Genetic studies of *Phellinus noxius* - Insights for managing brown root rot disease

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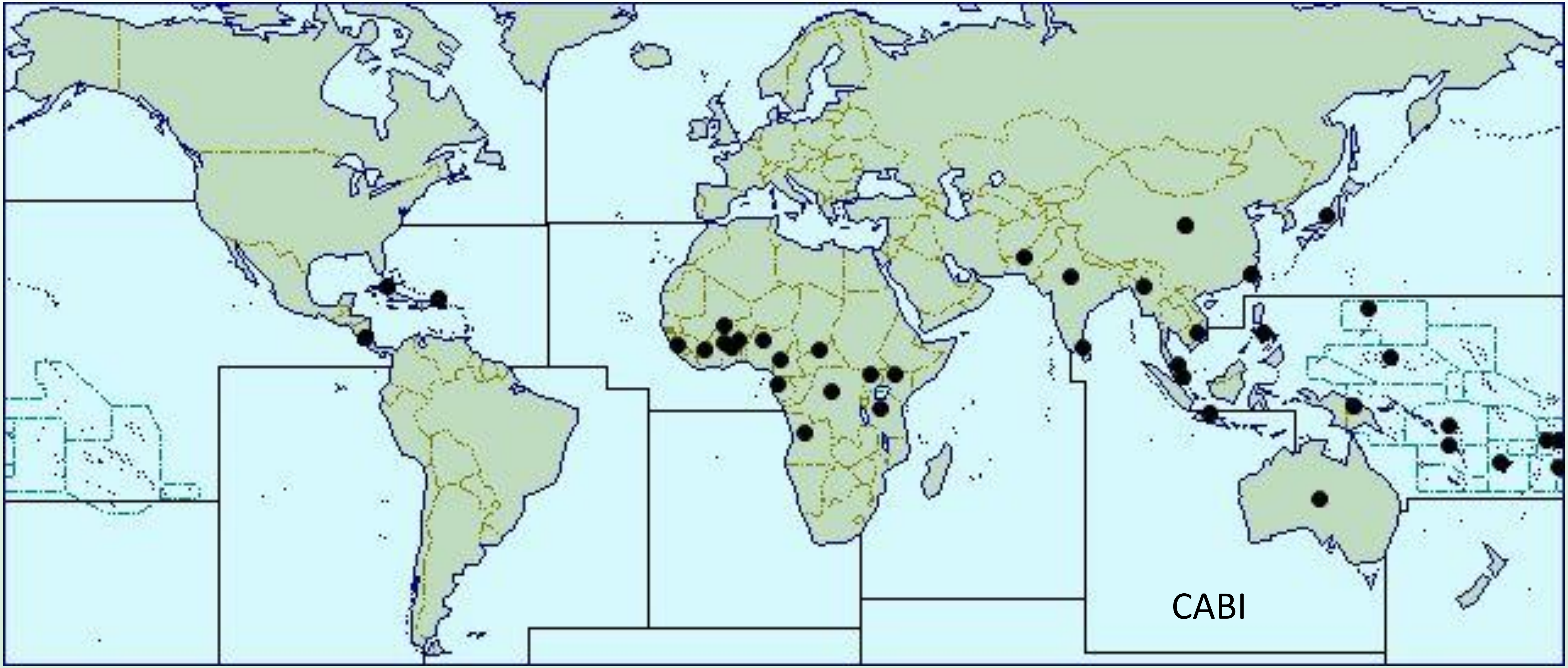
Reported global distribution of *Phellinus noxius*



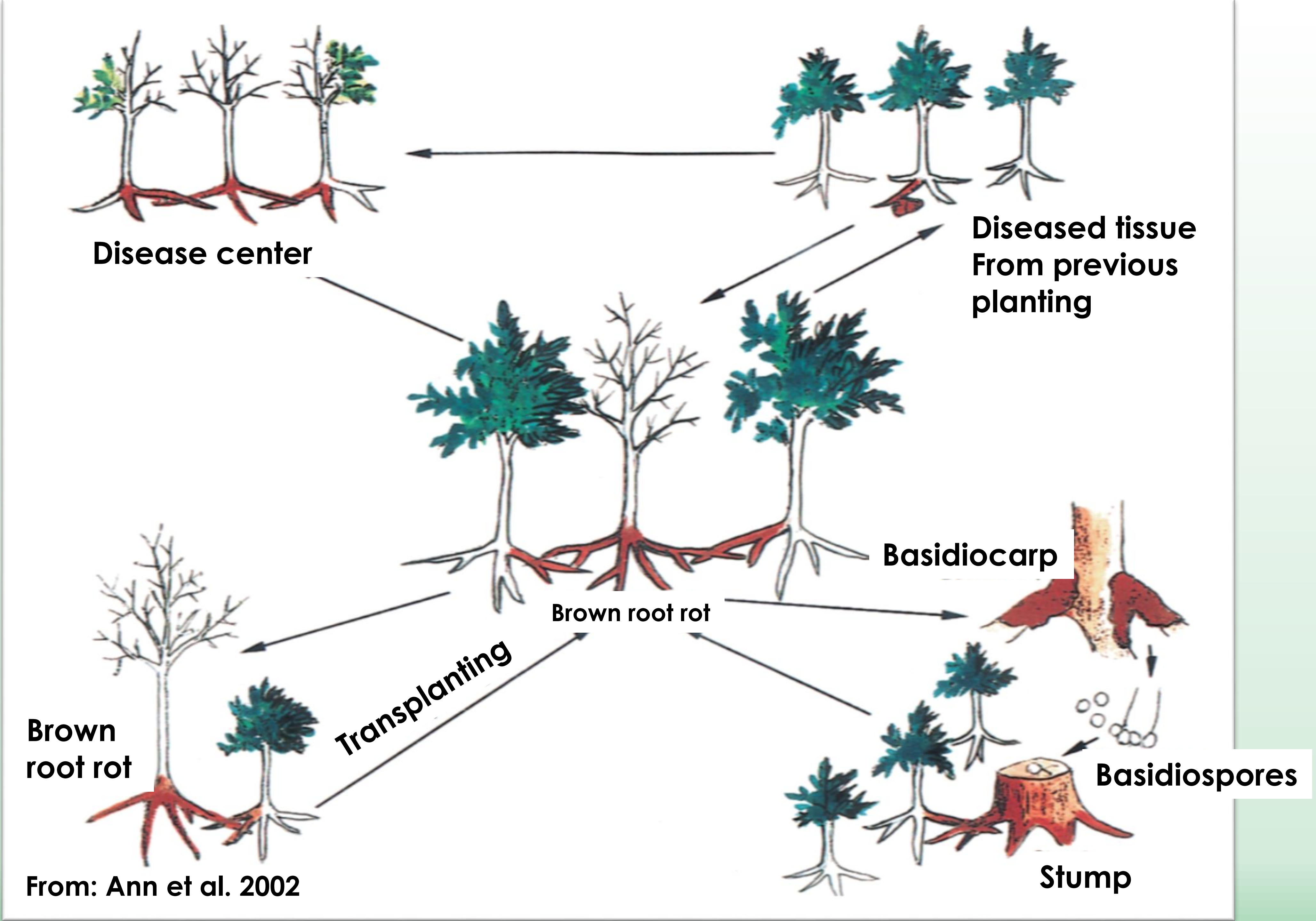
CABI

Wide host range of *Phellinus noxius*
~ 200 species from ~59 families

Example host species:

Araucariaceae - *Araucaria cunninghamii* (colonial pine)
Arecaceae - *Elaeis guineensis* (African oil palm)
Casuarinaceae - *Casuarina* equisetifolia (casuarina)
Clusiaceae - *Calophyllum inophyllum* (Alexandrian laurel); *Garcinia mangostana* (mangosteen)
Euphorbiaceae - *Hevea brasiliensis* (rubber)
Fabaceae - *Acacia confuse*; *Bauhinia variegata* (mountain ebony)
Meliaceae - *Swietenia mahagoni* (Cuban mahogany)
Lamiaceae - *Tectona grandis* (teak)
Moraceae - *Artocarpus altilis* (breadfruit); *Ficus microcarpa* (Indian laurel tree
Podocarpaceae - *Podocarpus macrophyllus* (Long-leaf podocarpus)
Rubiaceae - *Coffea* (coffee)
Salicaceae - *Salix babylonica* (weeping willow)
Sapindaceae - *Koelreuteria elegans*; *K. paniculata* (golden rain tree)
Sterculiaceae - *Theobroma cacao* (cocoa)
Theaceae - *Camellia sinensis* (tea)

Disease cycle of *Phellinus noxius*



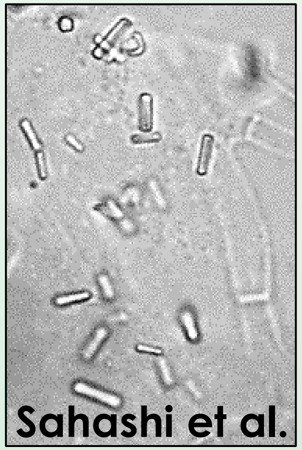
From: Ann et al. 2002

Ann, P.-J.; Chang, T.-T.;
Ko, W.-H. 2002.
Phellinus noxius brown root rot of fruit and ornamental trees in Taiwan.
Plant Disease 86: 820-826.

Dispersal and survival of *Phellinus noxius*

Ota et al.

