

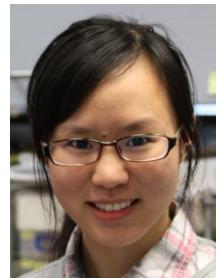


Fungal Community Associated with the Urban Trees and Prevention of Tree Disease

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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

Epiphytic fungi

Endophytes, weak parasites

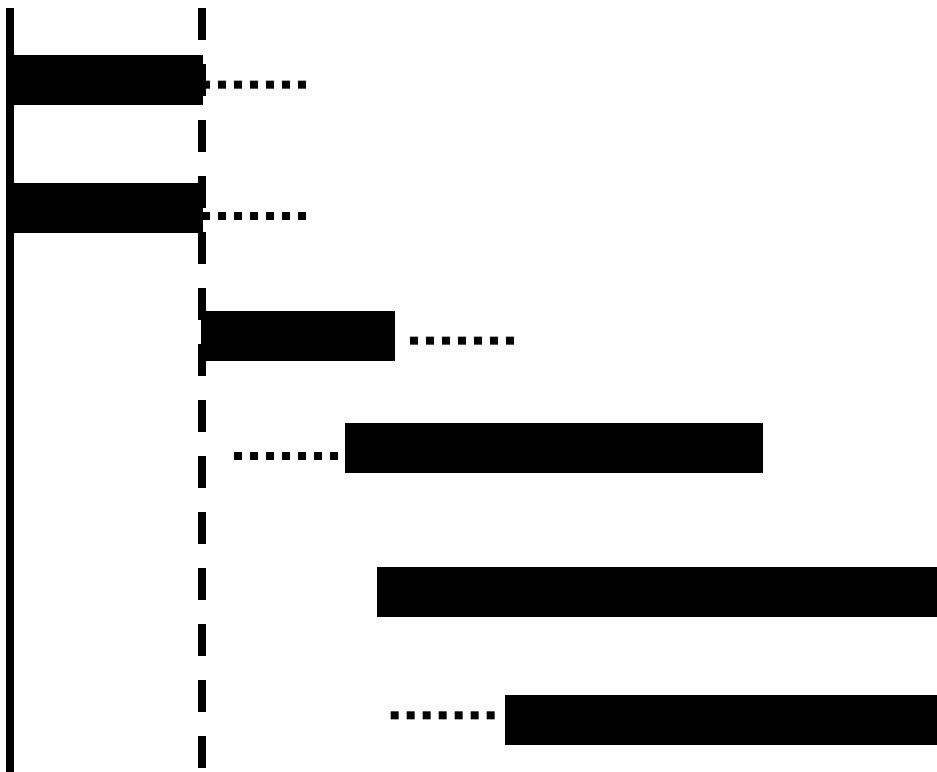
Pioneer saprotrophic fungi

Polymer-degrading fungi

Secondary opportunistic fungi

Degraders of recalcitrant compounds

Host death



Epiphytes

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



Image Courtesy of M. McGinnis
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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



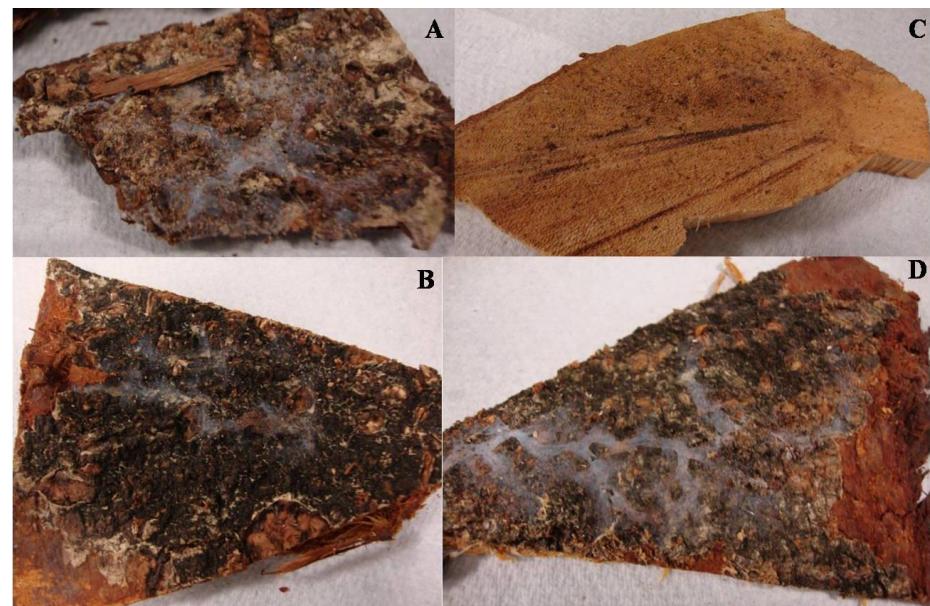
<http://www.anbg.gov.au/fungi/images/0122.jpg>

