaria*, *Colletotrichum*



Pioneer Saprotrophic Fungi

- Utilize sugars and other simple soluble nutrients
- Germinate and grow rapidly
- Cannot compete well with other fungi
- Includes *Mucor*, *Rhizopus*, *Pythium*



Polymer-degrading Fungi

- Extended growth on structural polymers (cellulose, hemicellulose, chitin)
- Defend resource against invaders either through sequestering nutrients or producing antibiotics



Includes *Fusarium*, *Chaetomium*,
Stachybotrys, *Trichoderma*

Fungi That Degrade Resistant Polymers

- Utilize cellulose, hemicellulose and lignin in dead plant material
- Includes wide range of saprotrophic and wood decay ascomycetes and basidiomycetes



Wood Decay—White Rot

- Cellulose, lignin and hemicellulose removed at approx. equal rates
- Wood becomes pale (bleached) and stringy
- Predominant form of wood rot
- Fungi are the only organisms able to completely degrade lignin through the production of relatively few enzymes
 - e.g., laccase, lignin peroxidase, manganese peroxidase



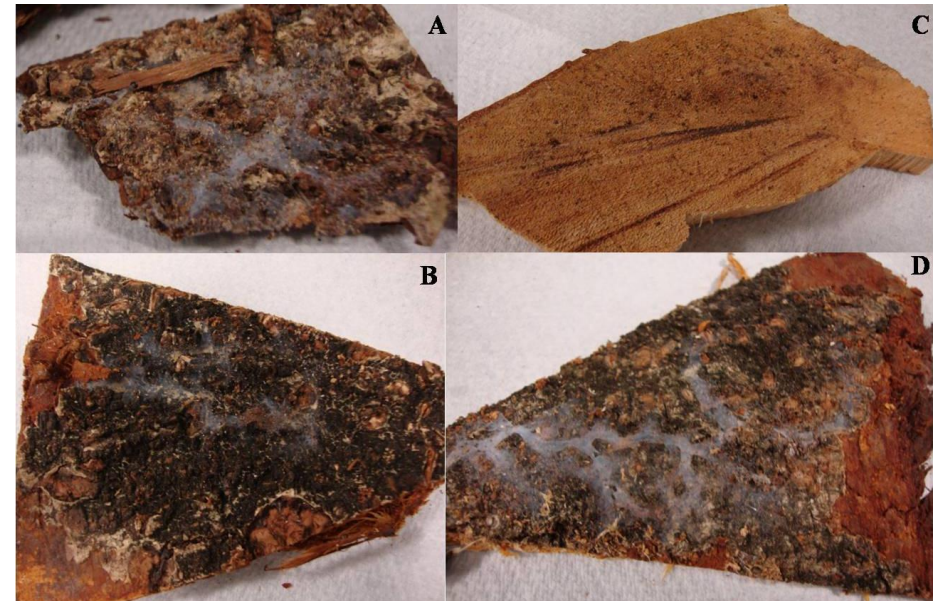
<http://www.anbg.gov.au/fungi/images/0122.jpg>

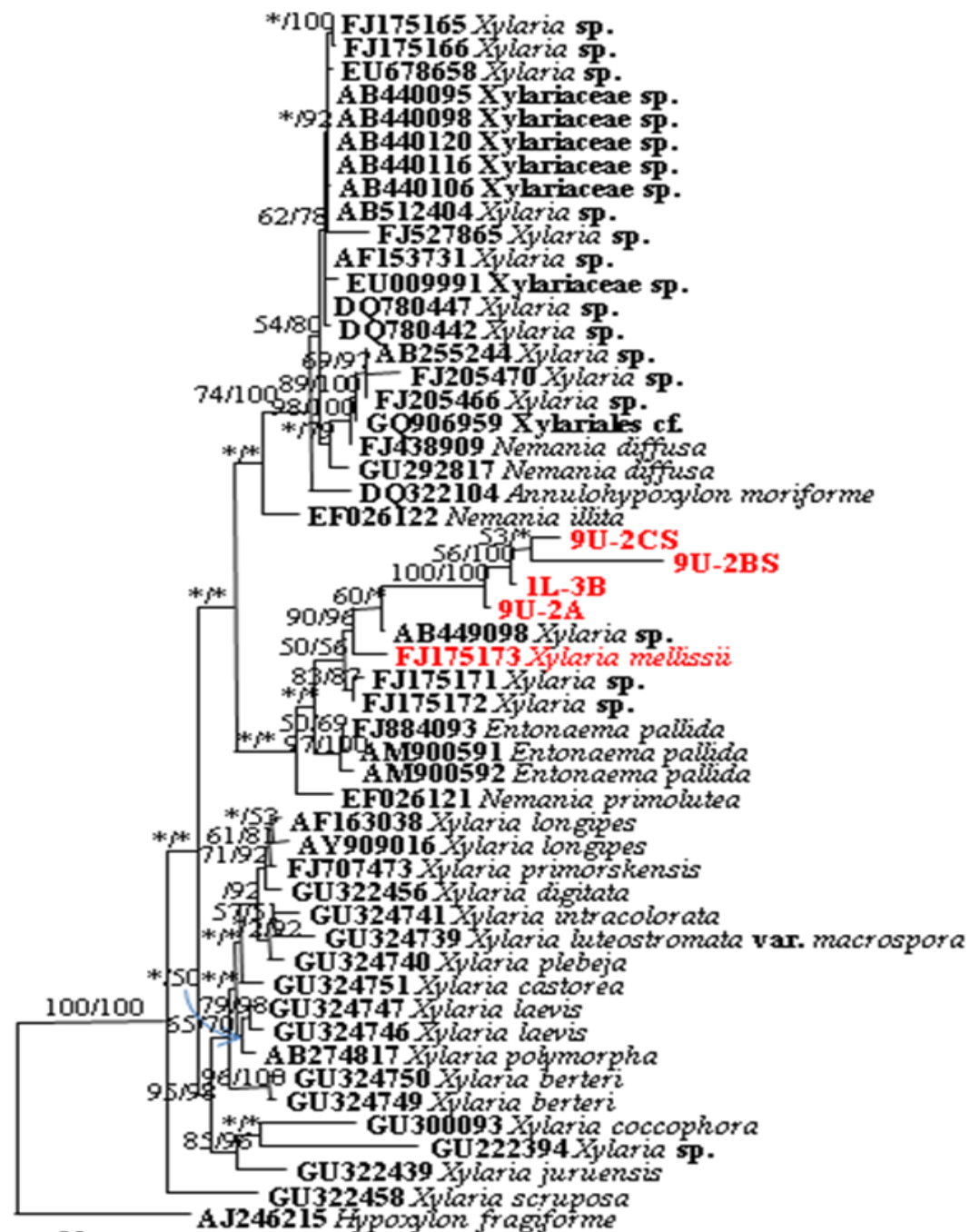
Wood Decay--Brown Rot

- Cellulose and hemicellulose are selectively removed by fungus, lignin is slightly modified
 - Cellulose degraded by oxidative process involving production of hydrogen peroxide
- Wood becomes dark and cubical
- Brown rot fungi comprise 10% of wood decay taxa, but 80% of brown rot fungi occur on conifers

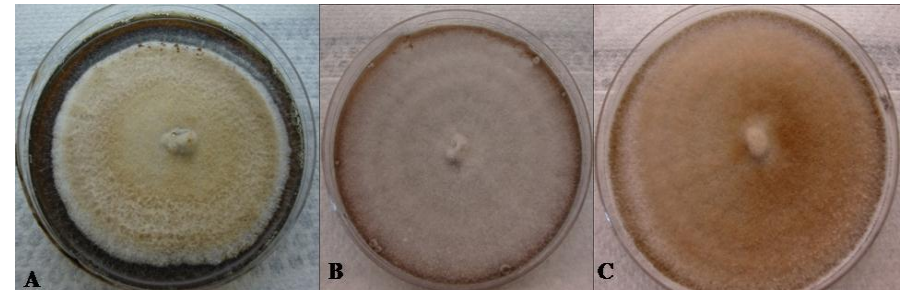


A Case Study





	Healthy samples	Infected samples
Sample No.	6	11
<i>Arthrographis cuboidea</i>	0	2
<i>Hypocrea</i> sp.	0	7
<i>Lasiodiplodia theobromae</i>	11	1
<i>Macrophomina phaseolina</i>	0	1
<i>Nodulisporium</i> sp.	0	3
<i>Penicillium</i> sp.	0	5
<i>Pseudofusicoccum stromaticum</i>	5	7
<i>Trichoderma</i> sp.	4	17
<i>Vuilleminia comedens</i>	0	1
<i>Xylaria mellissii</i>	0	4
Unidentified species	2	2



Photographs of *Nodulisporium* species obtained in this study.