Basidospores allow some dispersal and sexual recombination

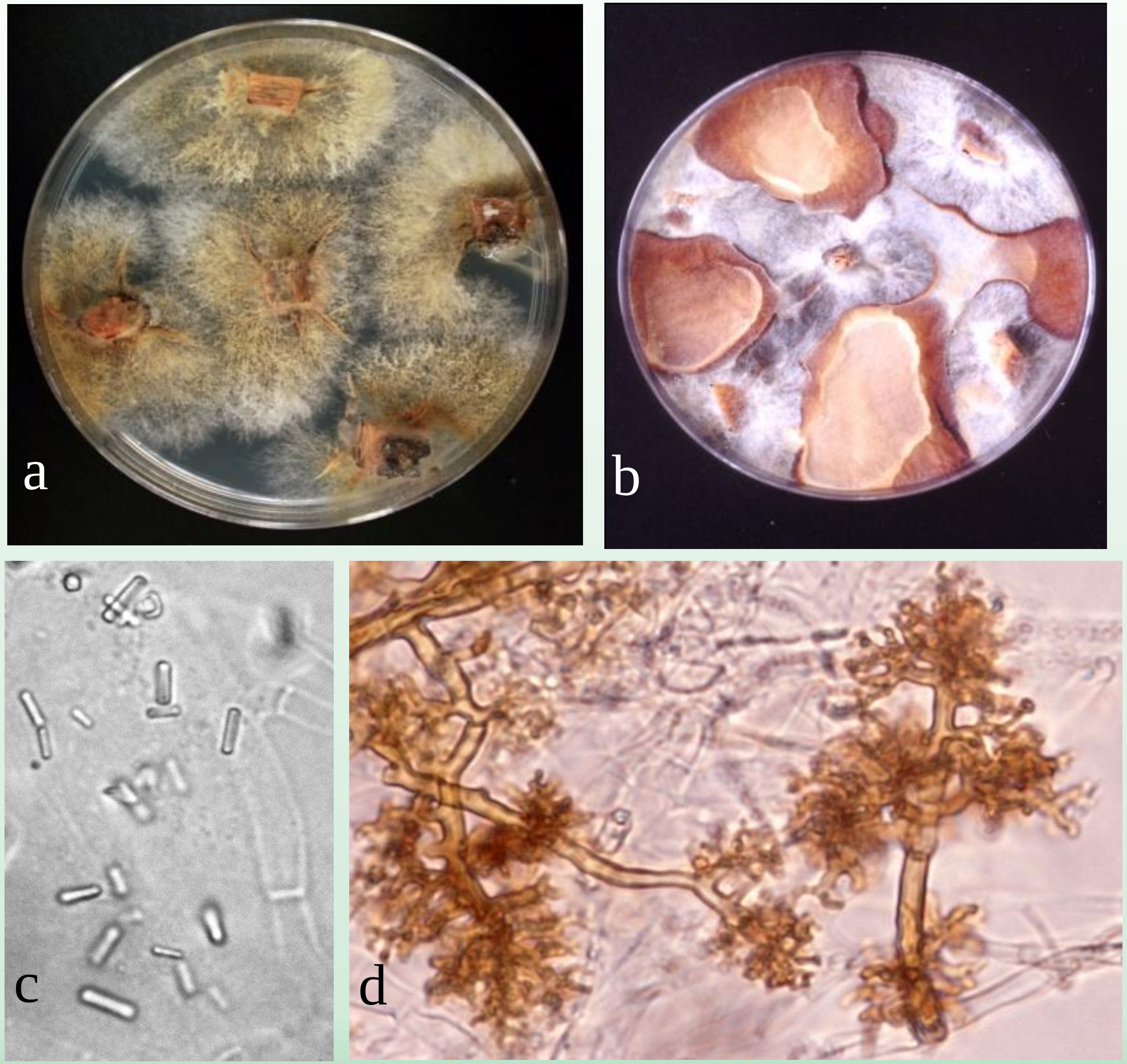


Sahashi et al.

Vegetative arthrocondia may contribute to survival and/or dispersal

P. noxius **can survive in dead roots for >10 years (Chang 1996)**

Cultural characteristics of *Phellinus noxius*

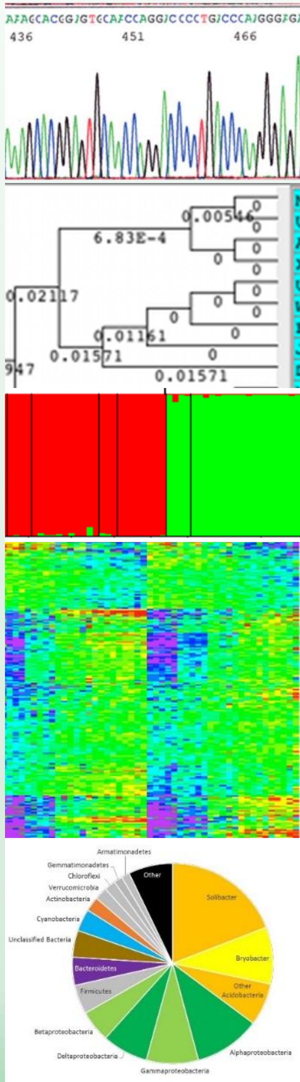


- **(a) Colonies growing up from small pieces of decayed wood.**
- **(b) Colony morphology on potato sucrose agar (PSA) with irregular dark brown patches.**
- **(c) Arthroconidia produced on PSA.**
- **(d) Staghorn-like hyphae produced on PSA.**

From: Norio Sahashi

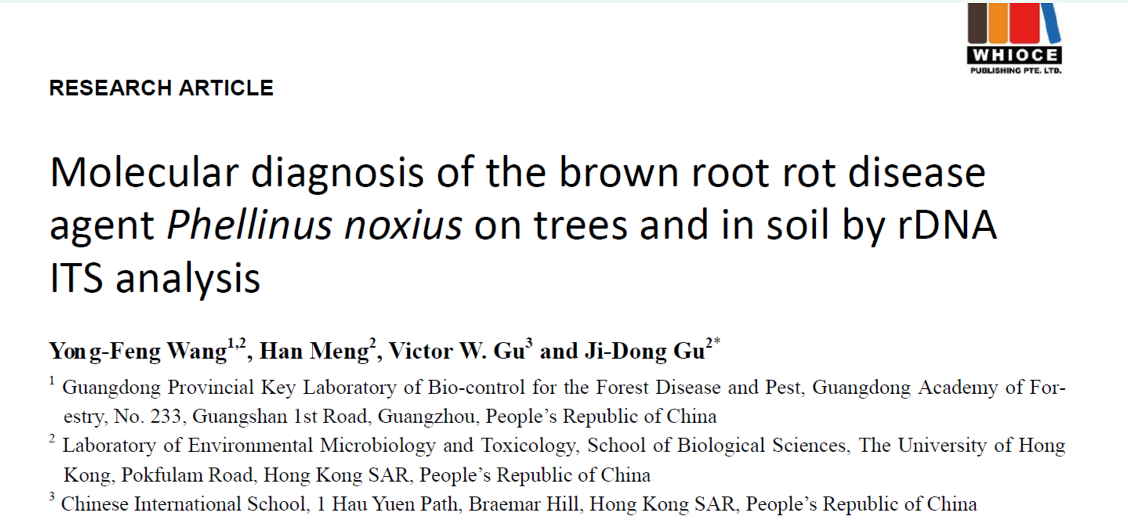
Genetic studies of *Phellinus noxius*

- **DNA-based diagnostics can identify *P. noxius***
- **Phylogenetic studies can determine genetic groups within *P. noxius***
- **Population genetic studies can determine gene flow and movement of *P. noxius***
- **Genomic/transcriptomic studies provide insights into pathogenicity genes and other genes of interest**
- **Soil metagenomic/metatranscriptomic studies examine soil levels of *P. noxius,* biocontrol agents, and other soil microbes and their interactions with environmental factors**



DNA sequence-based diagnostics are available to identify *Phellinus noxius* from wood and soil samples

(Wang et al. 2016; Tzean et al. 2016)



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RESEARCH ARTICLE

Molecular diagnosis of the brown root rot disease agent *Phellinus noxius* on trees and in soil by rDNA ITS analysis