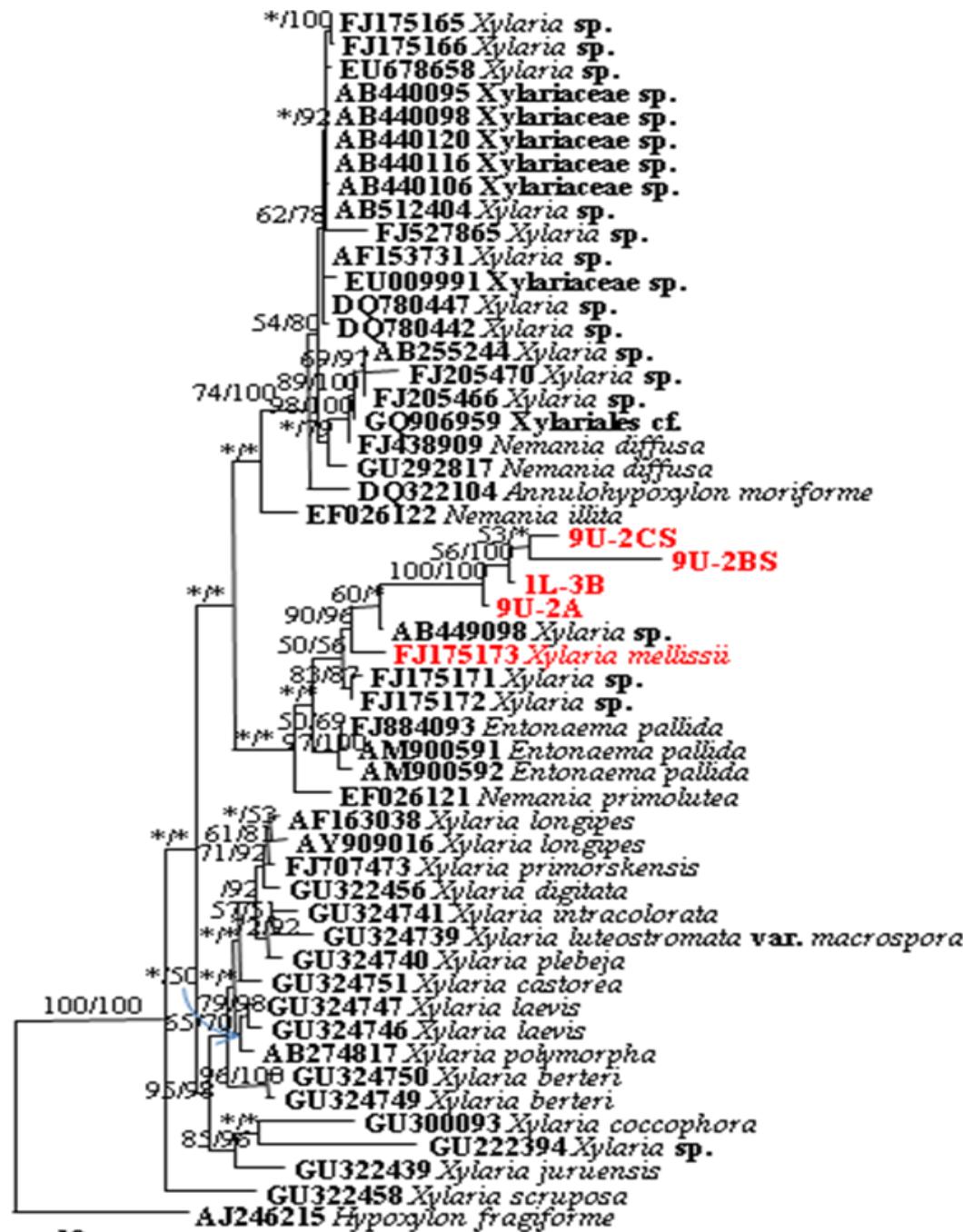
Wood Decay--Brown Rot

- 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

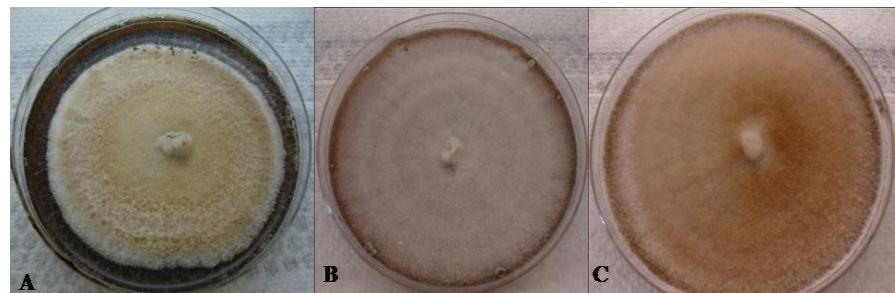


A Case Study





| | 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 |



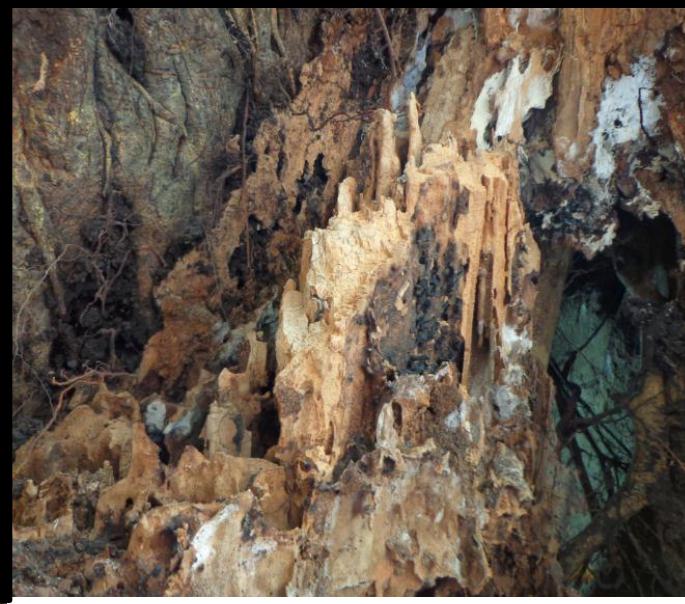
Photographs of *Nodulisporium* species obtained in this study.

A summary of fungal species obtained from the two sets of samples in this study

Lam Tsuen



Case study: Tree Branch





Infection of the Tree Branch



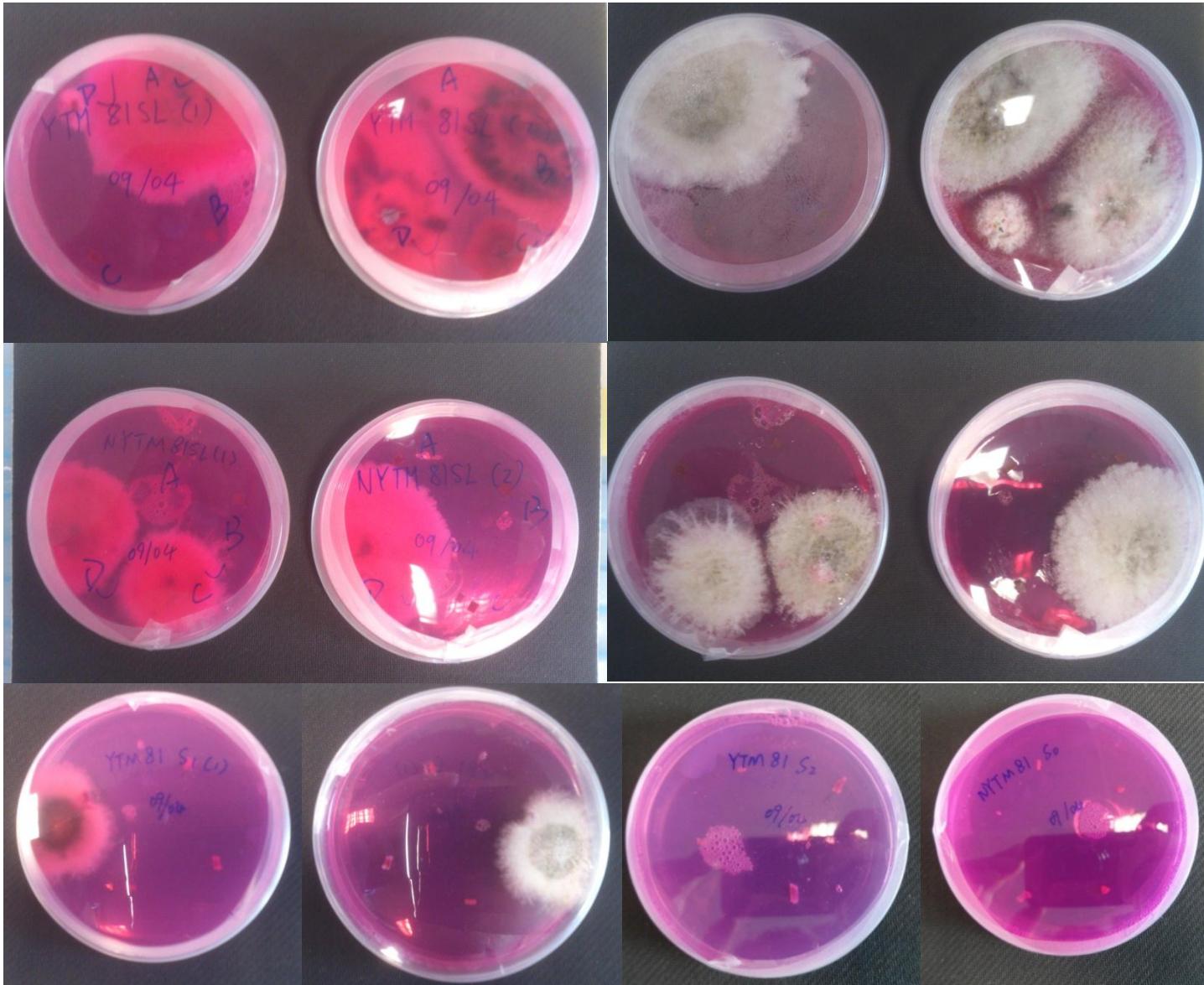
- A. *Hyphodontia flavipora*
- B. *Chondrostereum purpureum*
- C. *Physisporinus vitreus*
- D. *Hexagonia tenuis*