Lam Tsuen

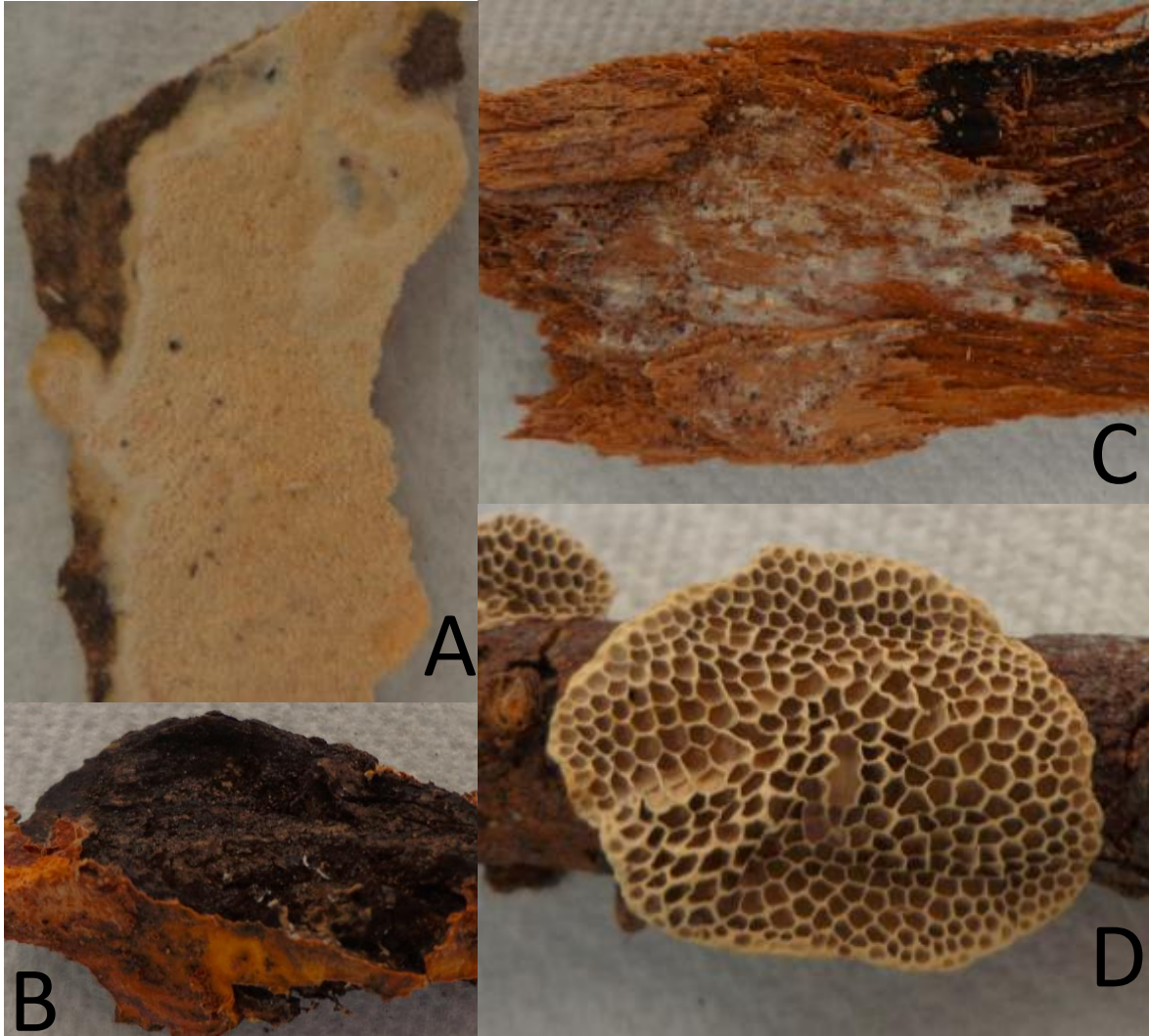


Case study: Tree Branch





Infection of the Tree Branch

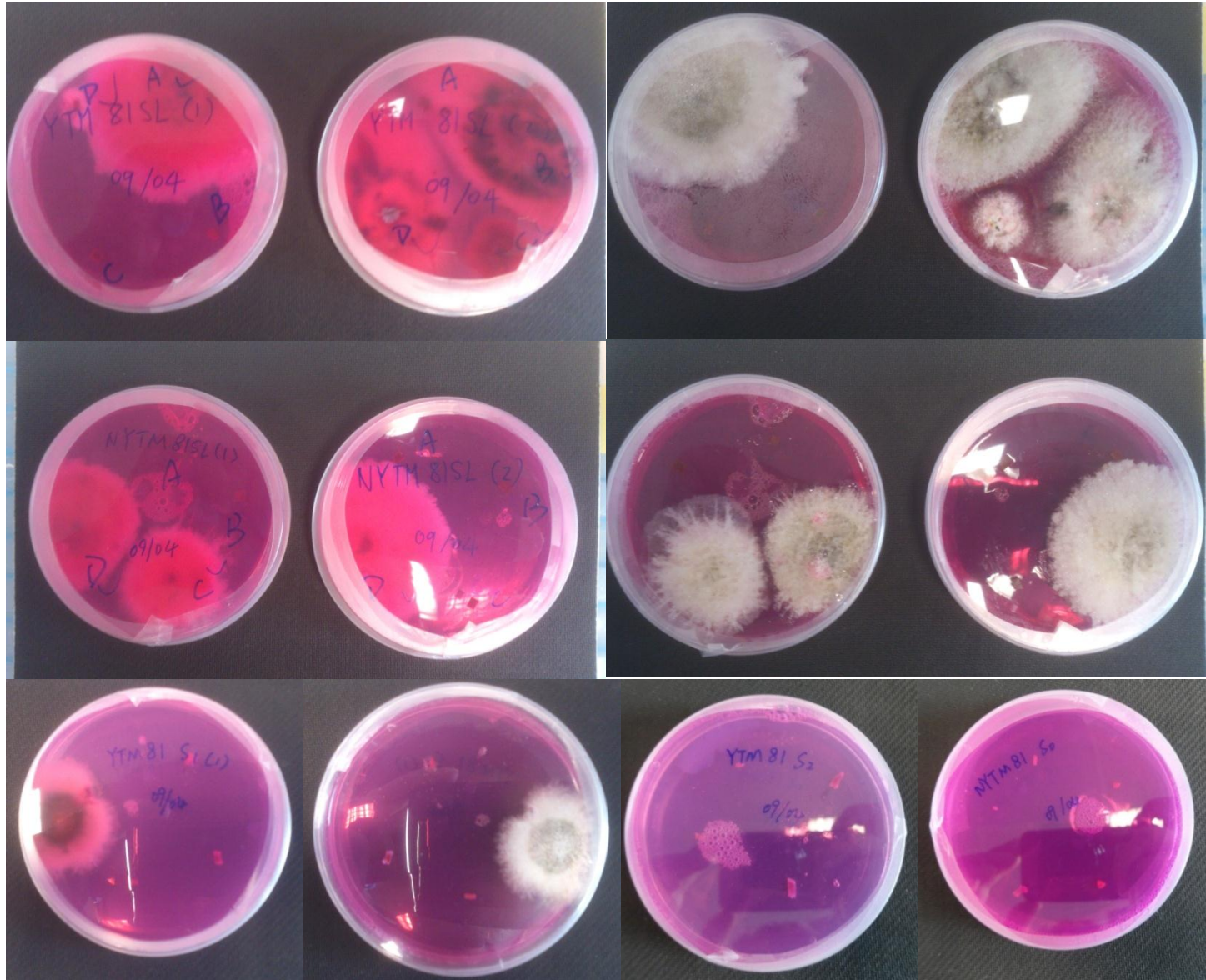


- A. *Hyphodontia flavipora*
- B. *Chondrostereum purpureum*
- C. *Physisporinus vitreus*
- D. *Hexagonia tenuis*

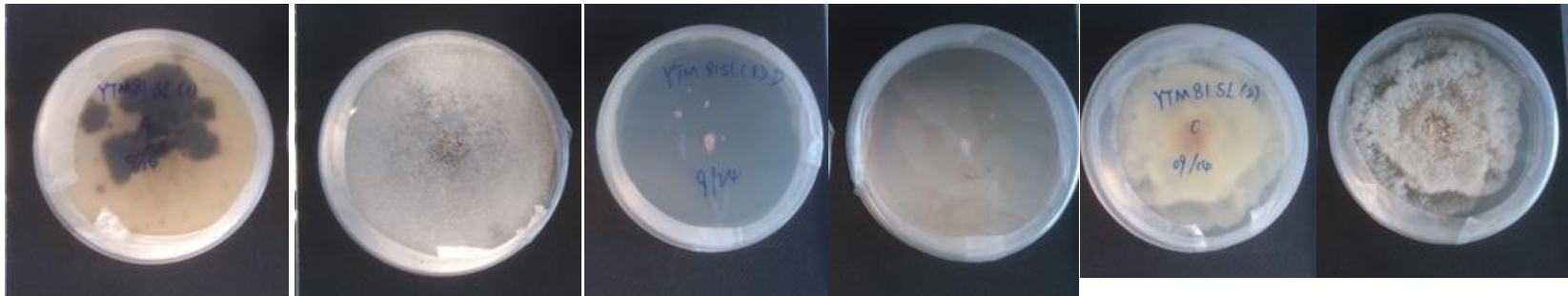
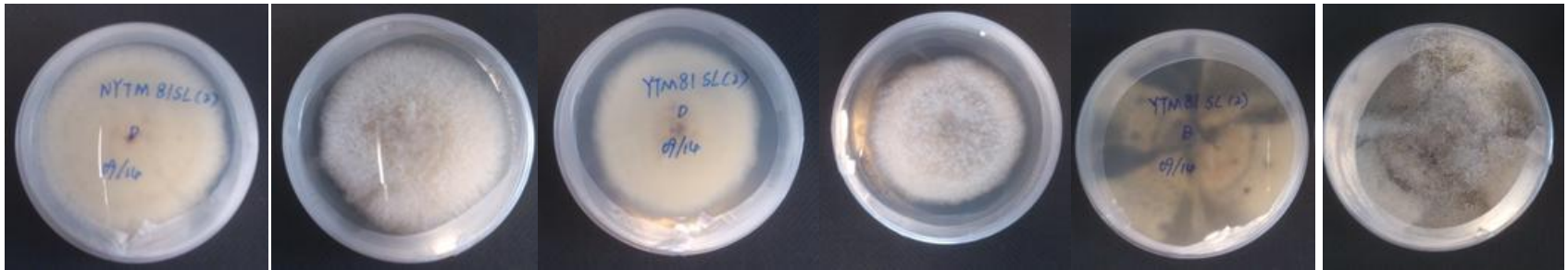
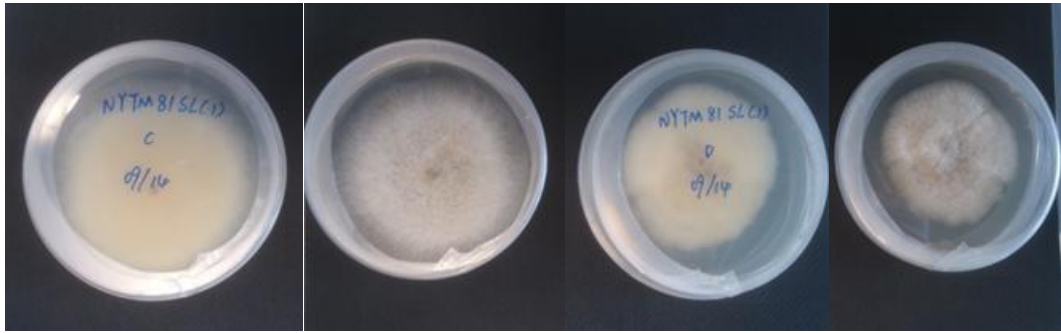
Further Study