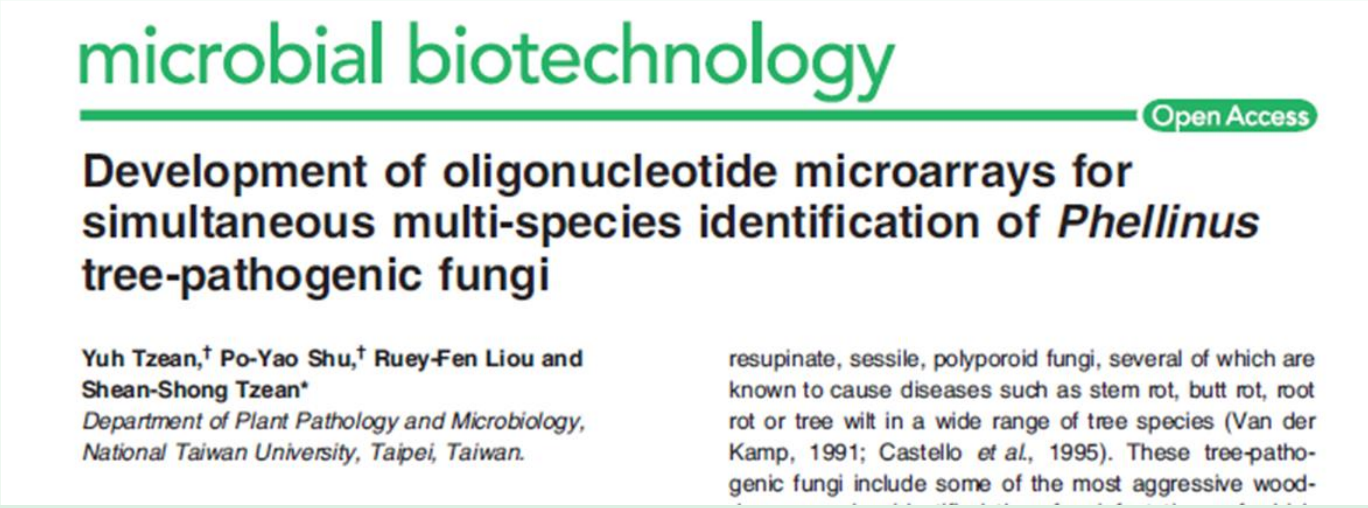
Yong-Feng Wang[1,2], Han Meng[2], Victor W. Gu[3] and Ji-Dong Gu[2*]

[1] Guangdong Provincial Key Laboratory of Bio-control for the Forest Disease and Pest, Guangdong Academy of Forestry, No. 233, Guangshan 1st Road, Guangzhou, People's Republic of China

[2] Laboratory of Environmental Microbiology and Toxicology, School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, People's Republic of China

[3] Chinese International School, 1 Hau Yuen Path, Braemar Hill, Hong Kong SAR, People's Republic of China

Applied Environmental Biotechnology (2016) 1: 81–91.



microbial biotechnology

Open Access

Development of oligonucleotide microarrays for simultaneous multi-species identification of *Phellinus* tree-pathogenic fungi

Yuh Tzean,† Po-Yao Shu,† Ruey-Fen Liou and Shean-Shong Tzean*

Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan.

resupinate, sessile, polyporoid fungi, several of which are known to cause diseases such as stem rot, butt rot, root rot or tree wilt in a wide range of tree species (Van der Kamp, 1991; Castello *et al.*, 1995). These tree-pathogenic fungi include some of the most aggressive wood-

Microbial Biotechnology (2016) 9: 235-244.

Population genetics: Examining mechanisms of *Phellinus noxius* spread in Taiwan (Chung et al. 2015)

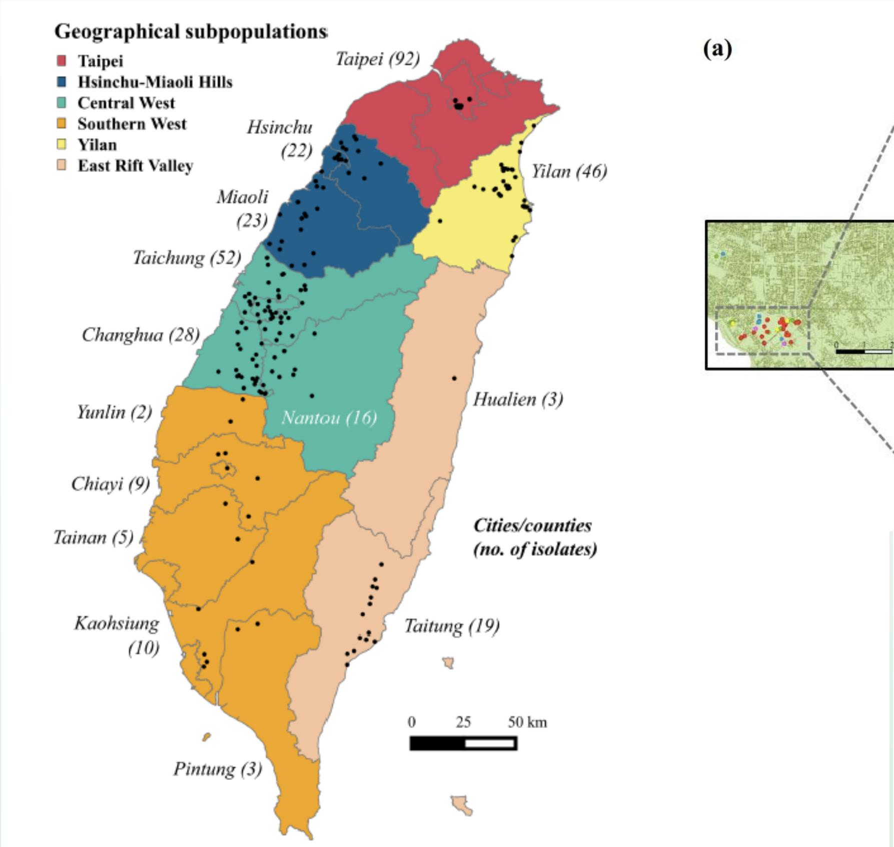
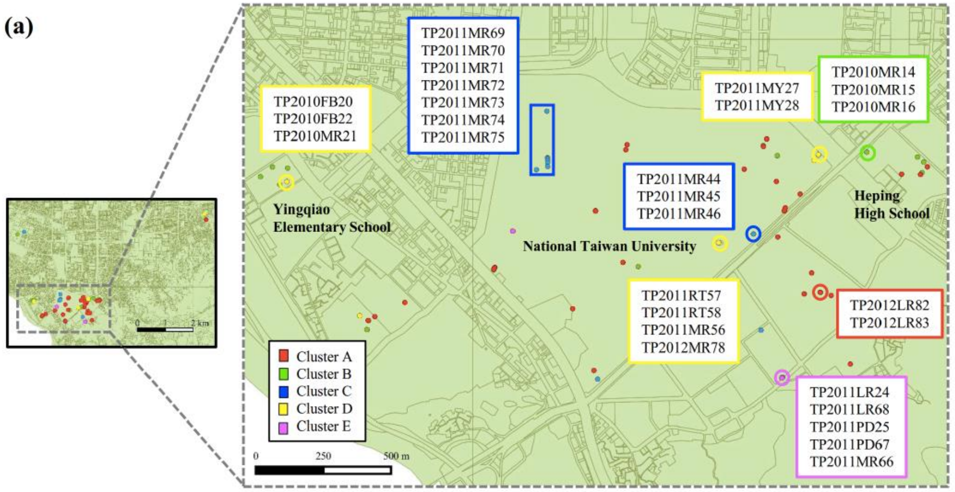
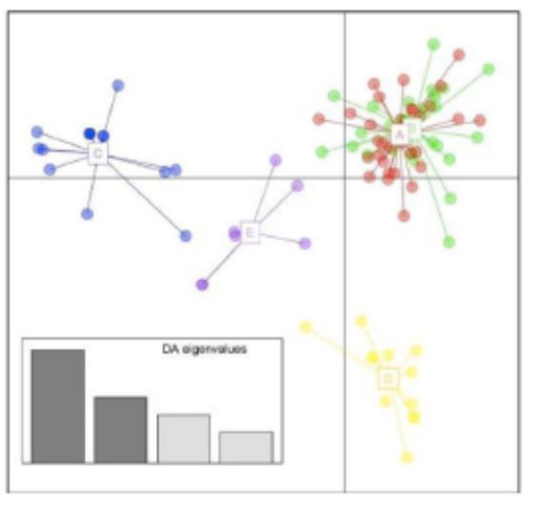
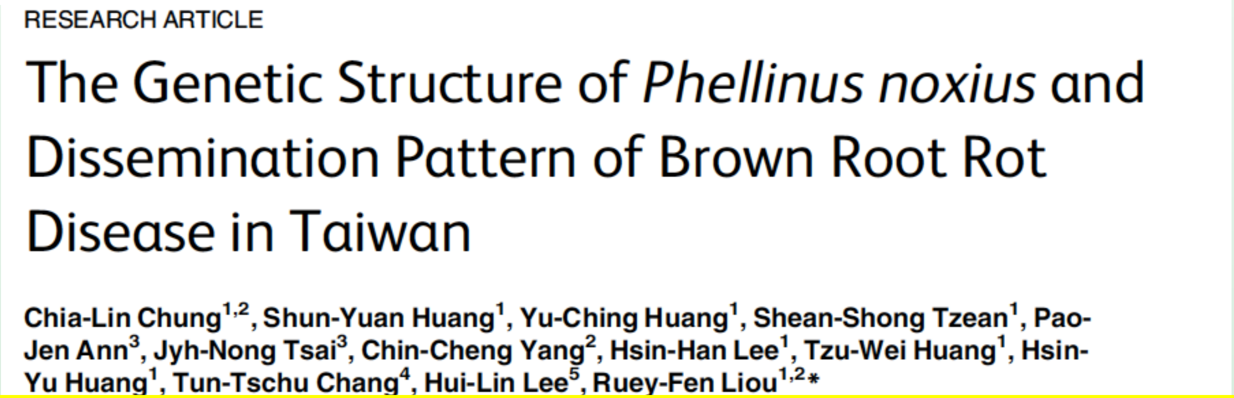


Fig 1. A total of 3291 isolates of *Phellinus noxius* were sampled from 14 cities/counties in Taiwan. The cities/counties and the numbers of *P. noxius*