B

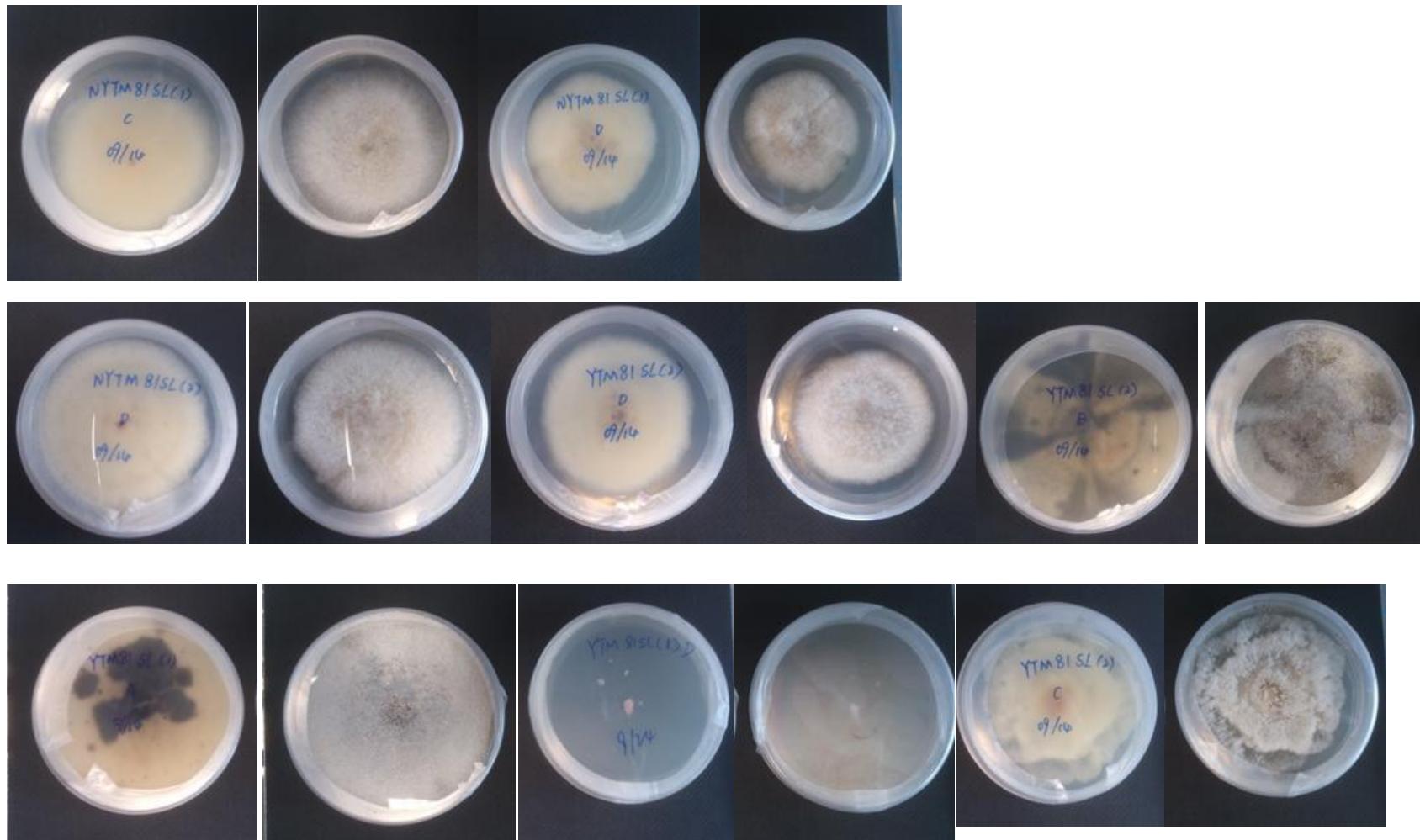
Further Study



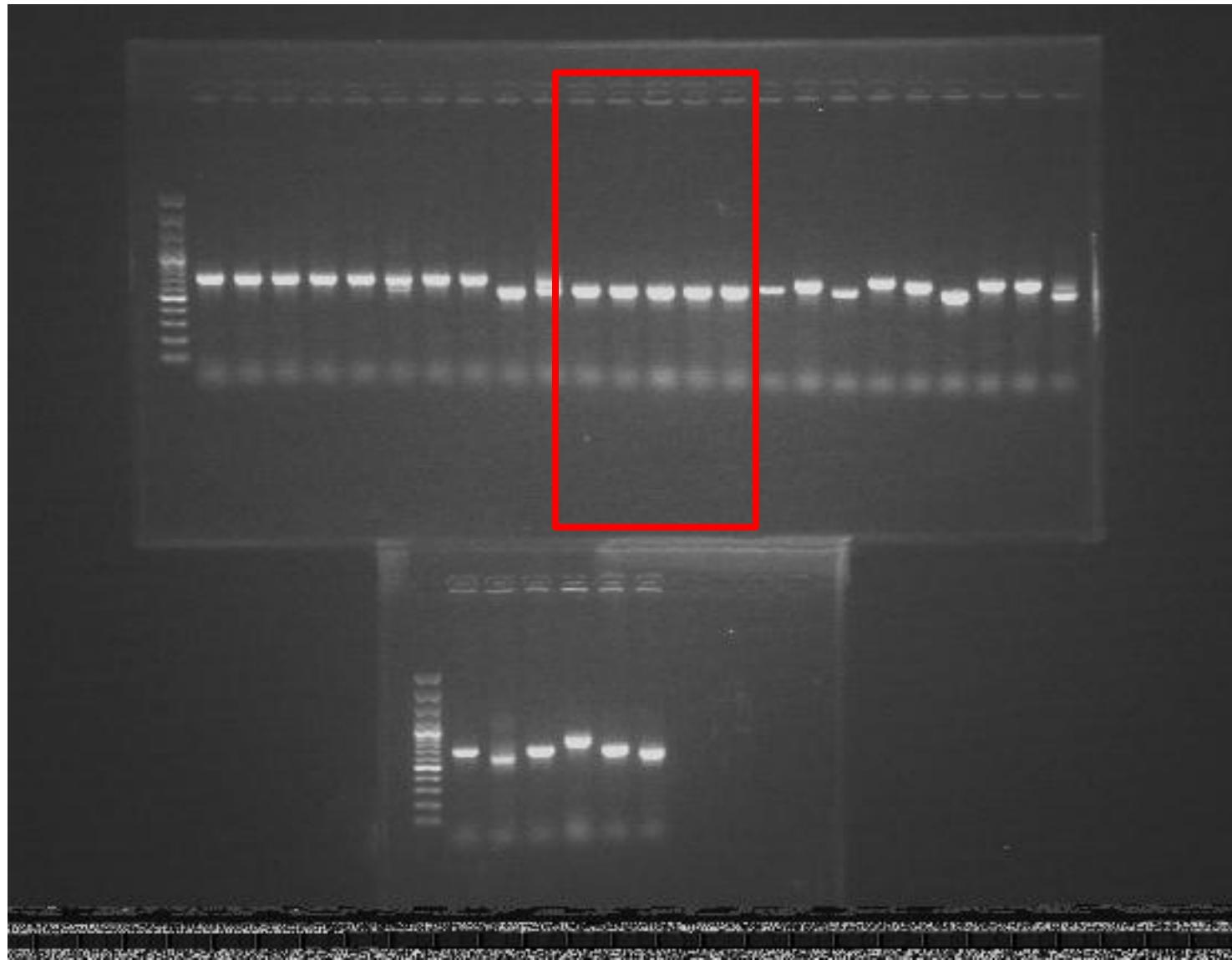
Isolation of fungi from local trees



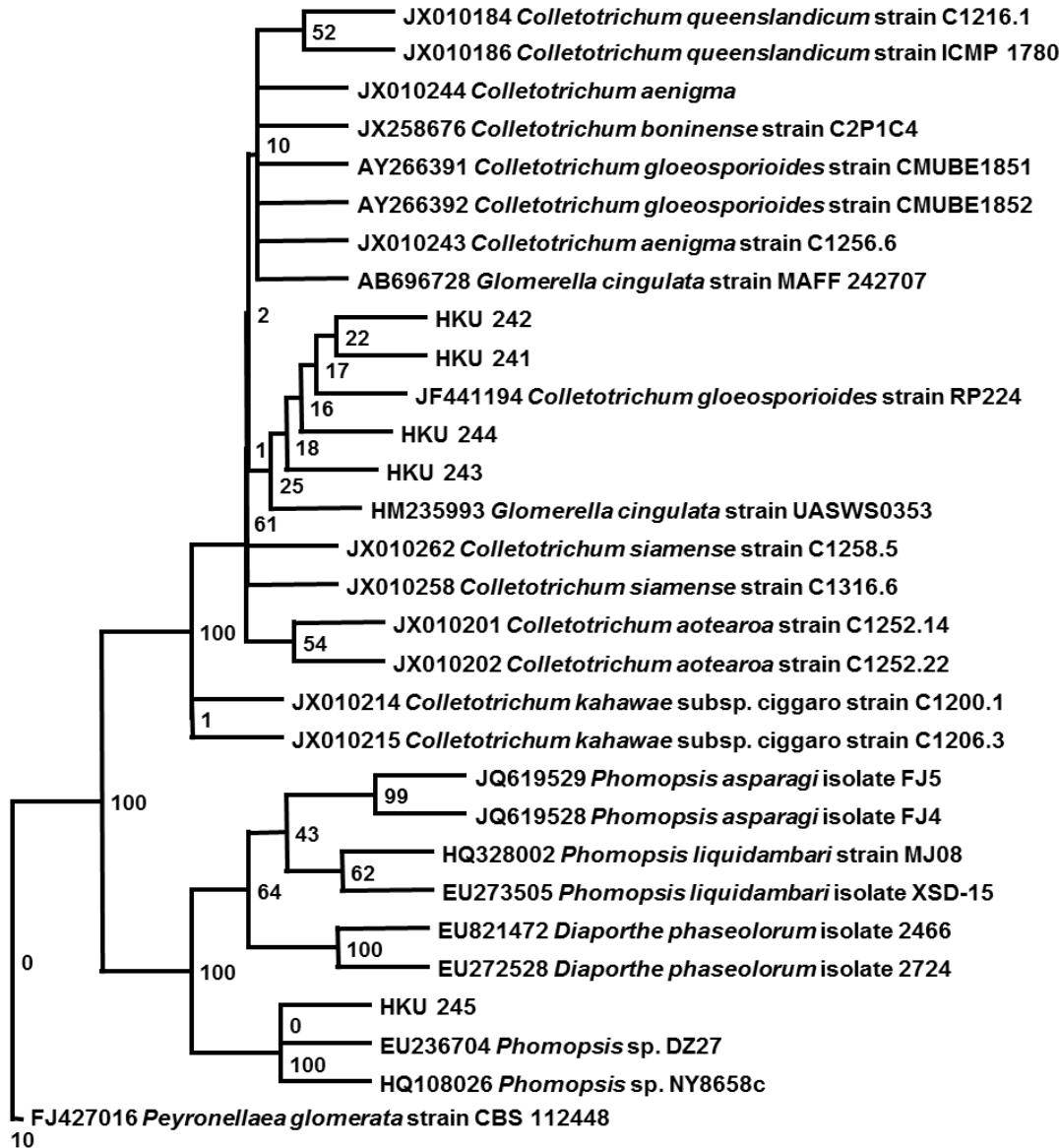
Isolation of fungi from local trees



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*

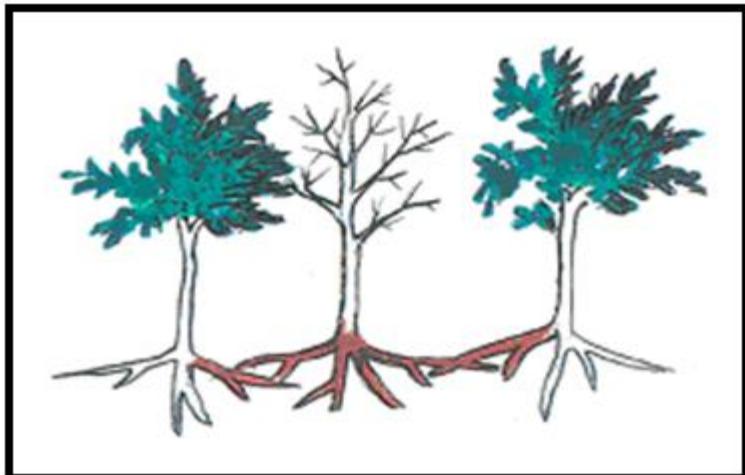
Host Ranges of *Phellinus noxius*

- 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.