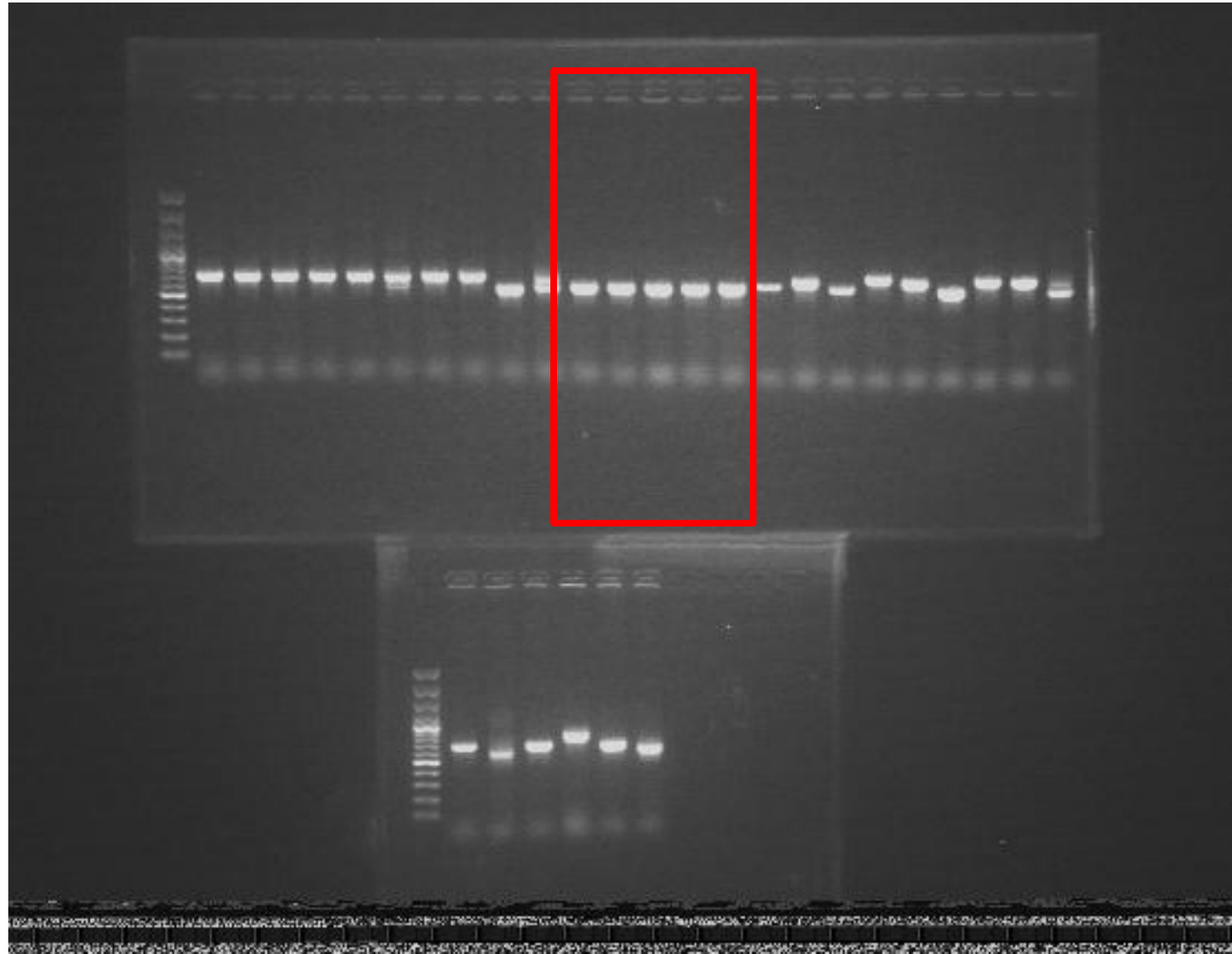
Isolation of fungi from local trees



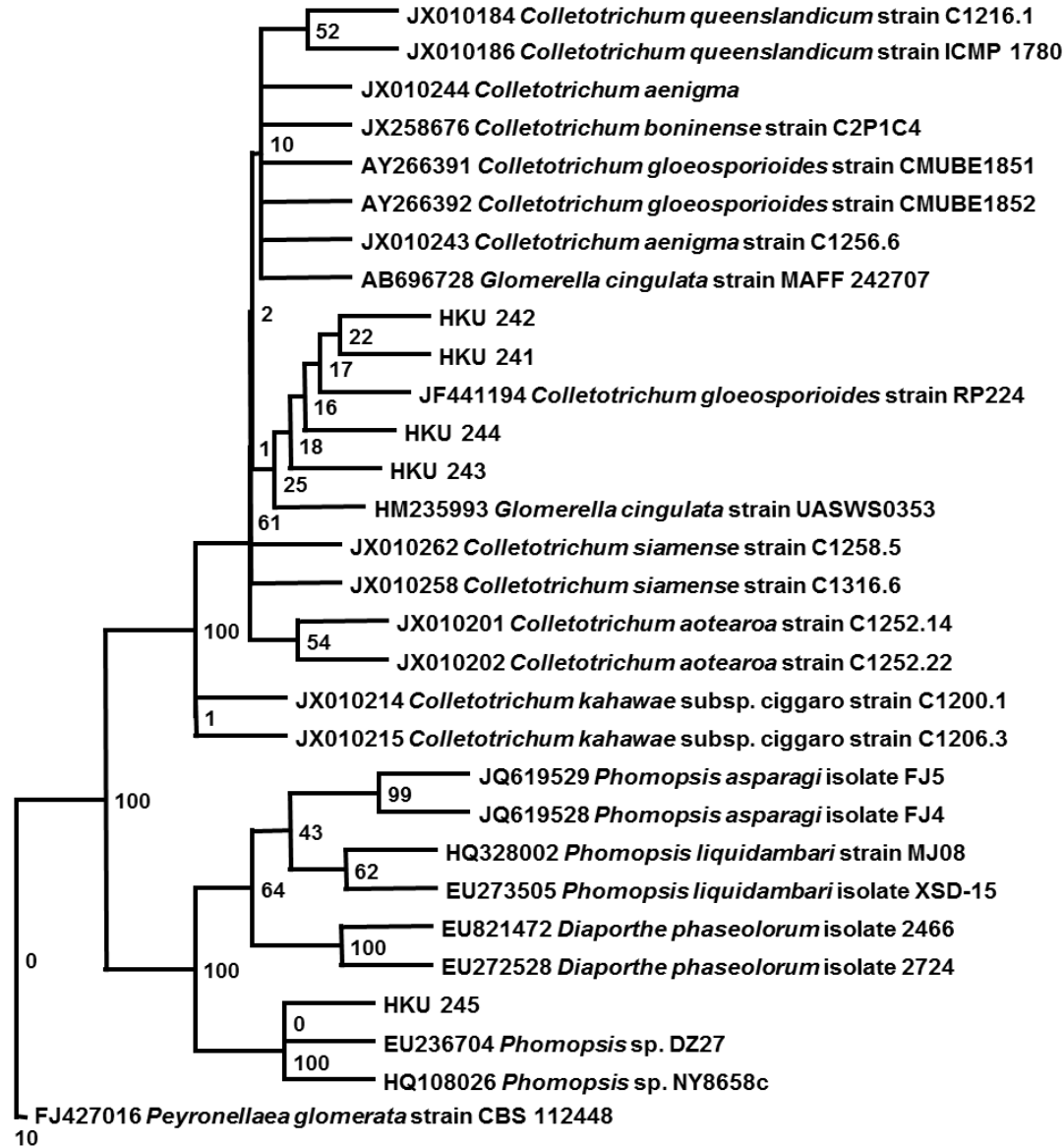
Isolation of fungi from local trees



Gel electrophoresis of extracted DNA



Results – Phylogenetic Tree



We see the trees (fungi), but what is the forest (community)?

- Community based analysis
- Culturability
- Mechanisms of degradation/infection

Brown Root Rot Disease and *Phellinus noxius*

- What is *P. noxius*?
- Host range of *P. noxius*
- Transmission pathways
- Symptoms of BRRD caused by *P. noxius*
- Decay caused *P. noxius* at microscopic levels
- Distribution of *P. noxius*

What is *Phellinus noxius*?