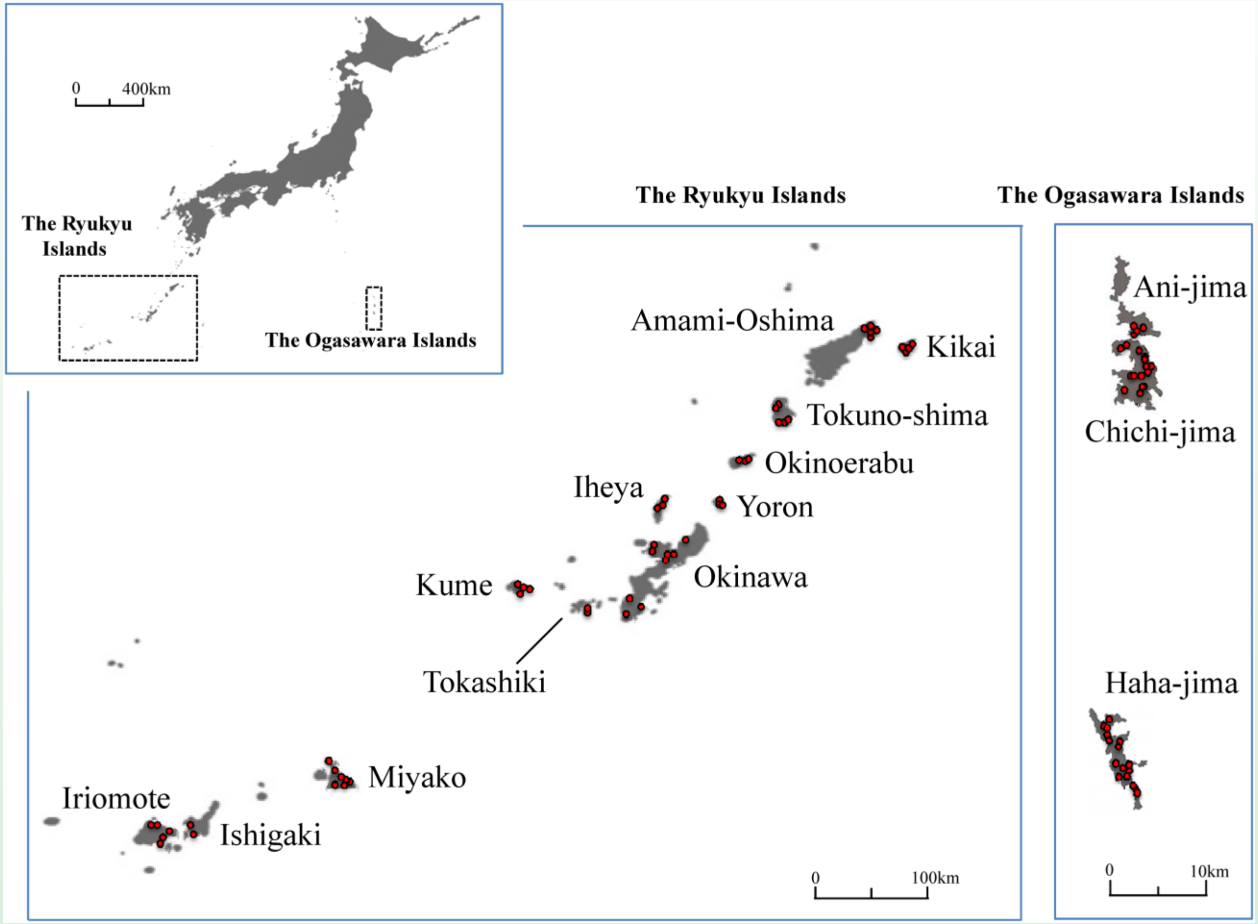
RESEARCH ARTICLE

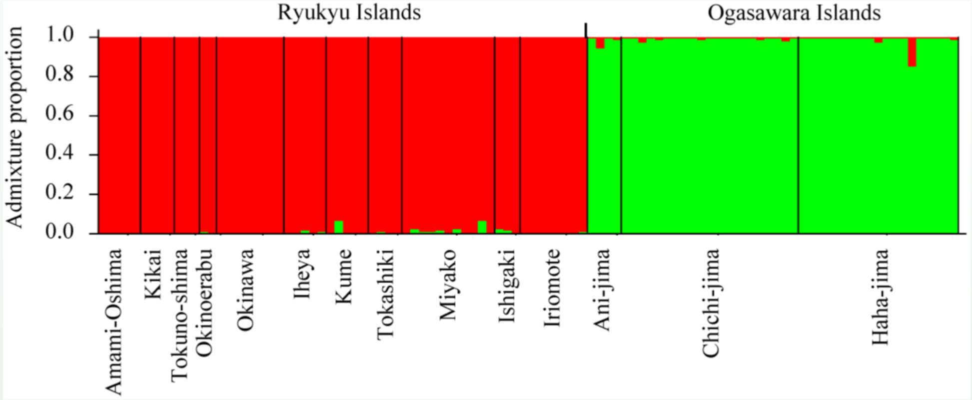
The Genetic Structure of *Phellinus noxius* and Dissemination Pattern of Brown Root Rot Disease in Taiwan

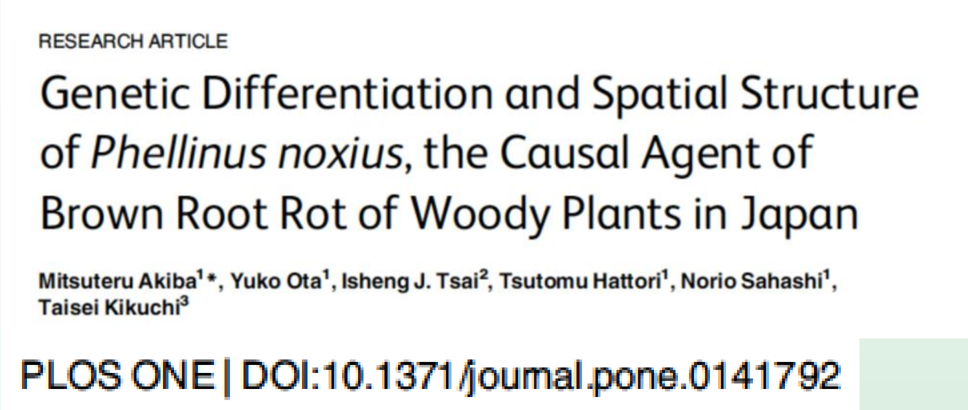
Chia-Lin Chung[1,2], Shun-Yuan Huang[1], Yu-Ching Huang[1], Shean-Shong Tzean[1], Pao-Jen Ann[3], Jyh-Nong Tsai[3], Chin-Cheng Yang[2], Hsin-Han Lee[1], Tzu-Wei Huang[1], Hsin-Yu Huang[1], Tun-Tschu Chang[4], Hui-Lin Lee[5], Ruey-Fen Liou[1,2]*

Tree-to-tree spread of *Phellinus noxius* is largely clonal, but basidiospore-derived spread has resulted in little differentiation among populations in Taiwan (Chung et al. 2015)

Population genetics: Genetic divergence in *Phellinus noxius* by islands observed in Japan (Akiba et al. 2015)






RESEARCH ARTICLE

Genetic Differentiation and Spatial Structure of *Phellinus noxius*, the Causal Agent of Brown Root Rot of Woody Plants in Japan

Mitsuteru Akiba[1]*, Yuko Ota[1], Isheng J. Tsai[2], Tsutomu Hattori[1], Norio Sahashi[1], Taisei Kikuchi[3]

PLOS ONE | DOI:10.1371/journal.pone.0141792

Phellinus noxius **populations are genetically distinct on the Ryukyu and Ogaswawara islands of Japan (Akiba et al. 2015).**

Is *Phellinus noxius* the same in different geographic regions?