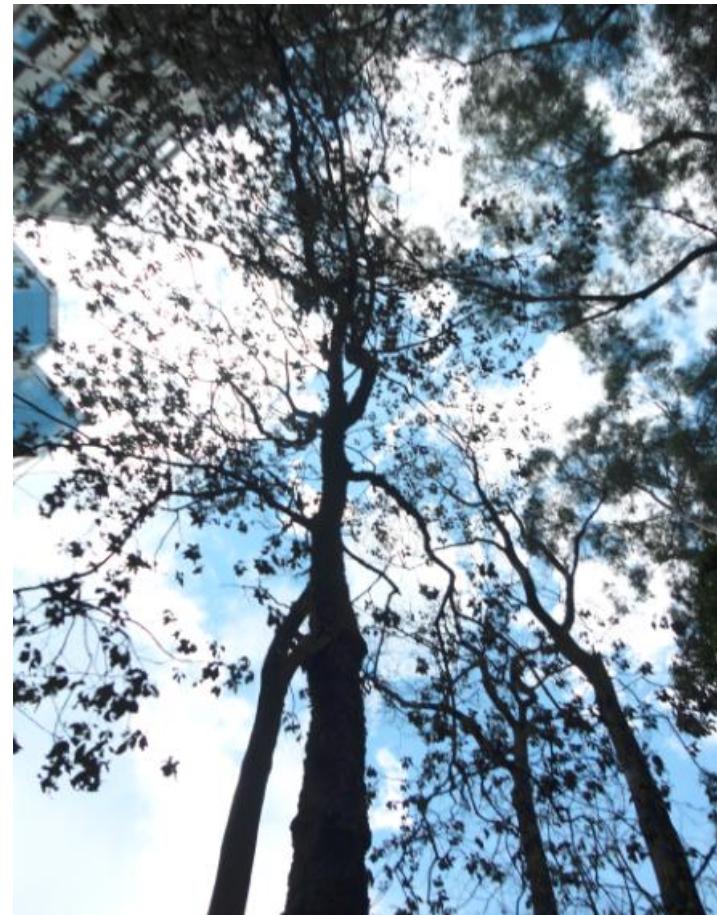
| Hosts | | Location |
|------------------------------|------|---|
| <i>Bombax ceiba</i> | 木棉 | Kwun Tong |
| <i>Celtis sinensis</i> | 朴 | Kwai Tsing |
| <i>Delonix regia</i> | 鳳凰木 | Eastern |
| <i>Ficus benjamina</i> | 垂葉榕 | Central & Western |
| <i>Ficus microcarpa</i> | 細葉榕 | Islands, Kwai Tsing, 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



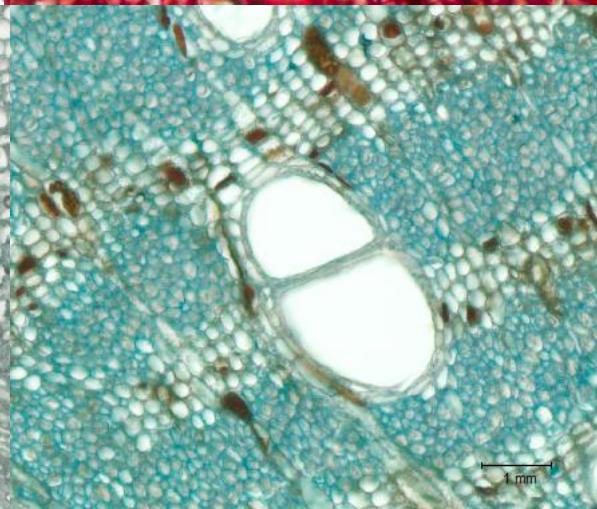
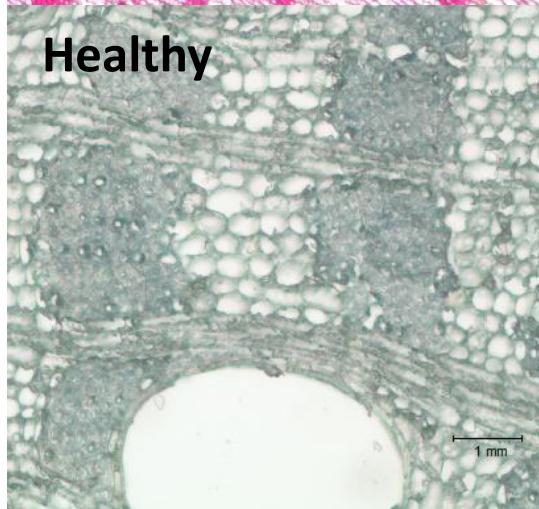
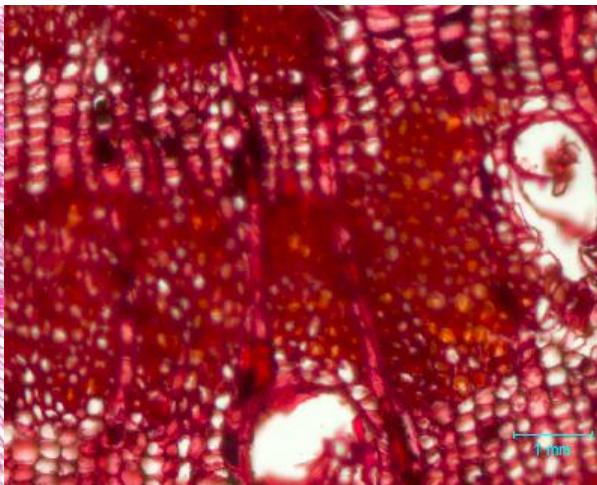
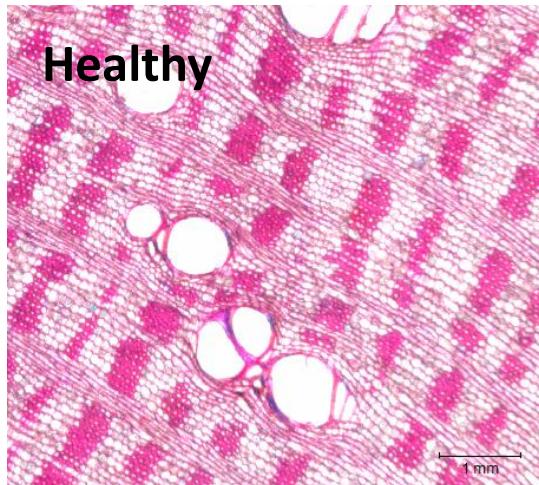
A 30-year-old flame tree (*Delonix regia*) in central Taiwan. A, 3 months before the appearance of quick decline; B, after the appearance of quick decline.
From Ann *et al* (2002)