- A fungus that causes brown root rot disease
- **Position in classification:**
Hymenochaetaceae, Hymenochaetales, Incertae sedis, Agaricomycetes, Agaricomycotina, Basidiomycota, Fungi



1. Fruiting body of *P. noxius*
2. Fresh fruiting body of *P. noxius*
3. White decay caused by *P. noxius*

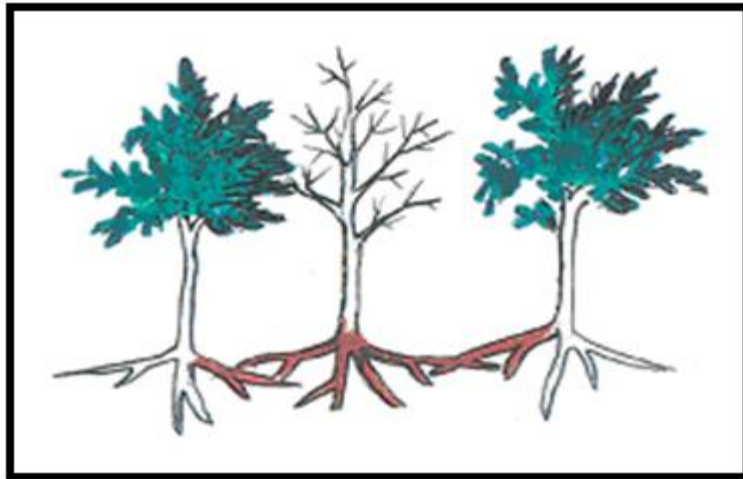
Host Ranges of *Phellinus noxius*

- Wide hosts: more than 200 tree species;
 - Occurrence in Hong Kong;
 - Both on living trees and stumps;
 - Currently, 27 trees in Hong Kong have been confirmed to be infected by *P. noxius*, including 10 tree species. Altogether, **13 strains** of *P. noxius* have been obtained.

Hosts		Location
<i>Bombax ceiba</i>	木棉	Kwun Tong
<i>Celtis sinensis</i>	朴	Kwai Tsing
<i>Delonix regia</i>	鳳凰木	Eastern
<i>Ficus benjamina</i>	垂業榕	Central & Western
<i>Ficus microcarpa</i>	細葉榕	Islands, Kwai Tsing, North, Sham Shui Po, Southern, Yau Tsim Mong
<i>Gleditsia fera</i>	华南皂莢	Wan Chai
<i>Aleurites moluccana</i>	石栗	Yau Tsim Mong
<i>Mangifera indica</i>	芒果	Central & Western
<i>Lophostemon confertus</i>	紅膠木	Yau Tsim Mong
<i>Vcmicia Montana Lour.</i>	木油桐	Yau Tsim Mong
Wood stump		Kwai Tsing, Central & Western

Transmission Pathways of *Phellinus noxius*

- Spreading through root-to-root connections mainly
 - ‘hot spot’
 - Infected trees cluster together



Adapted from Ann *et al.* (2002)



Symptoms of BRRD Caused by *Phellinus noxius*

- Comparison of early and late stages of infection



A 30-year-old flame tree (*Delonix regia*) in central Taiwan. A, 3 months before the appearance of quick decline; B, after the appearance of quick decline.

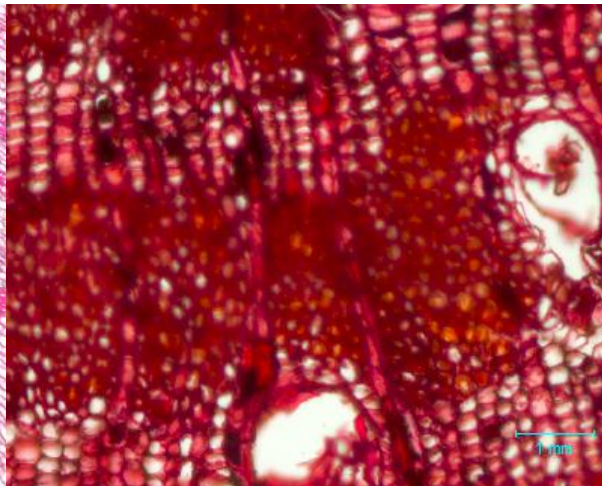
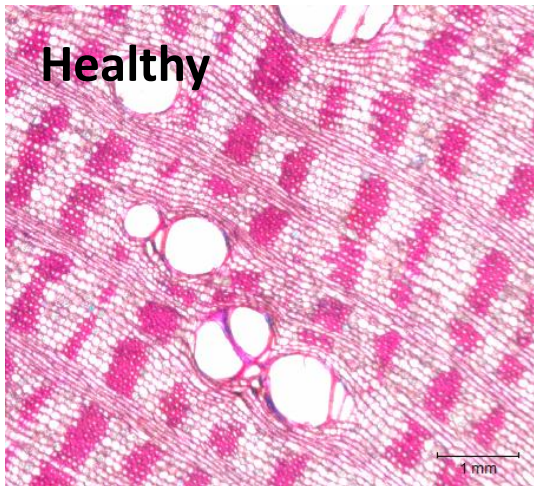
From Ann *et al* (2002)

Local Examples

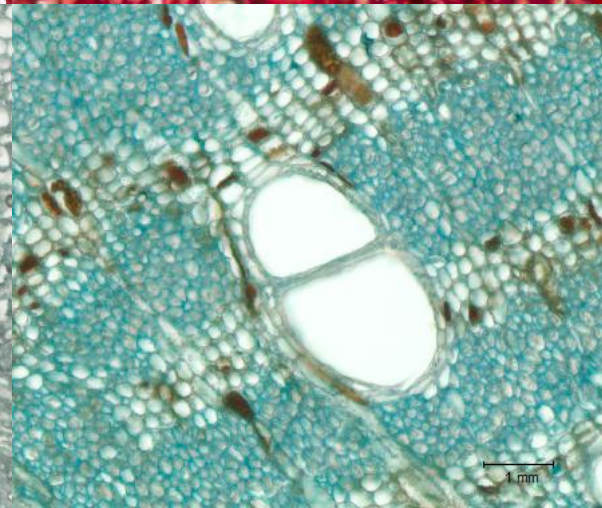
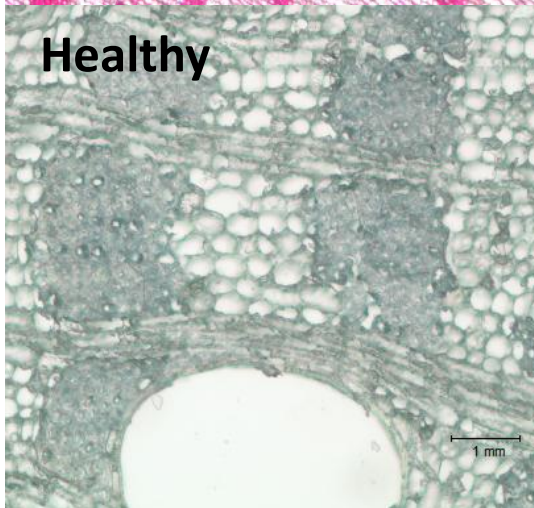


Decay Caused *Phellinus noxius* at Microscopic Levels

- Lignin degradation indicated with staining under light microscope

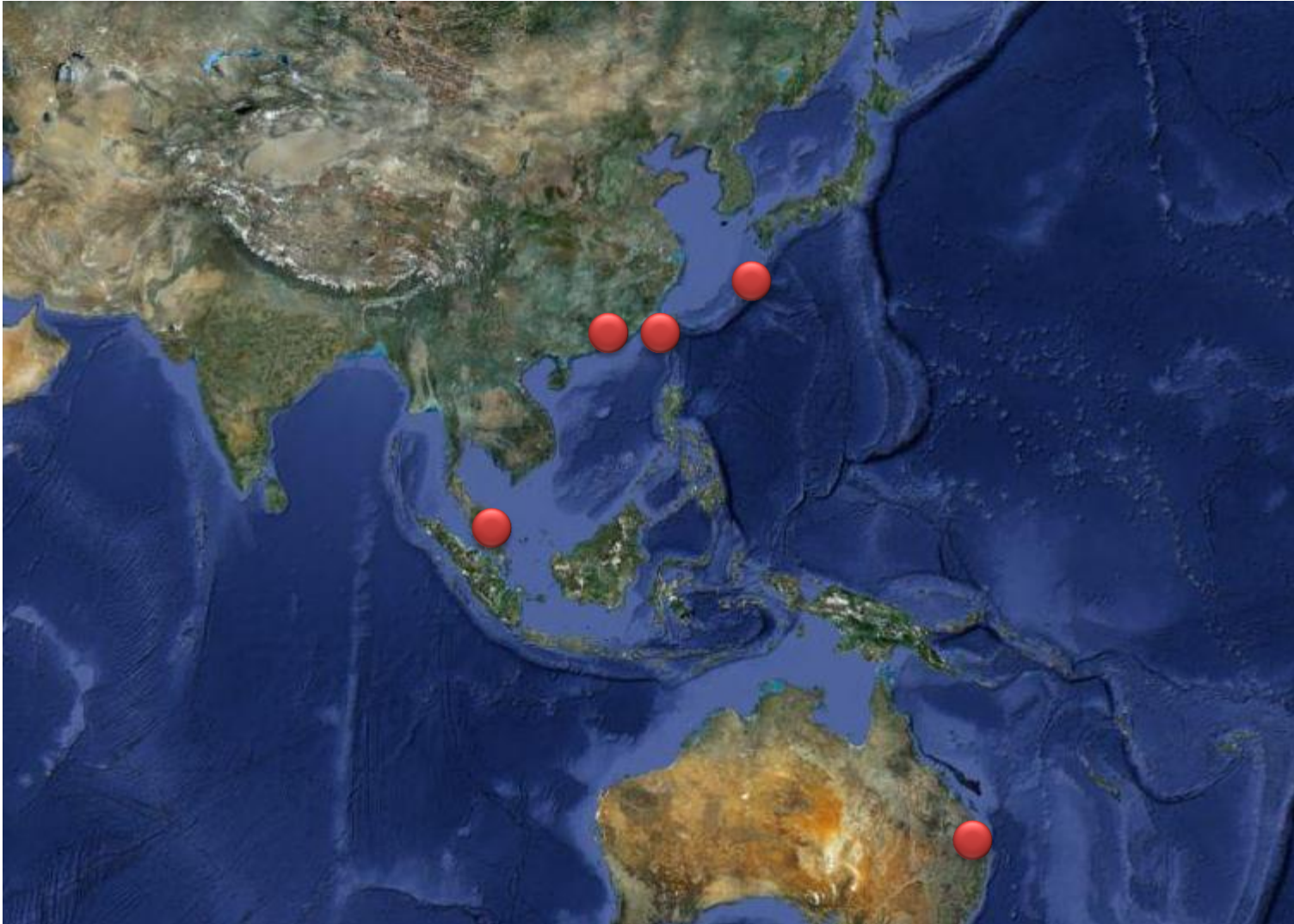


← Staining with Safranin-O, red show delignification (right);