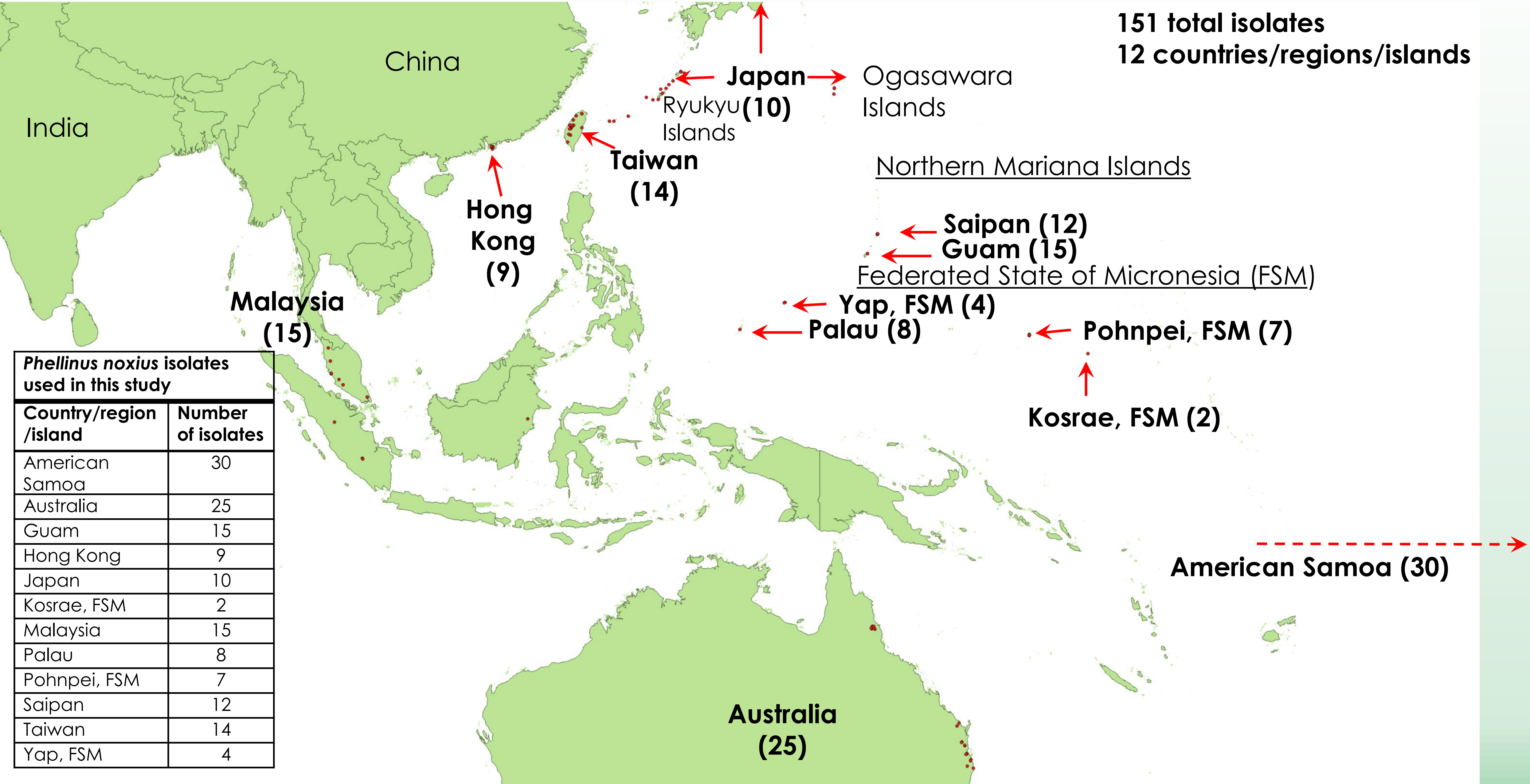
Different genetic groups may

- display different ecological behavior;
- have different environmental requirements;
- pose invasive threats; and
- represent unknown risks from hybridization

Current study – Genetic diversity of *Phellinus noxius* in eastern Asia, Australia, and Pacific islands

Phellinus noxius isolate collections:

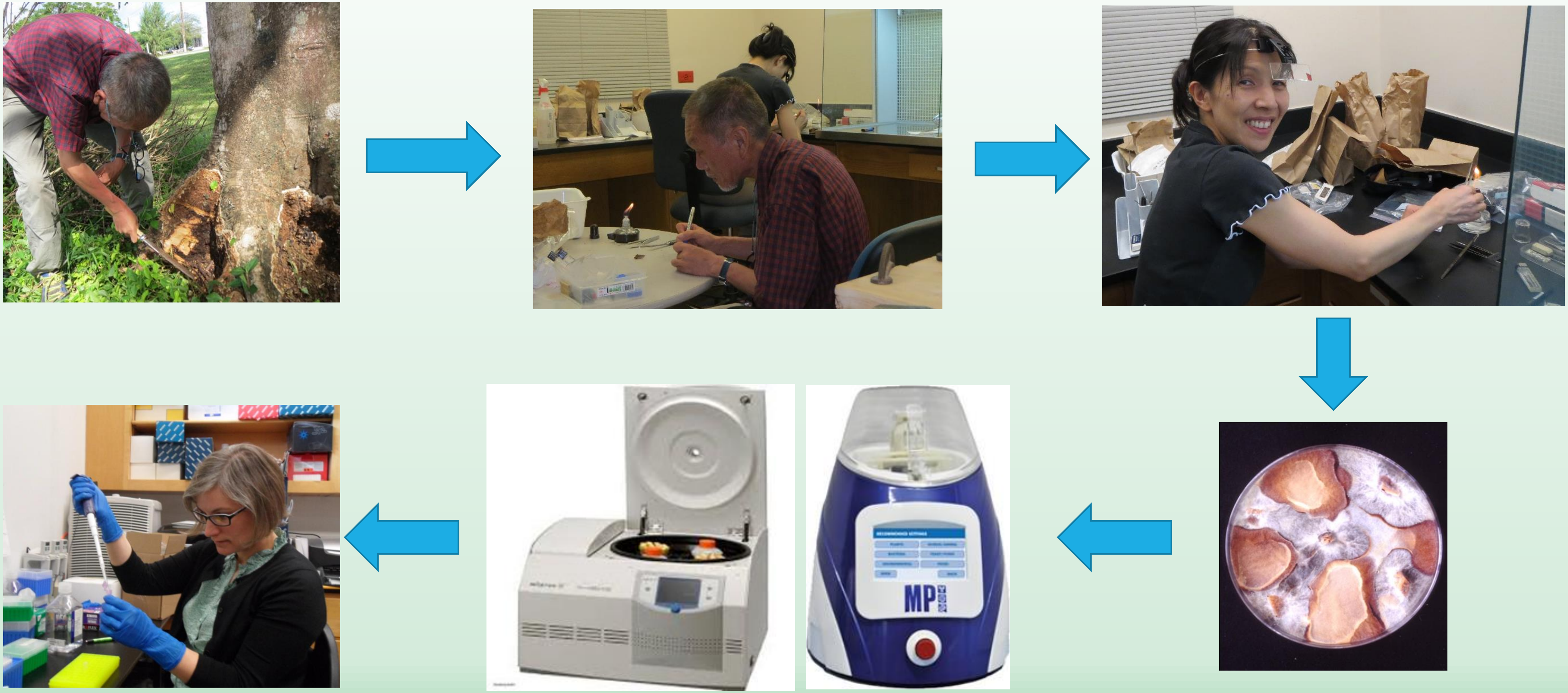
151 total isolates
12 countries/regions/islands



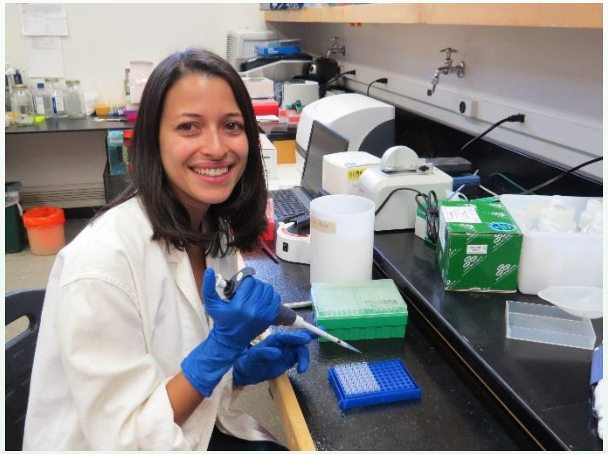
***Phellinus noxius* isolates used in this study**

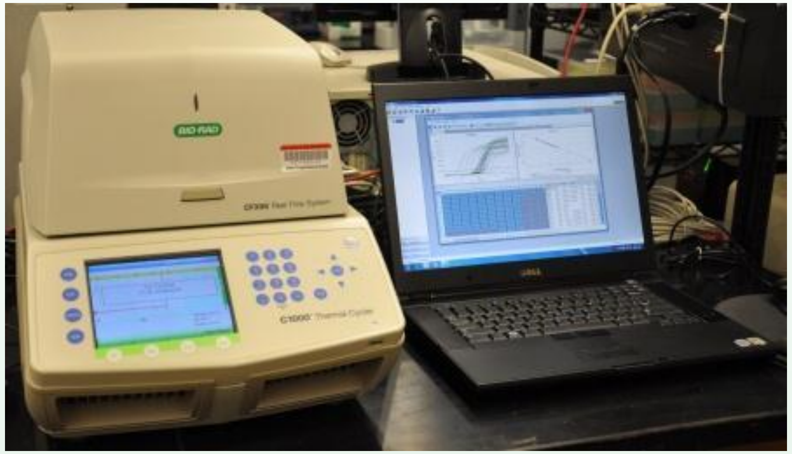
Country/region /island	Number of isolates
American Samoa	30
Australia	25
Guam	15
Hong Kong	9
Japan	10
Kosrae, FSM	2
Malaysia	15
Palau	8
Pohnpei, FSM	7
Saipan	12
Taiwan	14
Yap, FSM	4

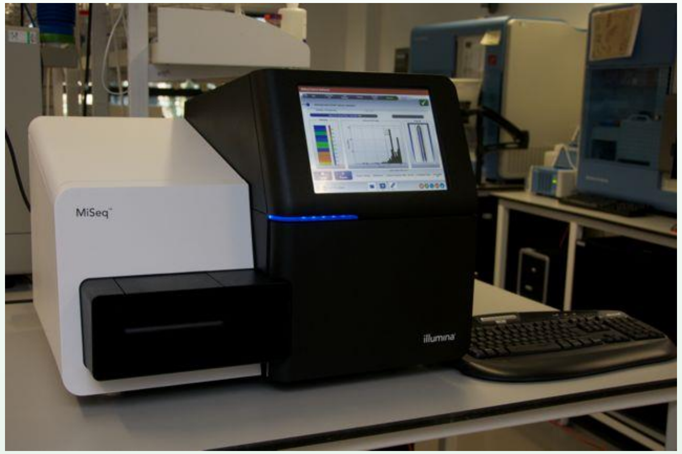
Phellinus noxius isolates were established in culture and DNA was extracted