Local Examples



Decay Caused *Phellinus noxius* at Microscopic Levels

- Lignin degradation indicated with staining under light microscope

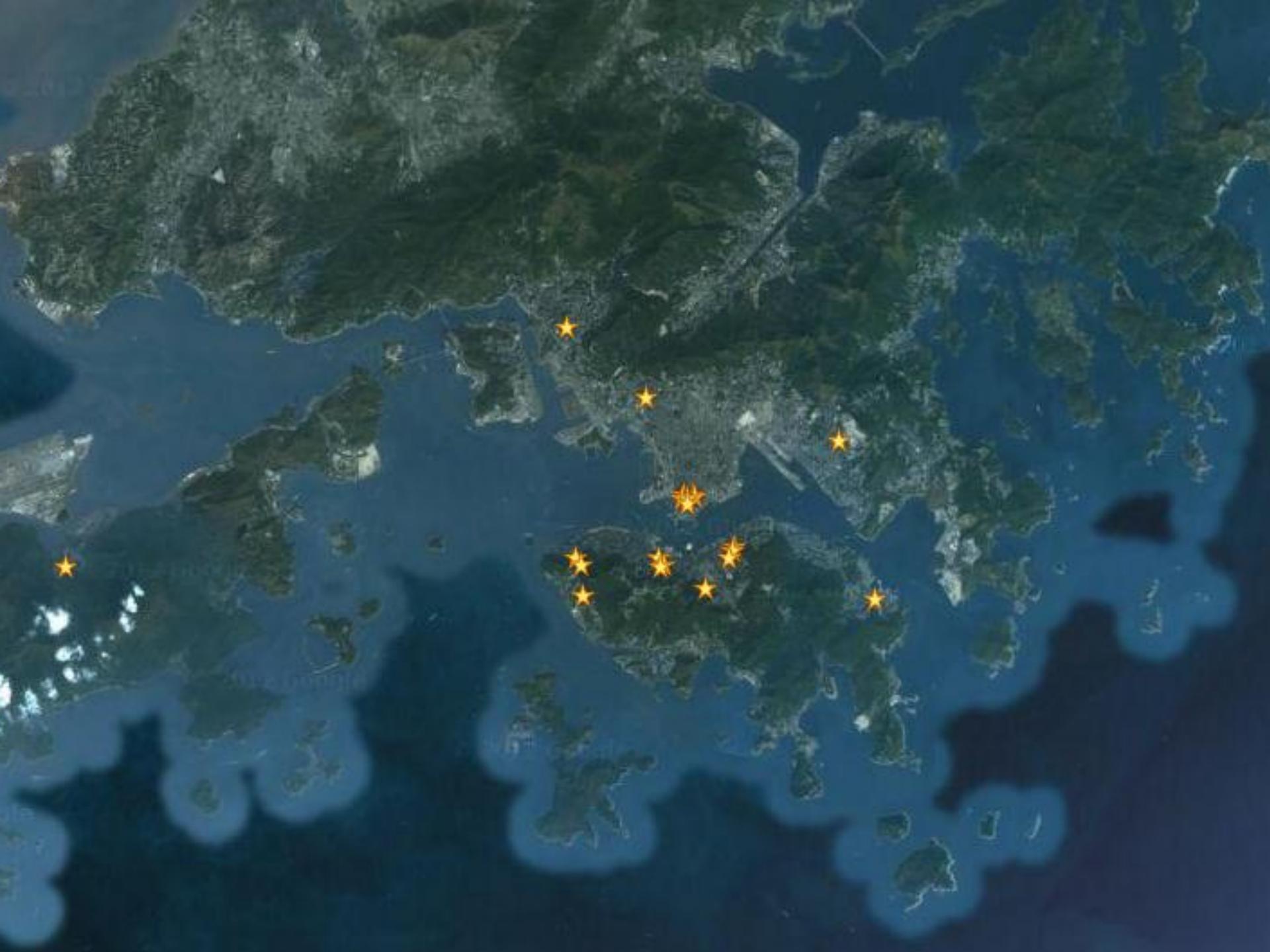


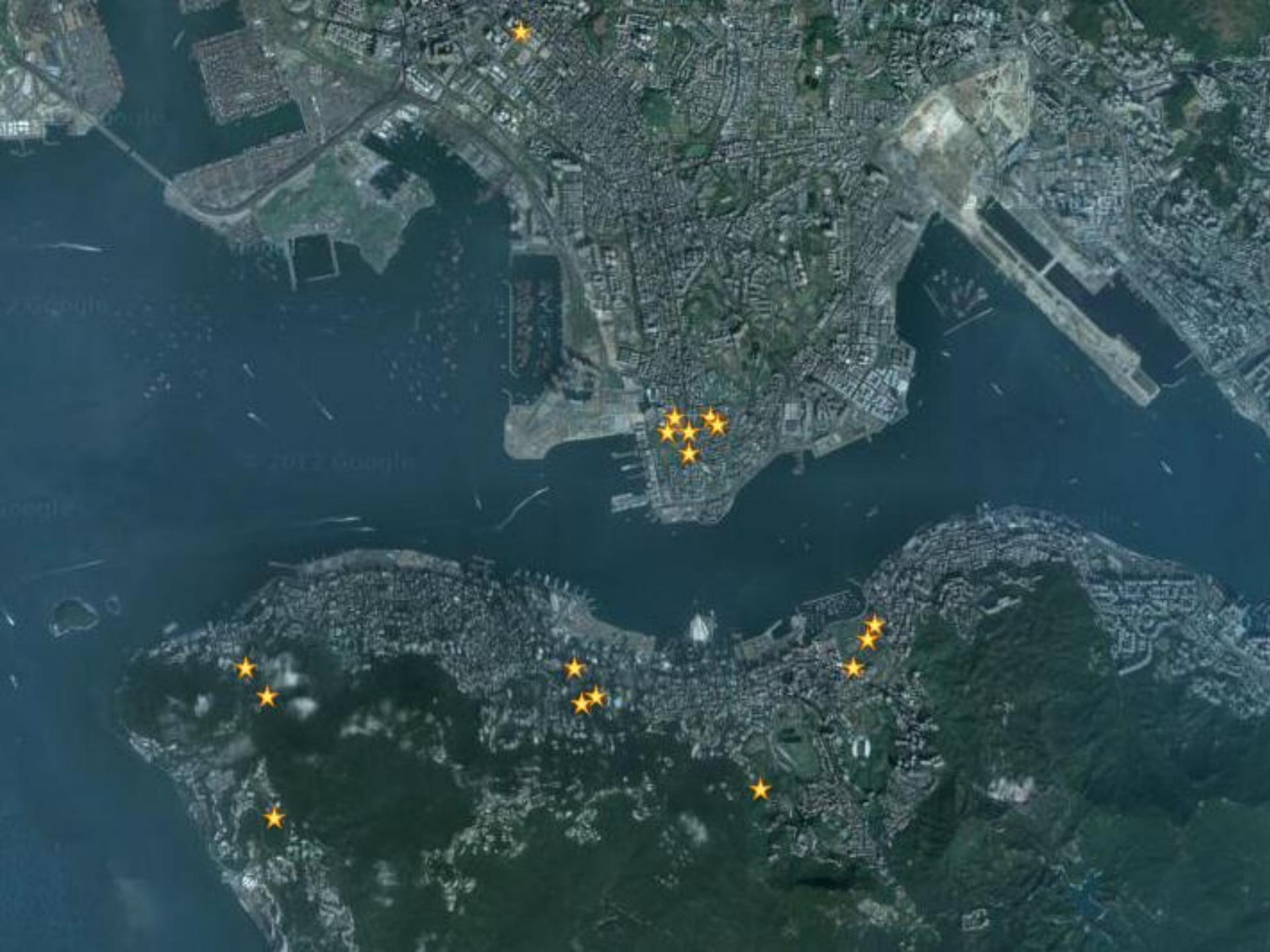
Distribution of Brown Root Rot Disease Caused by *Phellinus noxius*



BRR Caused by *Phellinus noxius* in Hong Kong



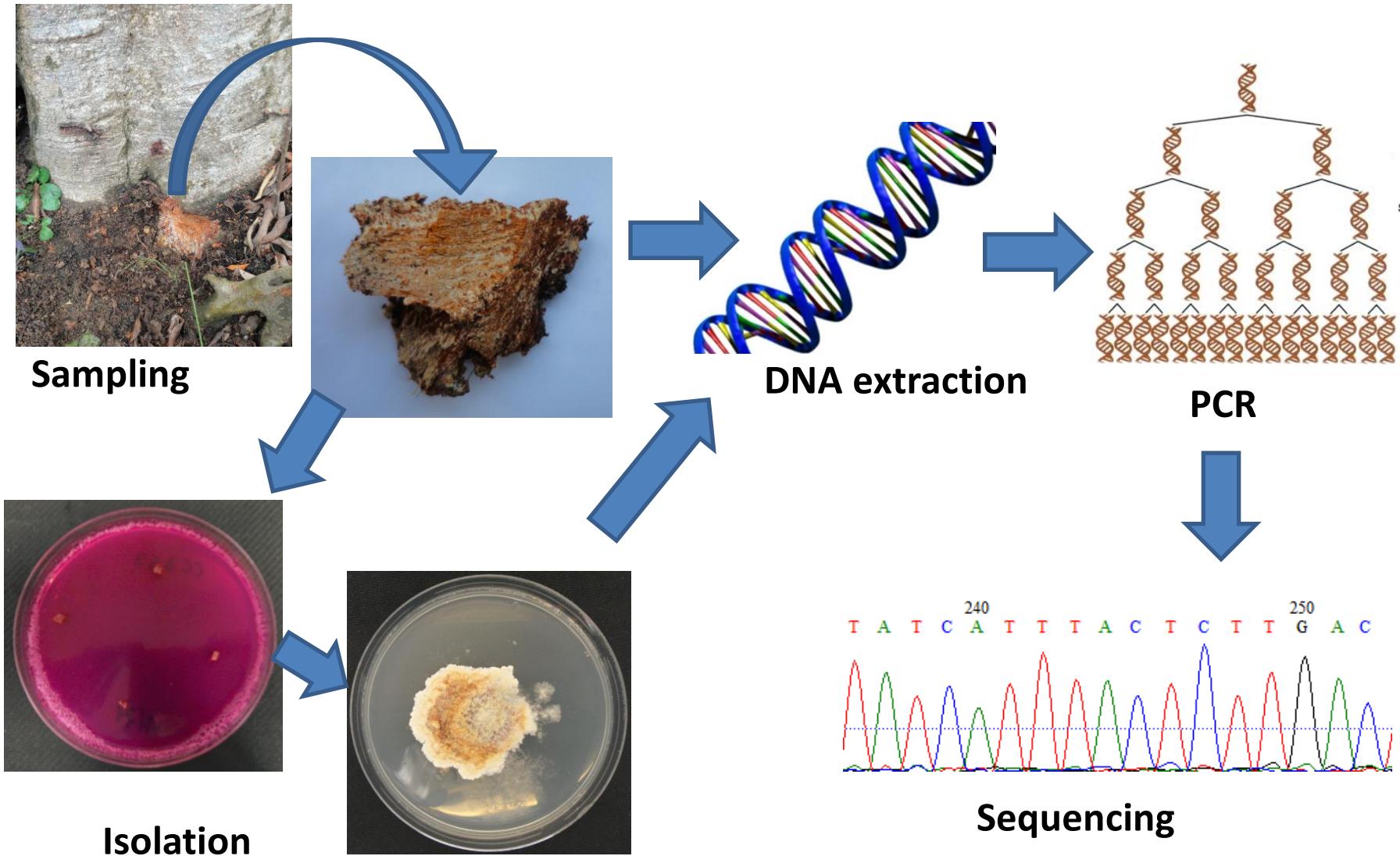




Laboratory Investigations of *P. noxius*

- 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*

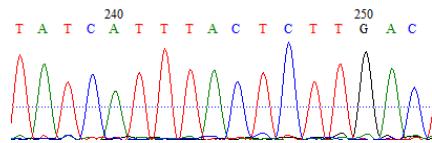
Lab Assay of *Phellinus noxius*



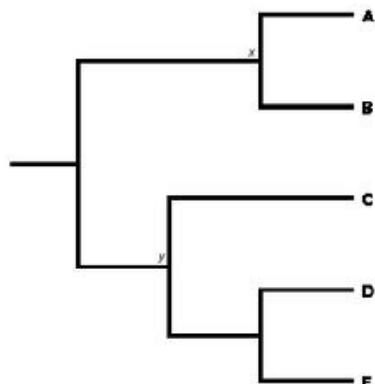
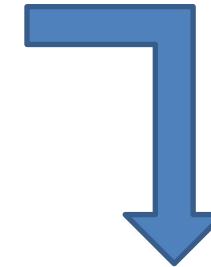
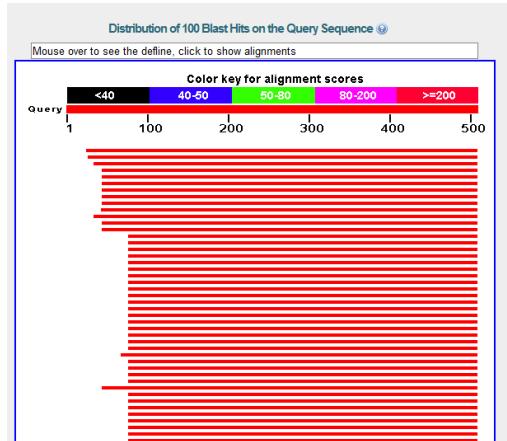
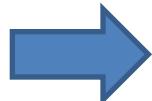
Isolates from Taiwan and Hong Kong