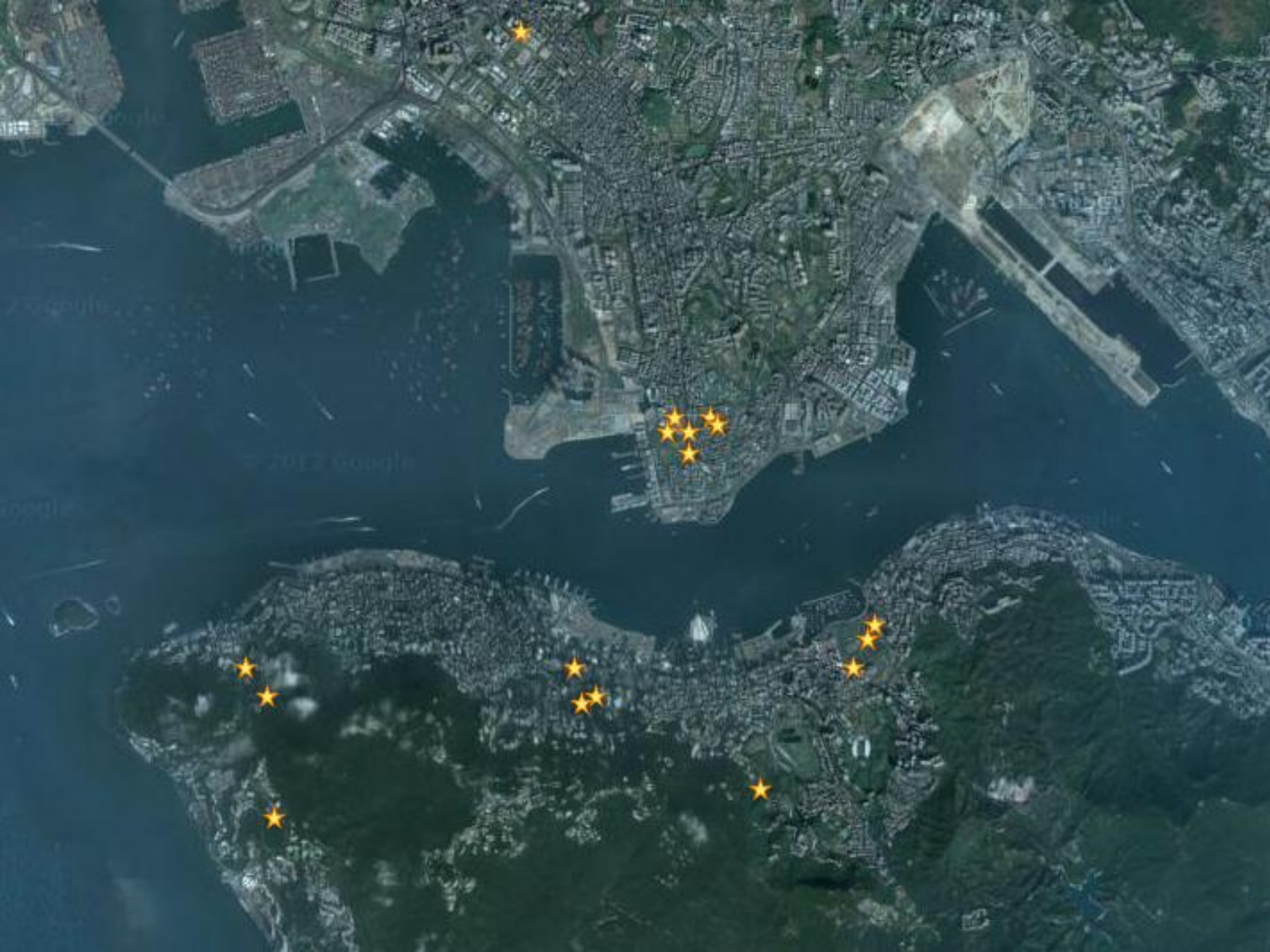
← Staining with Aniline Blue, blue show lignin degradation (right).