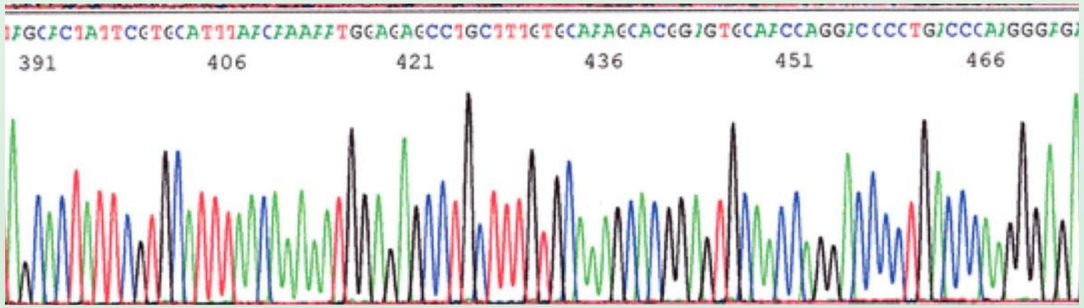


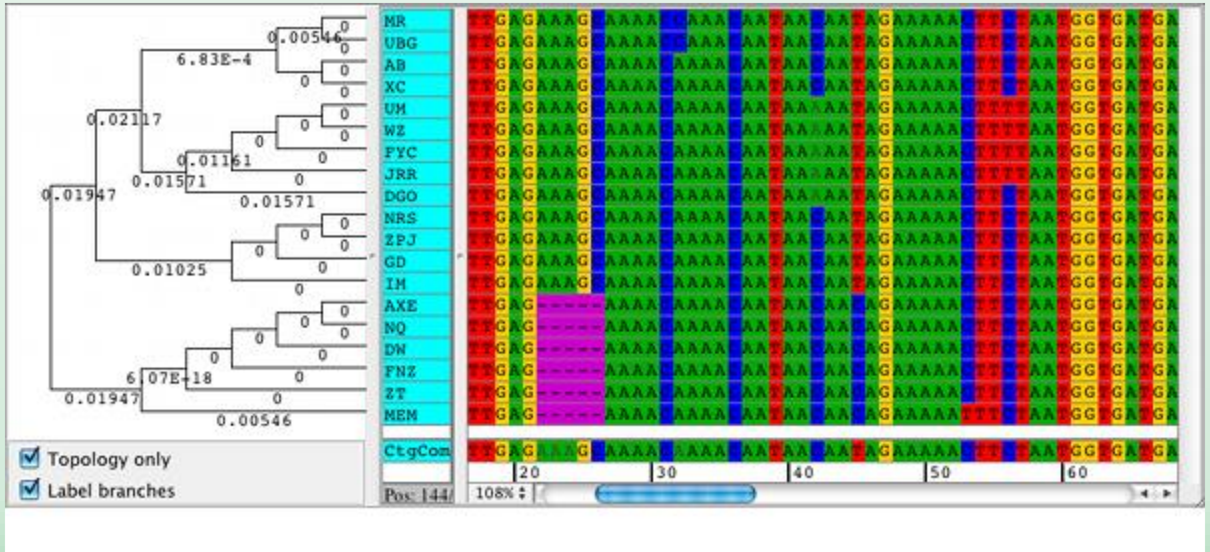
Phellinus noxius DNA is amplified by PCR, sequenced, and analyzed











☑ Topology only
☑ Label branches

Methods (cont.)

- **Sanger sequencing - 4 loci (3,846 total bp)**
  - Large Subunit (LSU; 26S)
  - Internal Transcribed Spacer (ITS)
  - Translation Elongation Factor 1-a (*tef1*)
  - RNA Polymerase II (*rpb2*)
- **RADSeq genomic sequencing**
  - Illumina Hi-Seq – 125pb paired end
- **Haplotype statistics – DNAsp**
- **Phylogenetics – Bayesian methods**
- **Parsimony networks in TCS**

Jane Stewart et al.,
Colorado State University

All four loci showed signals of genetic diversity within *Phellinus noxius*

Locus	Total bp	# haplotypes	Seg. sites	Π nucleotide diversity	Haplotype diversity
LSU	880	20	11	0.002	0.837
ITS	639	78	83	0.013	0.924
rpb2	861	86	103	0.014	0.983
tef1	1077	60	83	0.008	0.923

Jane Stewart et al., in preparation

LSU phylogeny highlights phylogenetic diversity within *Phellinus*

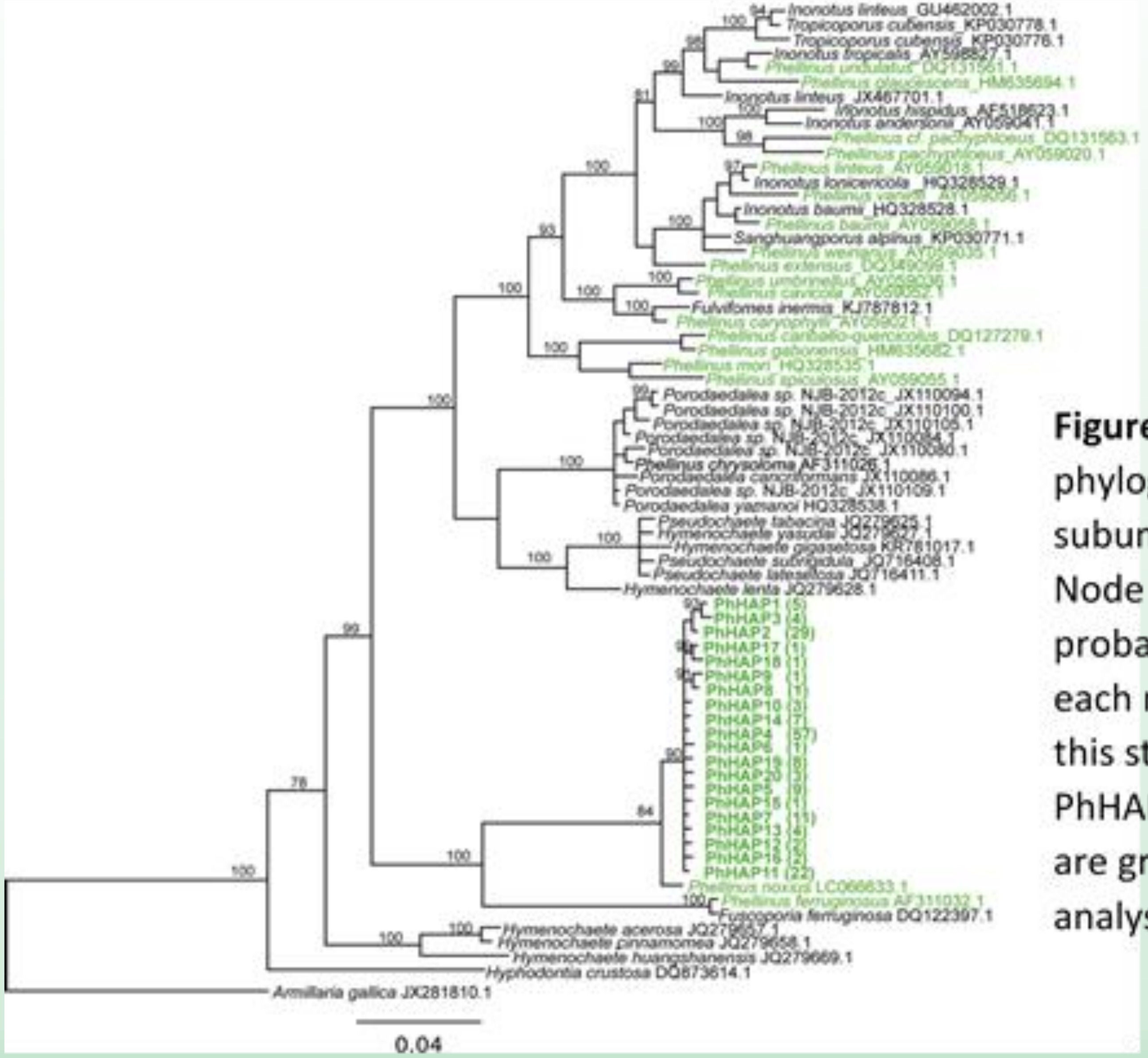
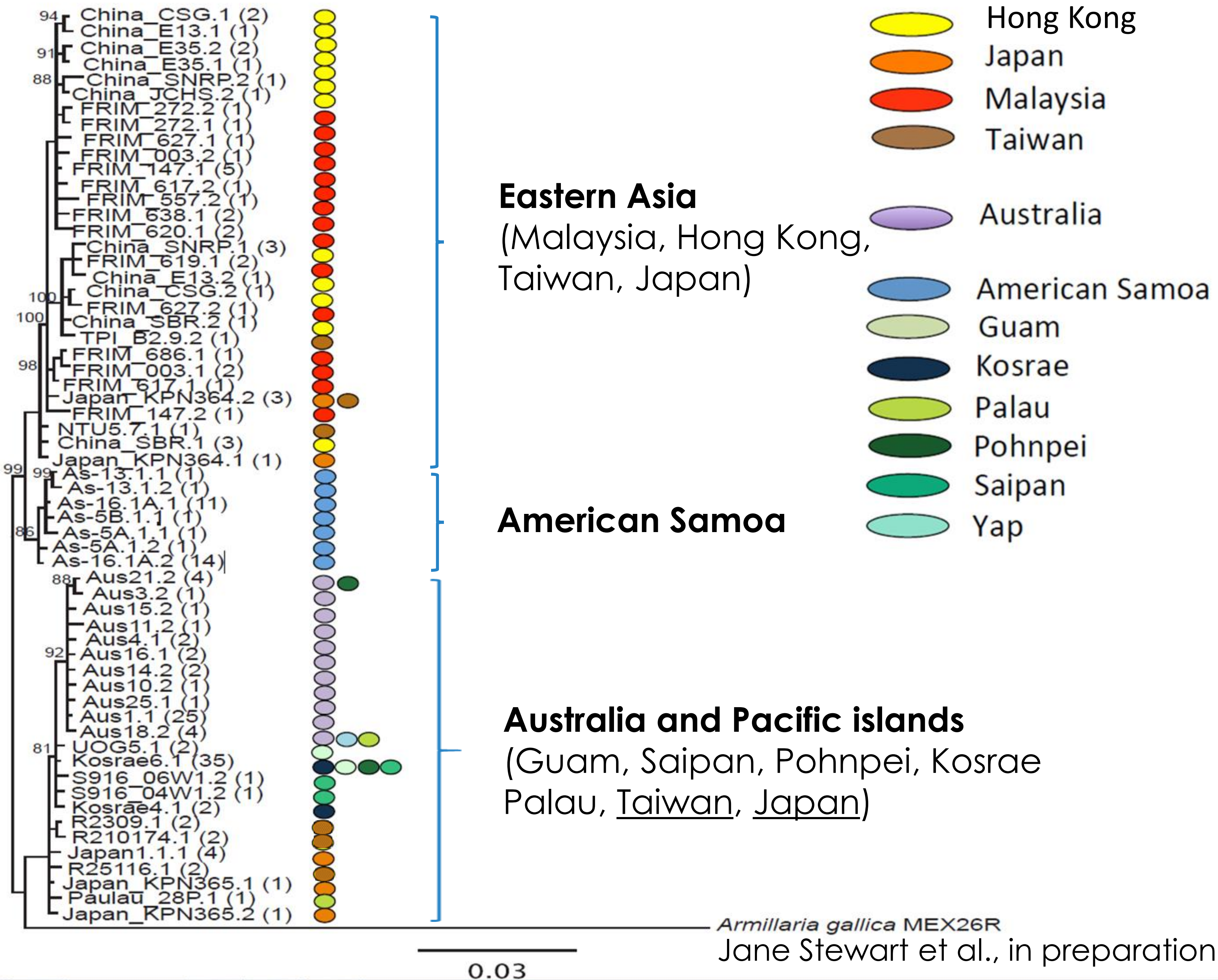