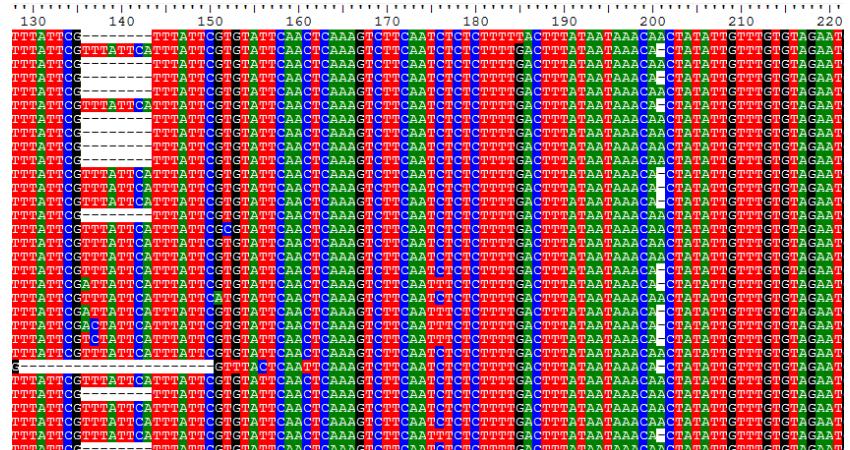
Lab Assay of *Phellinus noxius*



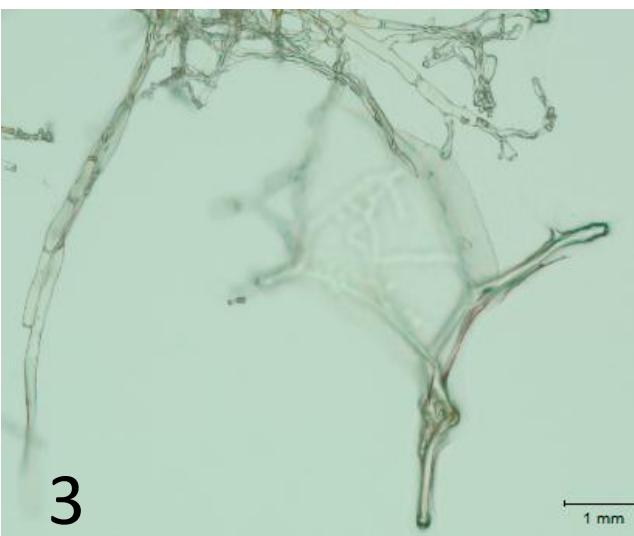
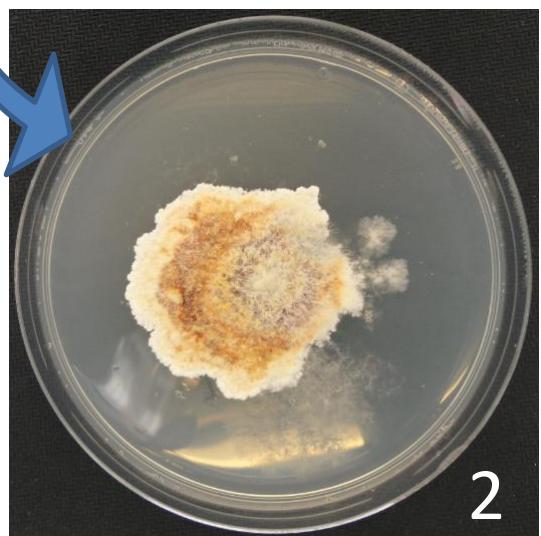
sequence