Distribution of Brown Root Rot Disease Caused by *Phellinus noxius*



BRR Caused by *Phellinus noxius* in Hong Kong

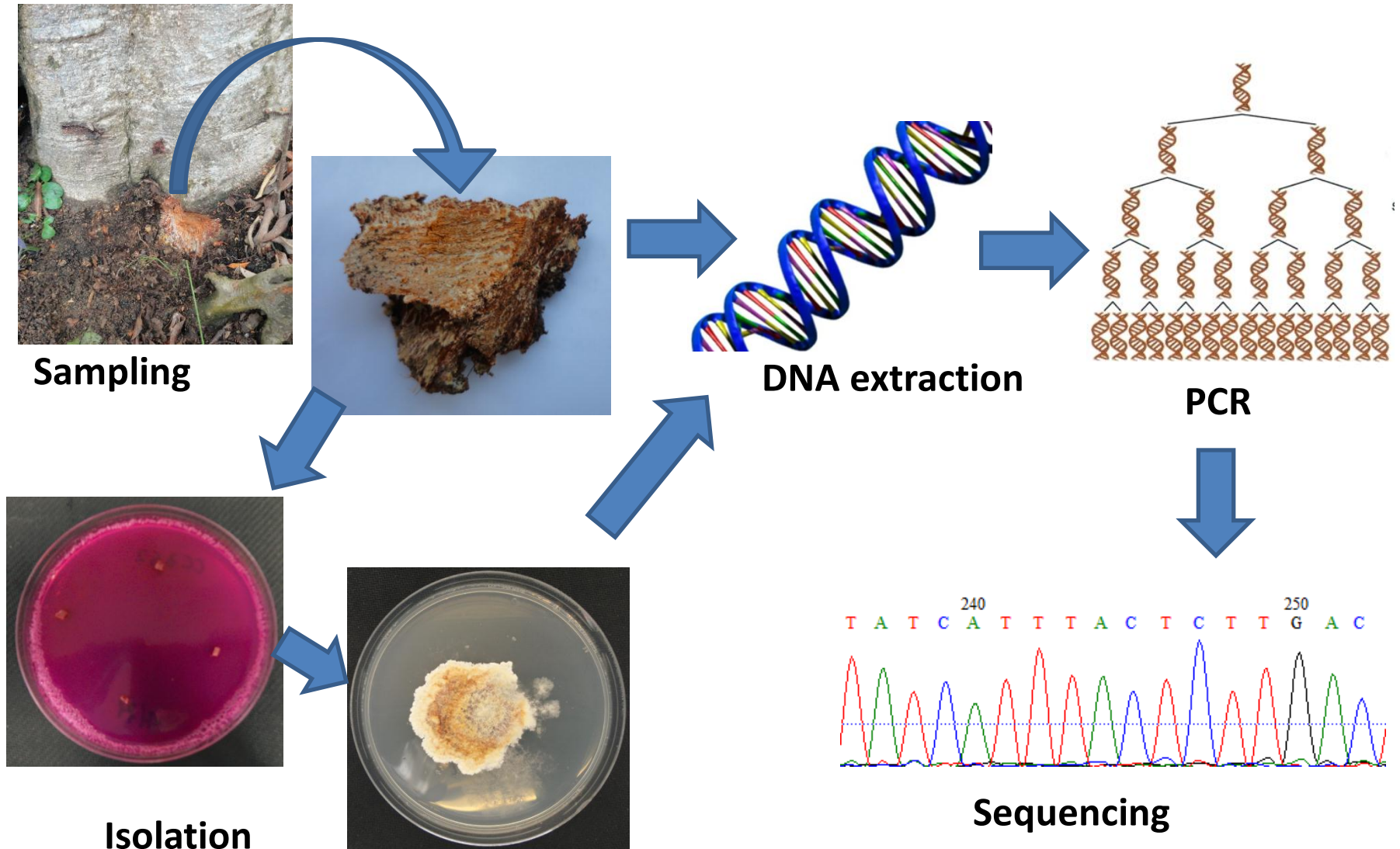




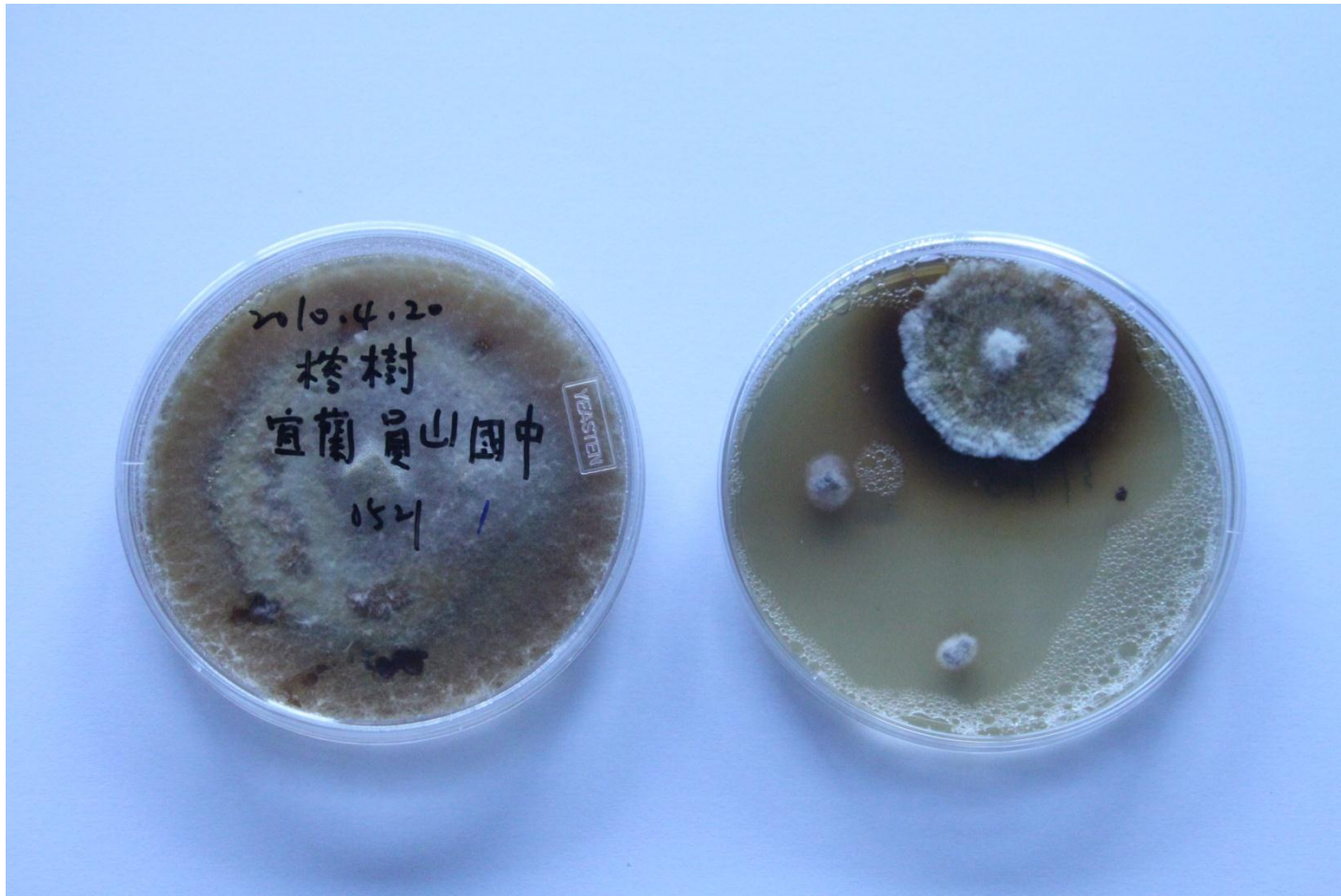
Laboratory Investigations of *P. noxius*

- Sampling
- Culture isolation on selective medium
- Identification based on morphology and phylogeny
- Phylogenetic analysis
- Testing of pH influence on growth and infection efficiency of *P. noxius*

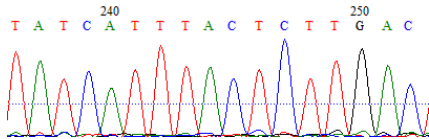
Lab Assay of *Phellinus noxius*



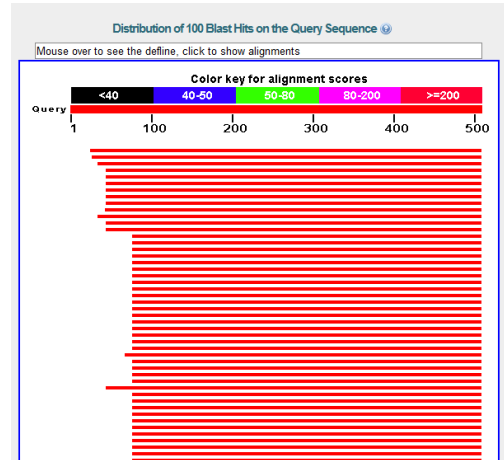
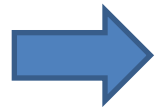
Isolates from Taiwan and Hong Kong



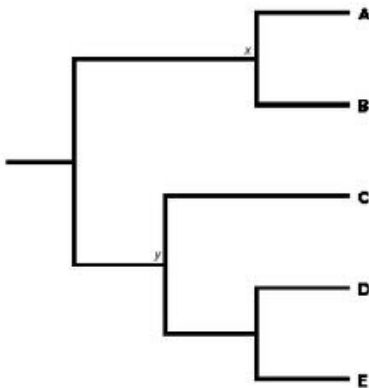
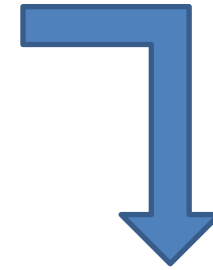
Lab Assay of *Phellinus noxius*



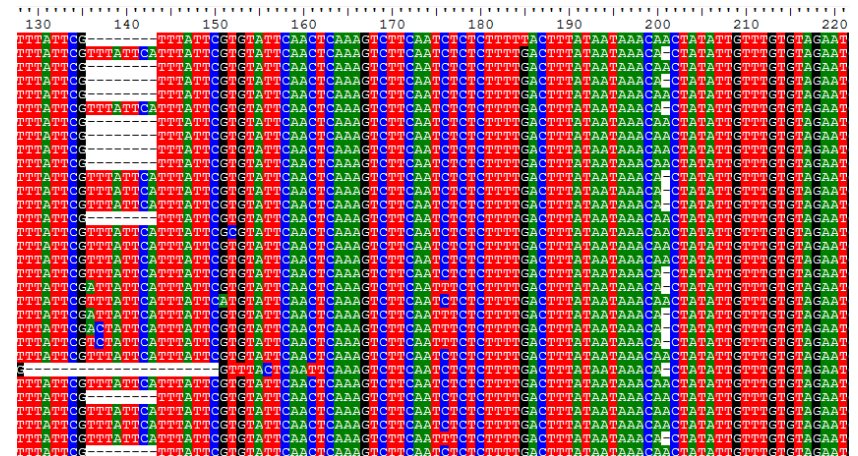
sequence



BLAST in NCBI



Phylogenetic analysis

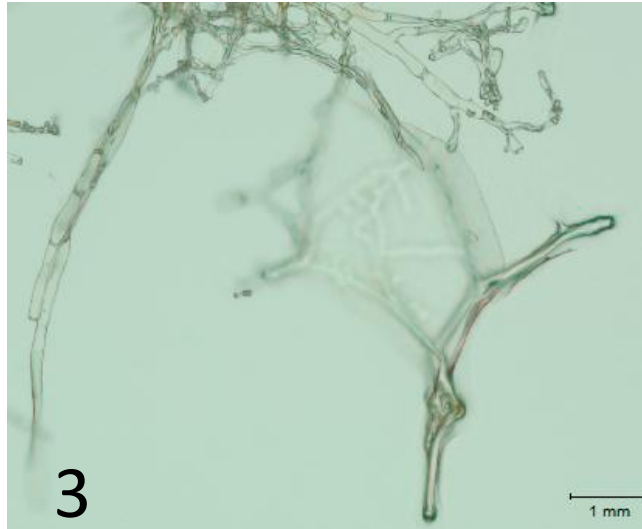
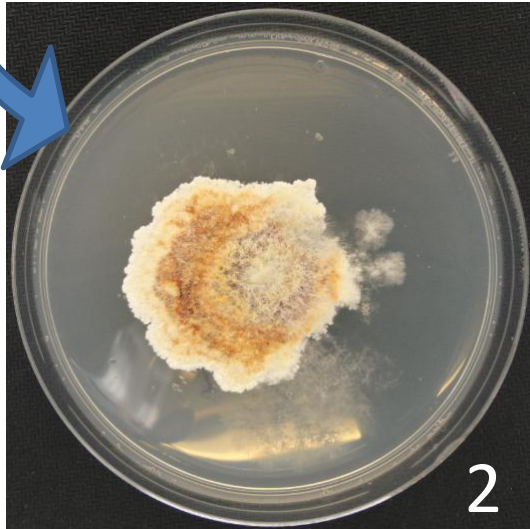