Figure 1: Bayesian phylogeny of the large subunit (LSU; 25S) of rDNA. Node support as posterior probability is labeled above each node. Isolates from this study are labeled as PhHAP. Note: these isolates are grouped together in this analyses.

Jane Stewart et al., in preparation

tef1-based phylogeny of *Phellinus noxius*: Three distinct clades



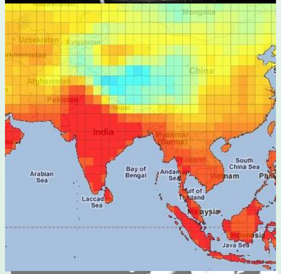
Eastern Asia
(Malaysia, Hong Kong, Taiwan, Japan)

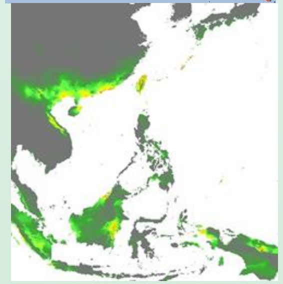
American Samoa

Australia and Pacific islands
(Guam, Saipan, Pohnpei, Kosrae Palau, <u>Taiwan</u>, <u>Japan</u>)

Jane Stewart et al., in preparation

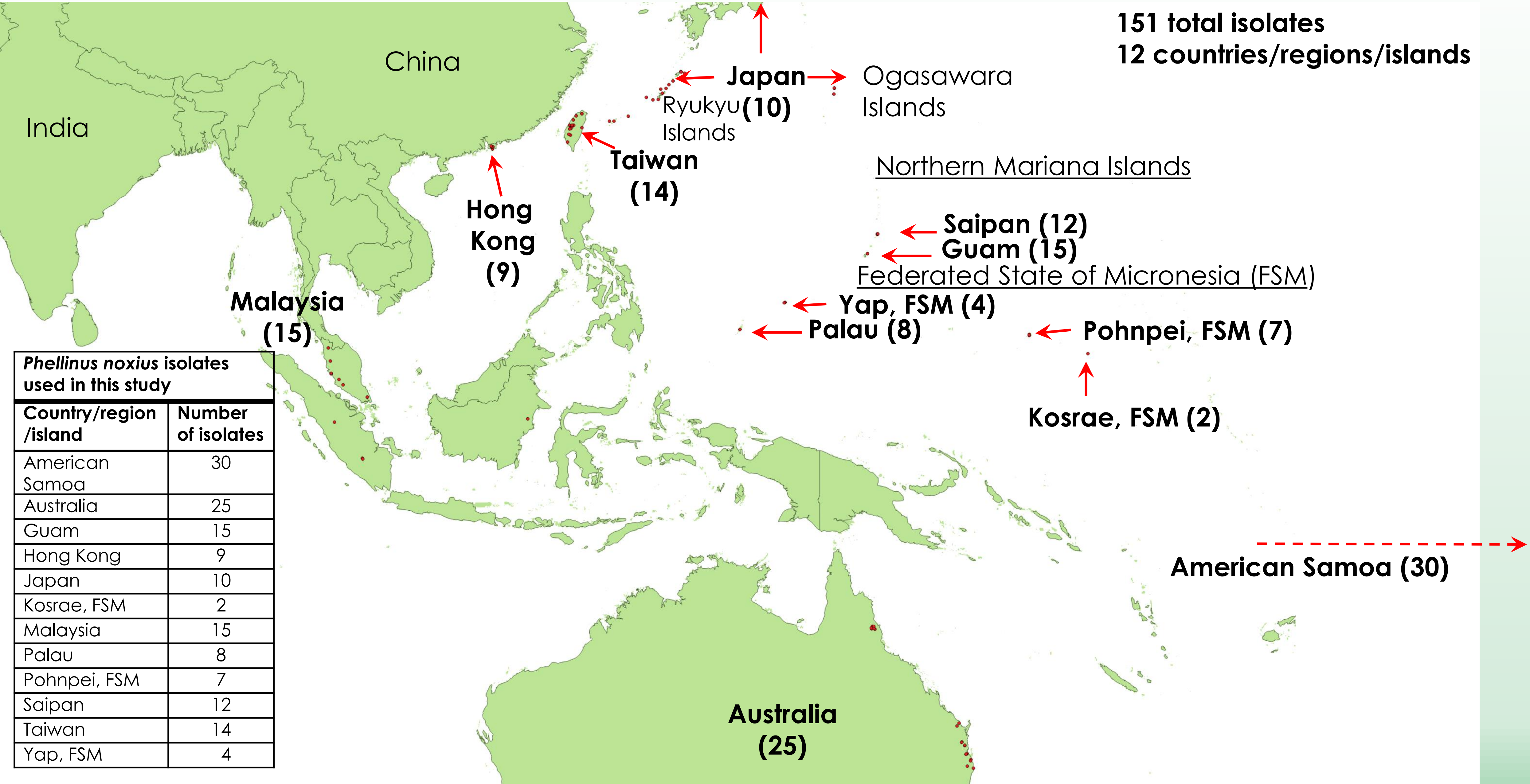
Bioclimatic modeling to predict climatic niche (potential distribution) for *Phellinus noxius* and its genetic groups





- Precise location data (GPS points) are needed for confirmed *P. noxius* (or different genetic groups)
- Global climate layer (grid) for diverse climate data
- Bioclimatic modeling program