Phylogenetic analysis

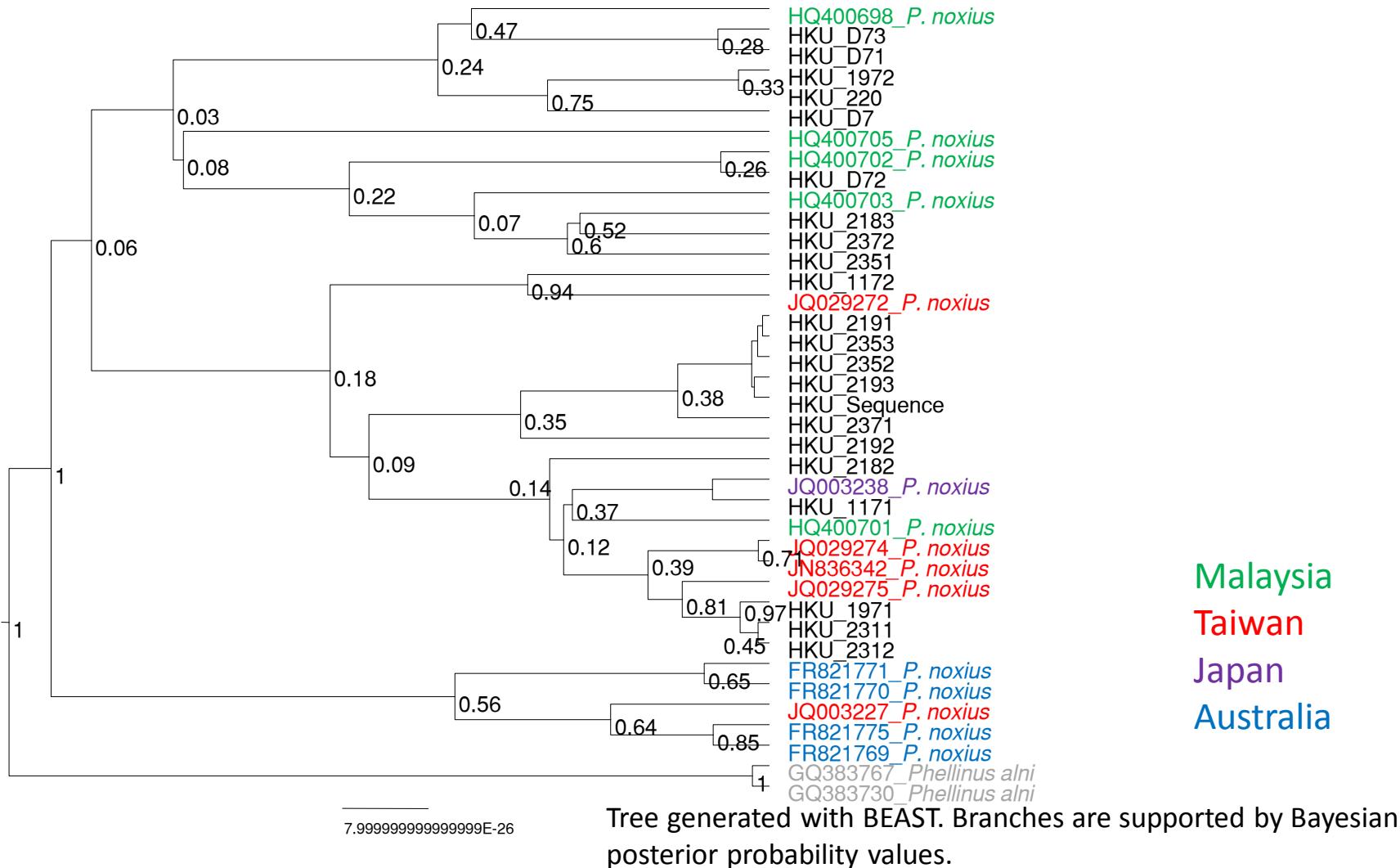


Biological Features: Mycelia on Different Scales

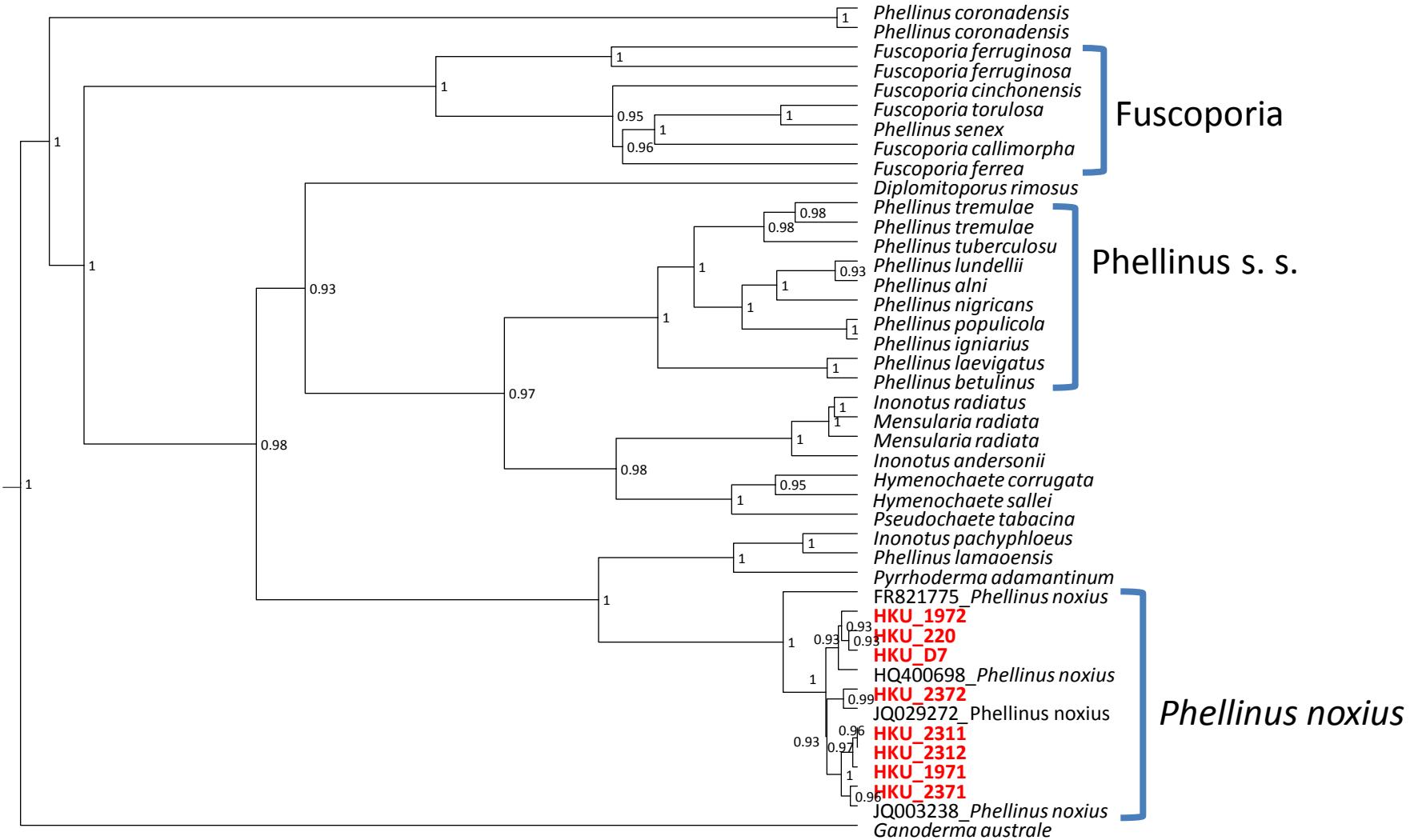


1. Mycelia on wood tissues show brown lines and cause white rot;
2. Mycelia on PDA culture, show irregular patches;
- 3 & 4. Mycelia under light microscope.

Phylogenetic Relationship among *P. noxius* from Different Regions



Classification of *P. noxius* Based on ITS



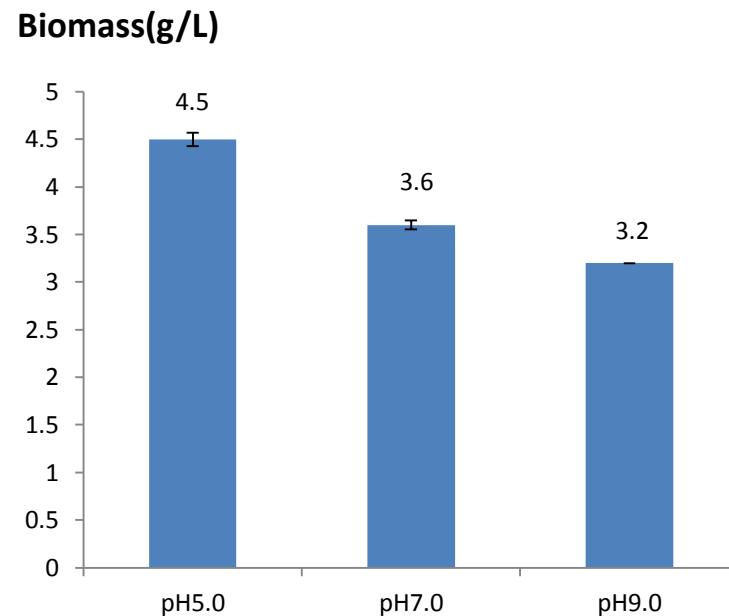
2.0E-21

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 at different pH level in Malt Extract Broth

Biomass pH 5.0

16.67g

Biomass pH 7.0

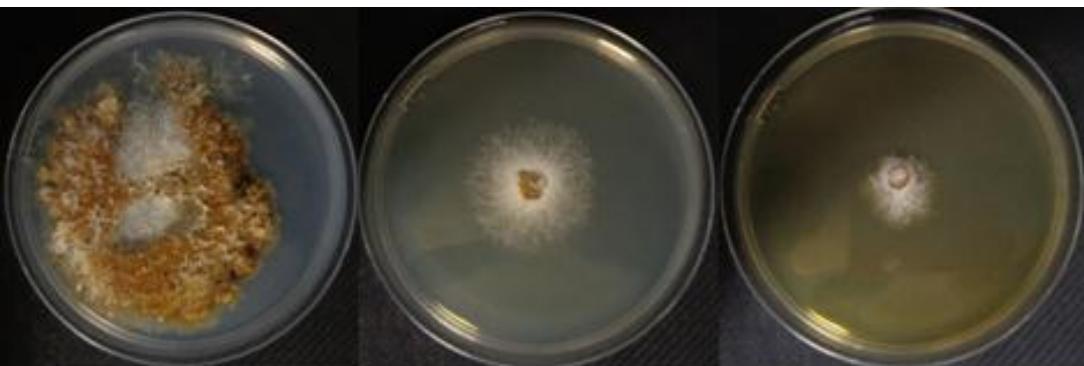
14.28g

Biomass pH 9.0

13.71g



Effect of different pH conditions on *Phellinus noxius*



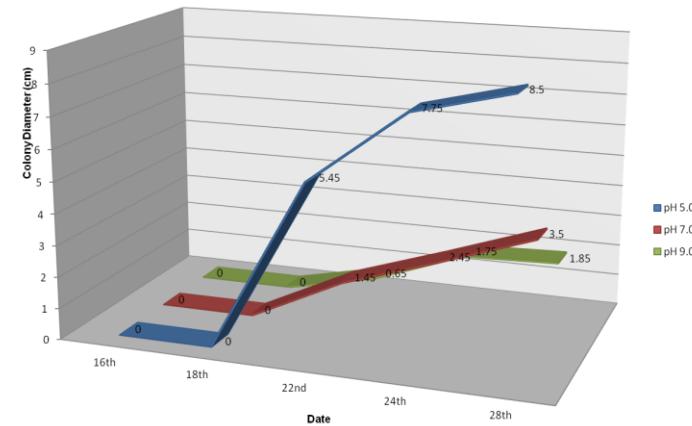
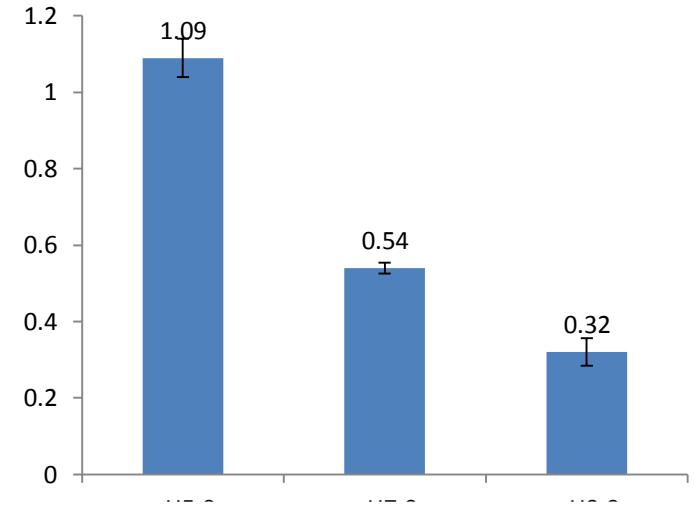
pH5.0