Multiple sequences alignment

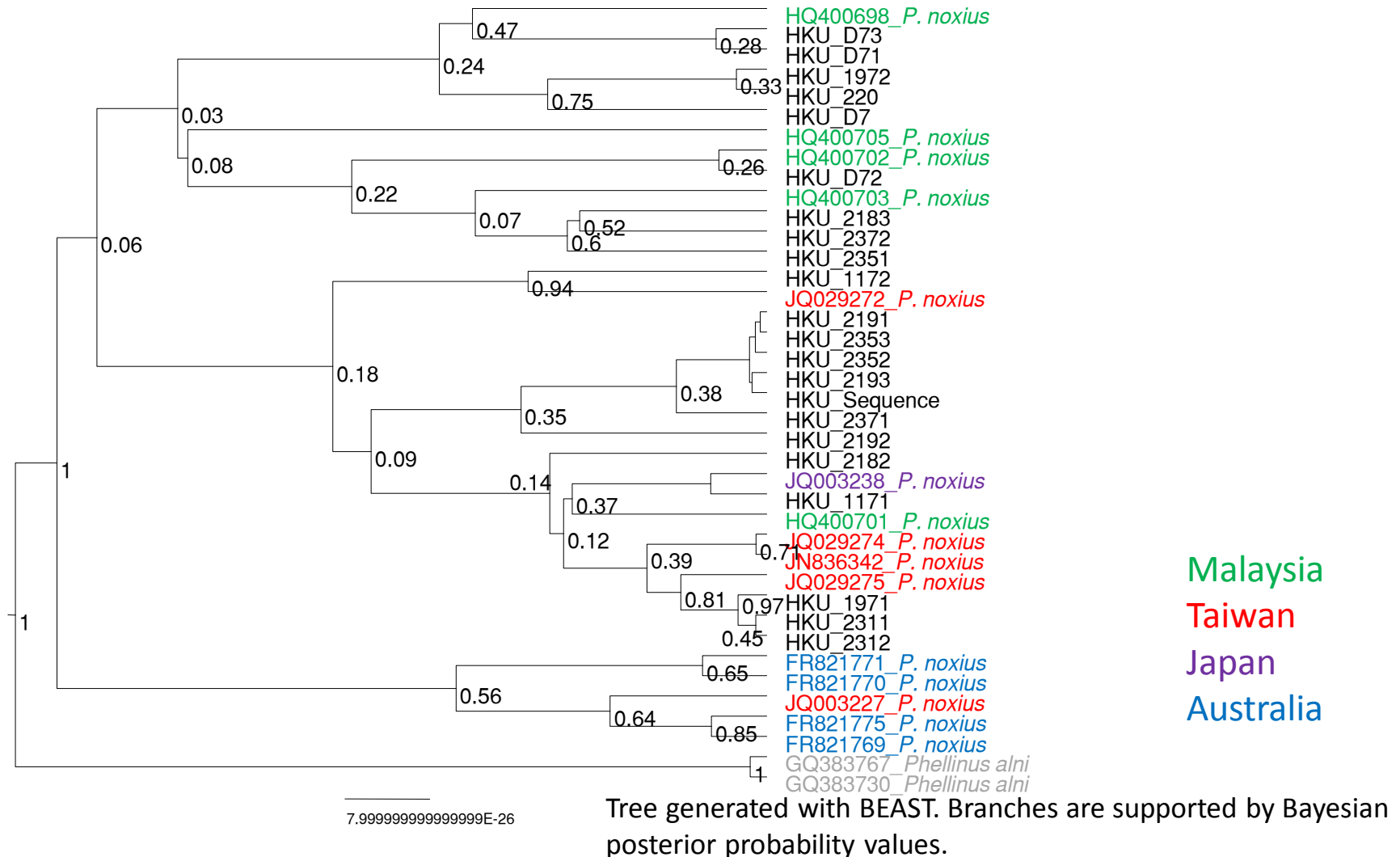
Biological Features: Mycelia on Different Scales



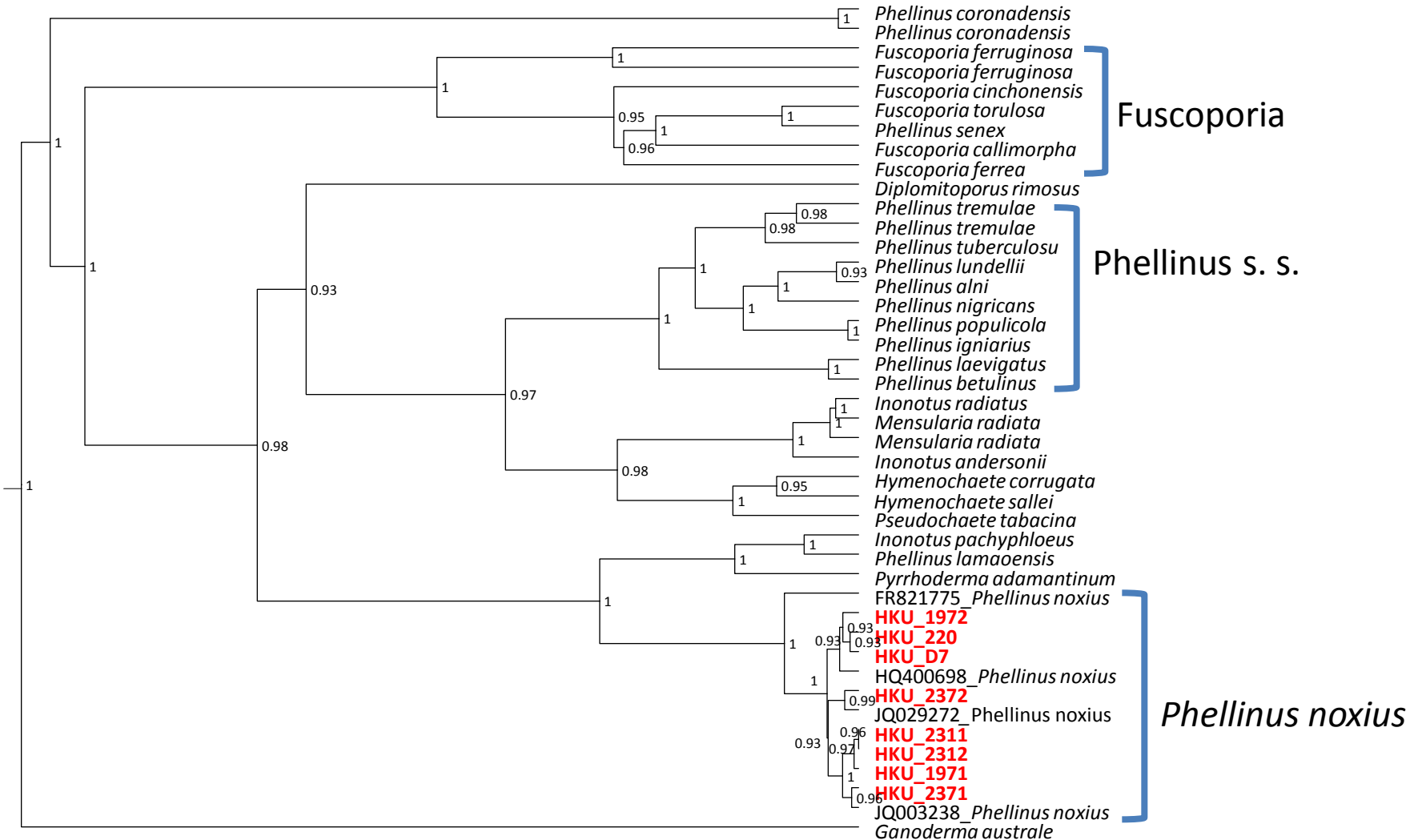
1. Mycelia on wood tissues show brown lines and cause white rot;
2. Mycelia on PDA culture, show irregular patches;
- 3 & 4. Mycelia under light microscope.



Phylogenetic Relationship among *P. noxius* from Different Regions



Classification of *P. noxius* Based on ITS



2.0E-21

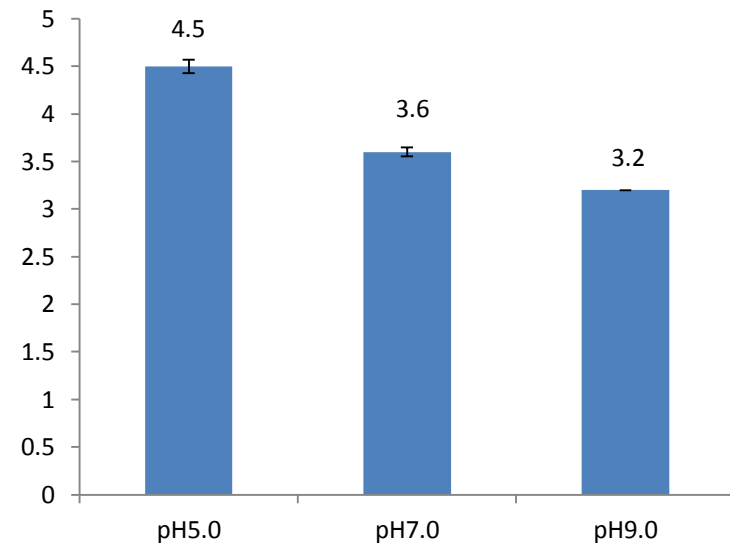
Tree generated with BEAST. Branches are supported by Bayesian posterior probability values.

Effect of different pH conditions on *Phellinus noxius*



Phellinus noxius grown in Malt Extract Broth medium at different pH levels

Biomass(g/L)



Biomass at different pH level in Malt Extract Broth

**Biomass
pH 5.0**

16.67g

**Biomass
pH 7.0**

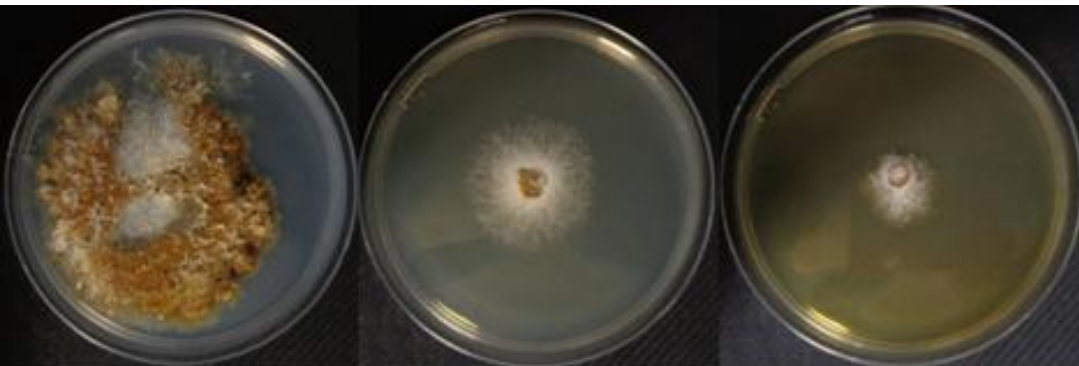
14.28g

**Biomass
pH 9.0**

13.71g



Effect of different pH conditions on *Phellinus noxius*



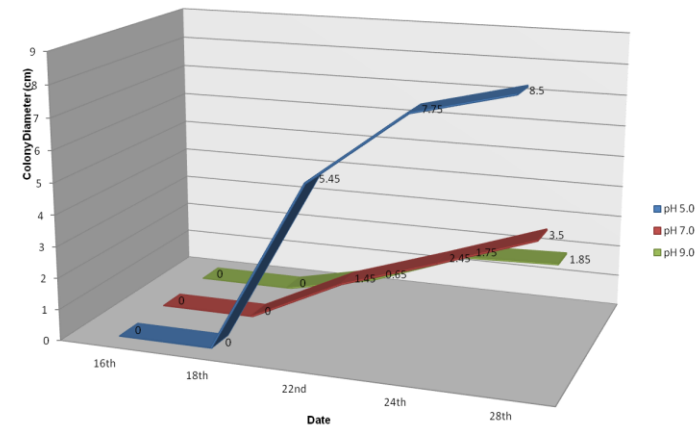
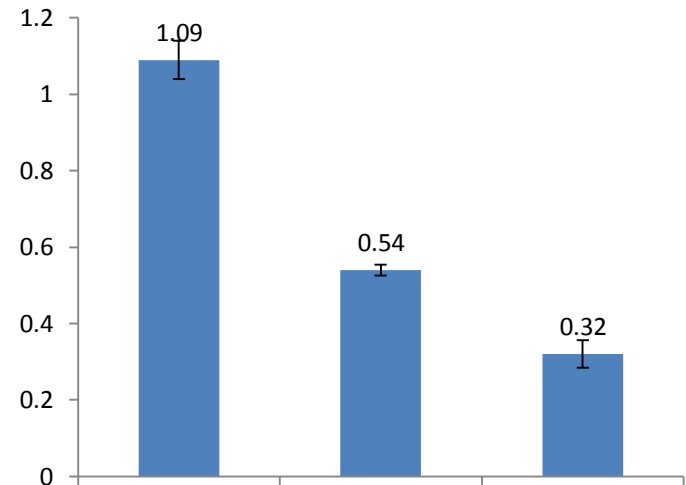
pH5.0

pH7.0

pH9.0

Phellinus noxius grown on Malt Extract Agar at different pH levels

Growth Rate (cm/day)



Growth rate on Malt Extract Agar at different pH levels



Influence of pH to *P. noxius*: Infection efficiency

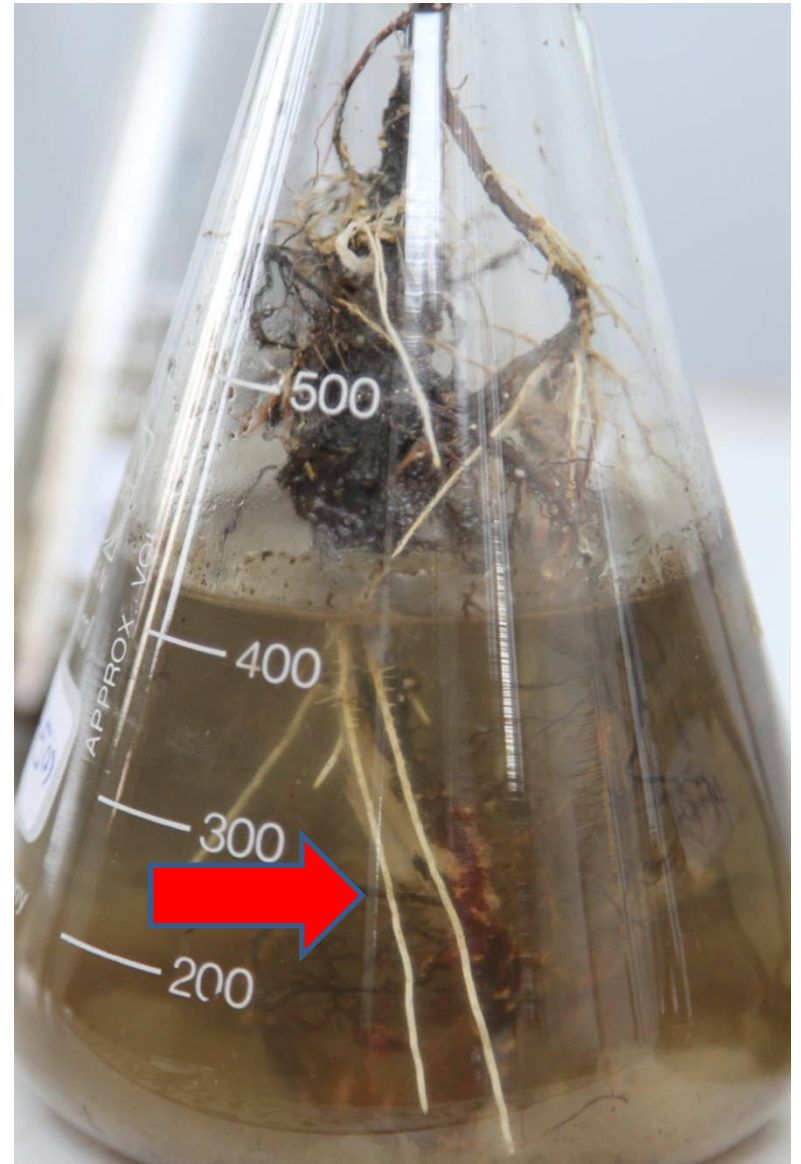


pH 5

pH6

pH7

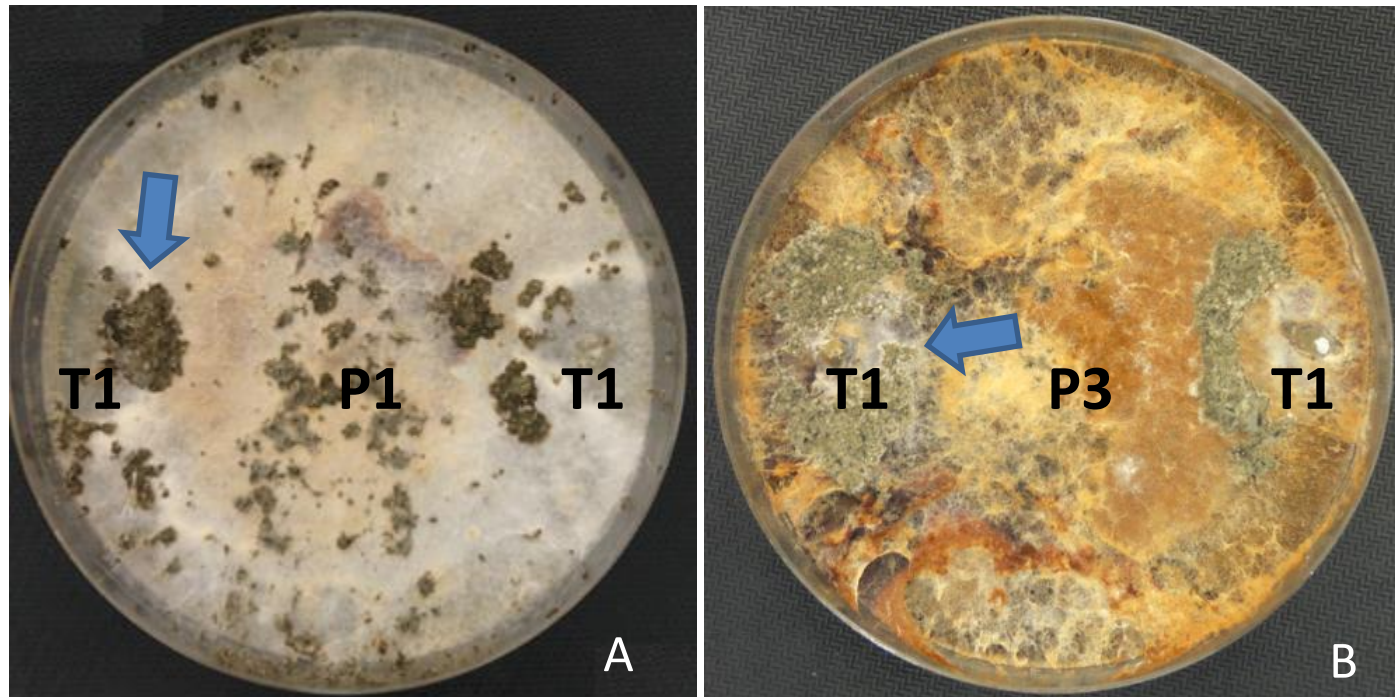
pH8



Biological Control

- Isolation of local *Trichoderma* spp.
 - Characterization
- Sources and efficacy
 - Culturing conditions and biocontrol effectiveness
- Applications in tree management
 - *in situ* testing

Inhibition of *Phellinus noxius* by *Trichoderma harzianum*



Trichoderma harzianum were inoculated on both sides of *Phellinus noxius* on PDA media. Blue arrows show the spores generated by *T. harzianum*.

A. P1-T1

B. P3-T1

P1: *Phellinus noxius* P3: *Phellinus noxius* T1: *Trichoderma harzianum*



Conclusions

- Biodeteriogens
 - Common agents responsible for wood-decaying
 - BRRD by *P. noxius* does not mean they contribute to degradation of dead wood above ground
- Sources and infectivity
 - *P. noxius* may have a common origin in Asia
 - Are local isolates capable of infection? when?
- Tree protection and risk management
 - Use biocontrol to decrease the risk
 - Minimize pathogen and promote the health of the hosts

Future work

- Phylogenetic analysis with multi-locus;
 - to find out the origin of *P. noxius* and stop the path of transmission
- Infectivity study
 - explore the initial infection mechanisms to interfere the infection efficiency by *P. noxius* to prevent brown root rot disease
- Biological control
 - Use antagonistic fungi to treat BRRD caused by *P. noxius*, such as *Trichoderma* spp.