pH7.0

pH9.0

Phellinus noxius grown on Malt Extract Agar at different pH levels

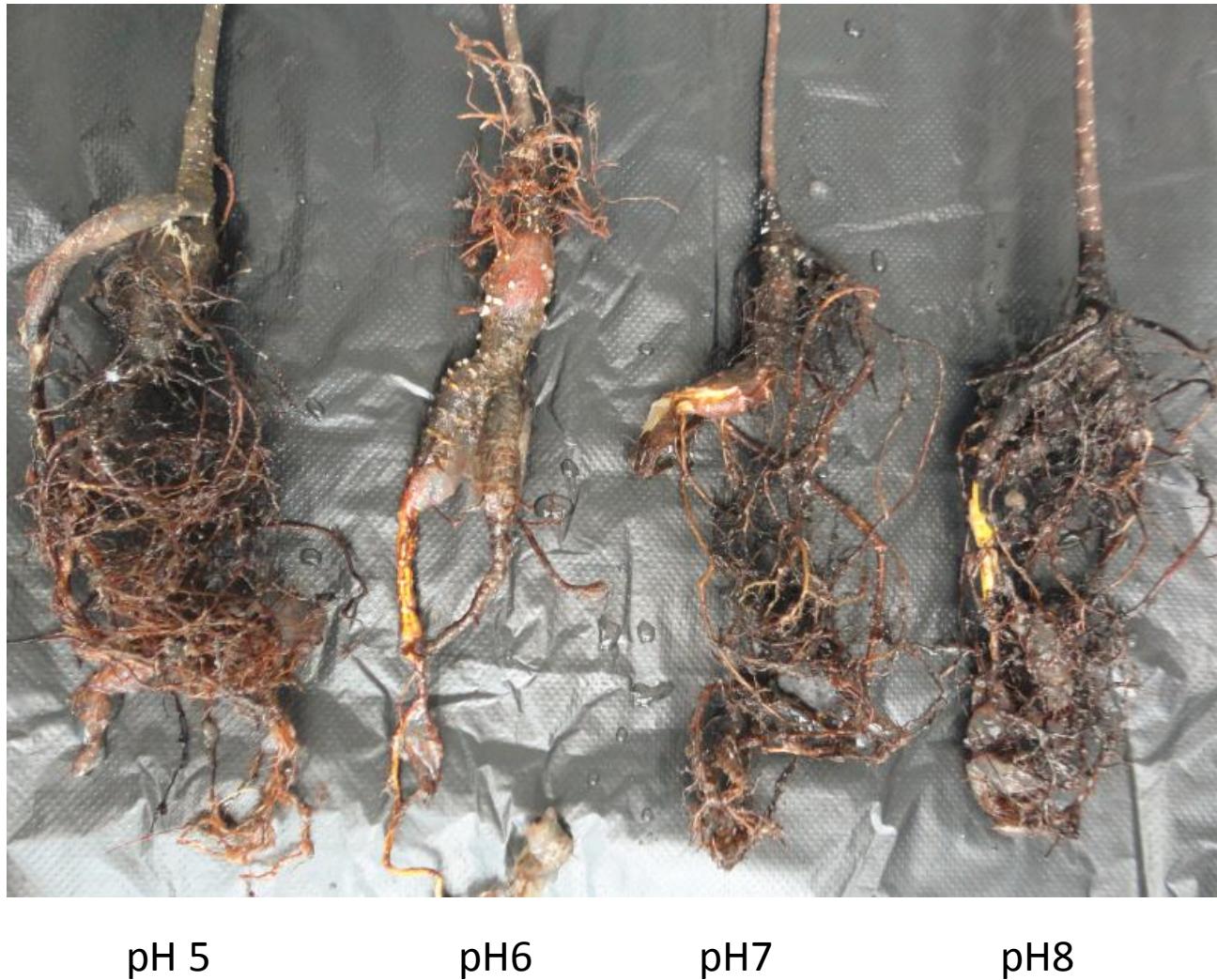
Growth Rate (cm/day)

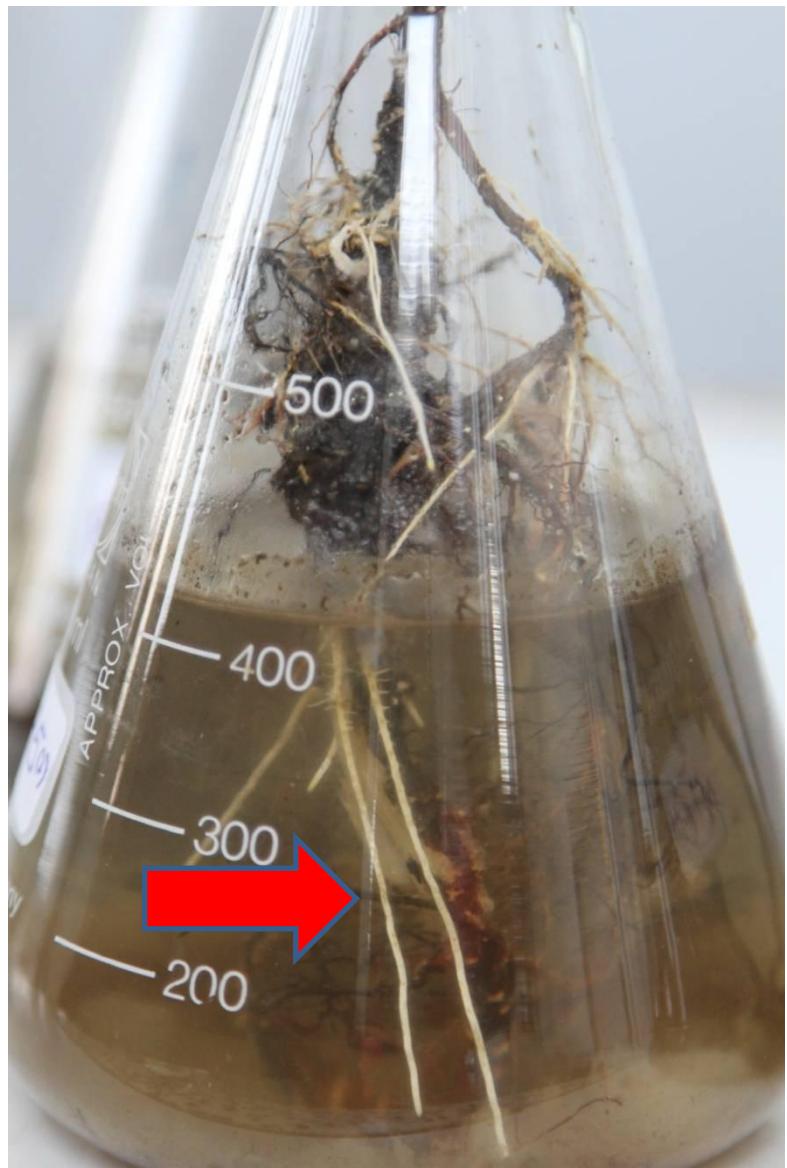


Growth rate on malt extract Agar at different pH levels



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

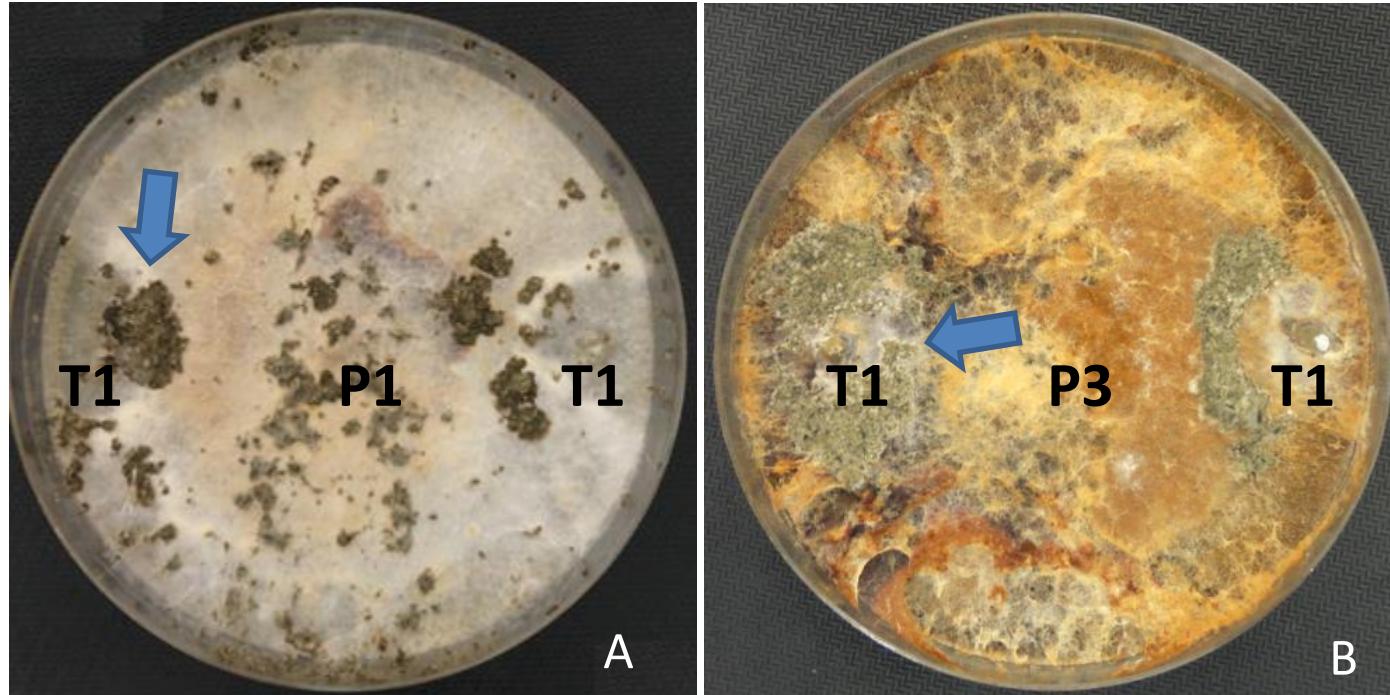




Biological Control

- 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.