GPS points for confirmed occurrences of *Phellinus noxius* or genetic groups



151 total isolates
12 countries/regions/islands

***Phellinus noxius* isolates used in this study**

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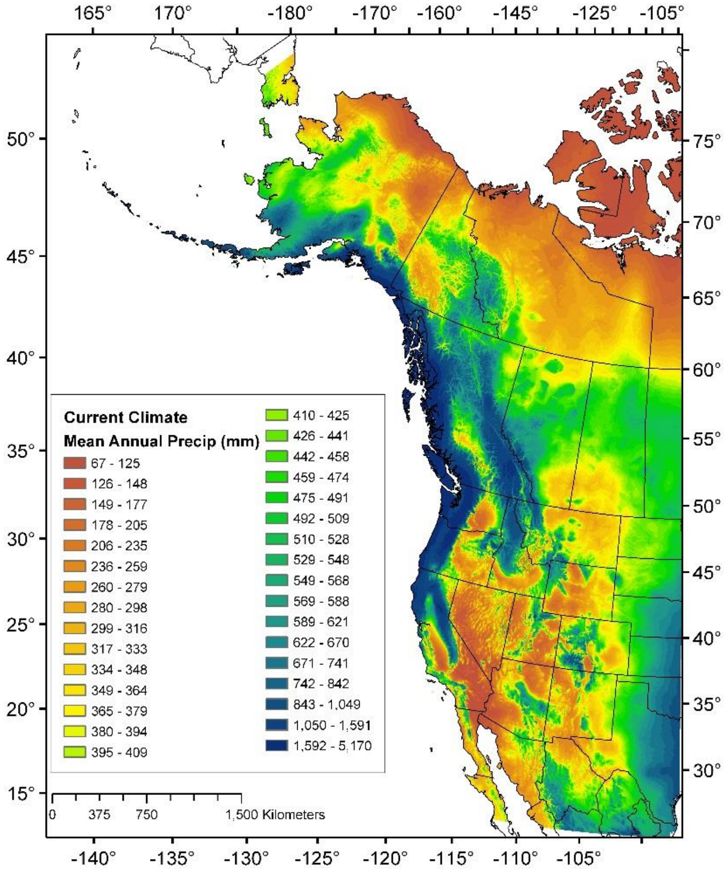
Climate data are available from several sources:

Example sources for climate data:

- **WorldClim**
  http://www.worldclim.org/
- **Daymet**
  http://www.daymet.org/
- **Rehfeldt et al. (2006)**
  http://forest.moscowfsl.wsu.edu/climate/index.html

Examples of climate data:

- Annual mean temp.
- Annual precipitation
- Mean diurnal range
- Max. temp. warmest month
- Min. temp coldest month
- Mean temp. wettest qtr.
- Mean temp. driest qtr.
- Precip. wettest qtr.
- Precip. driest qtr.
- Precip. wettest month
- Precip driest month
- etc.



Rehfeldt et al. 2004

Current Mean Annual Precipitation for western North America

Bioclimatic modelling, environmental niche modelling, species distribution modelling, or climate envelop modelling

Several different bioclimatic modelling programs are available.

We used Maximum Entropy or MaxEnt bioclimatic modelling, because it performs well with

1) limited occurrence points; and

2) presence-only data!

***Phellinus noxius* isolates used in this study**

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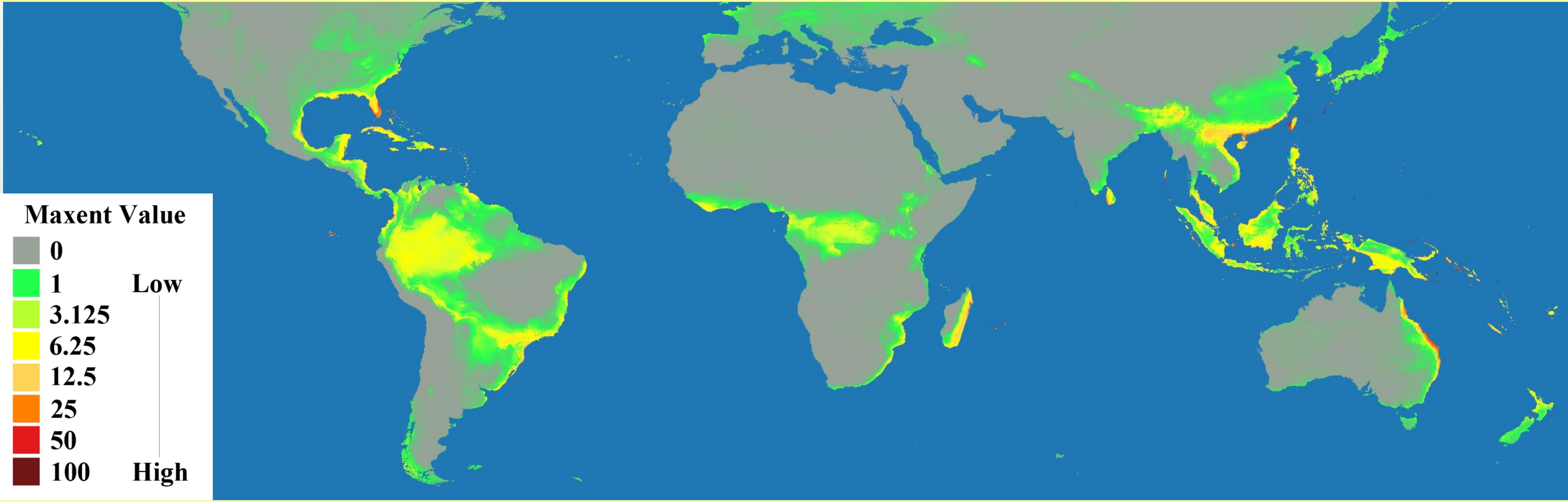
Saipan	12
Taiwan	14
Yap, FSM	4

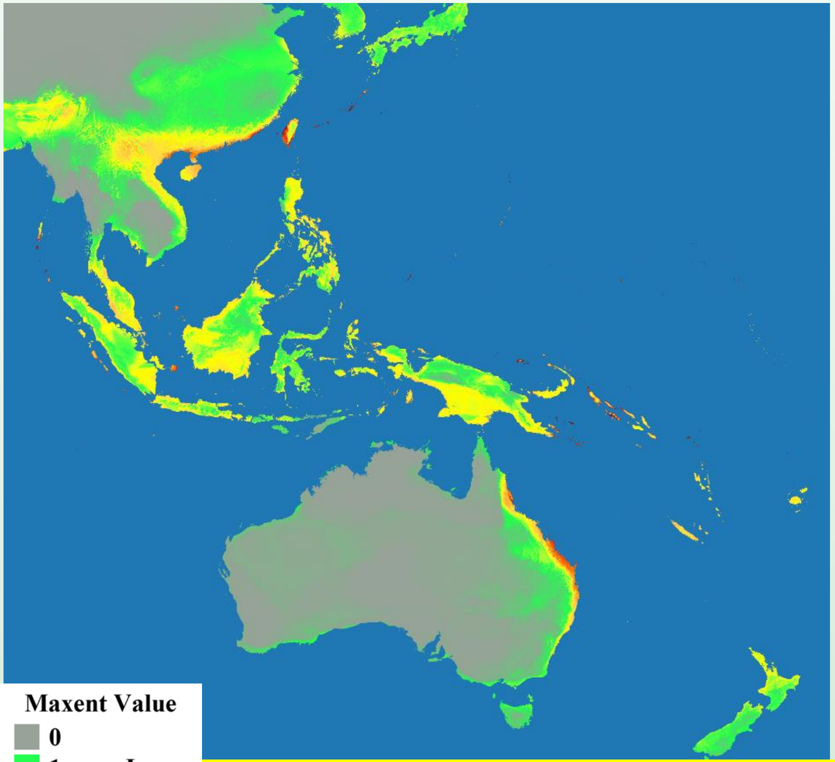
MaxEnt bioclimatic prediction of potential distribution (suitable climate space) for *Phellinus noxius* and geographic areas at risk from invasion

based on all locations of all *P. noxius* genotypes:

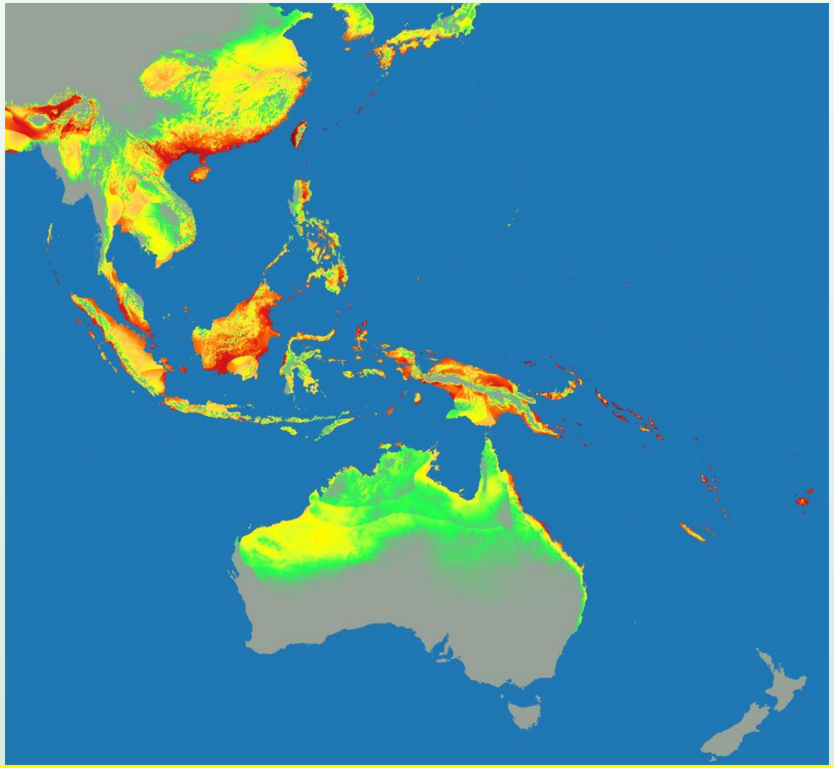


MaxEnt bioclimatic predictions for different genetic groups of *Phellinus noxius*
Potential distribution and geographic areas at risk from invasion

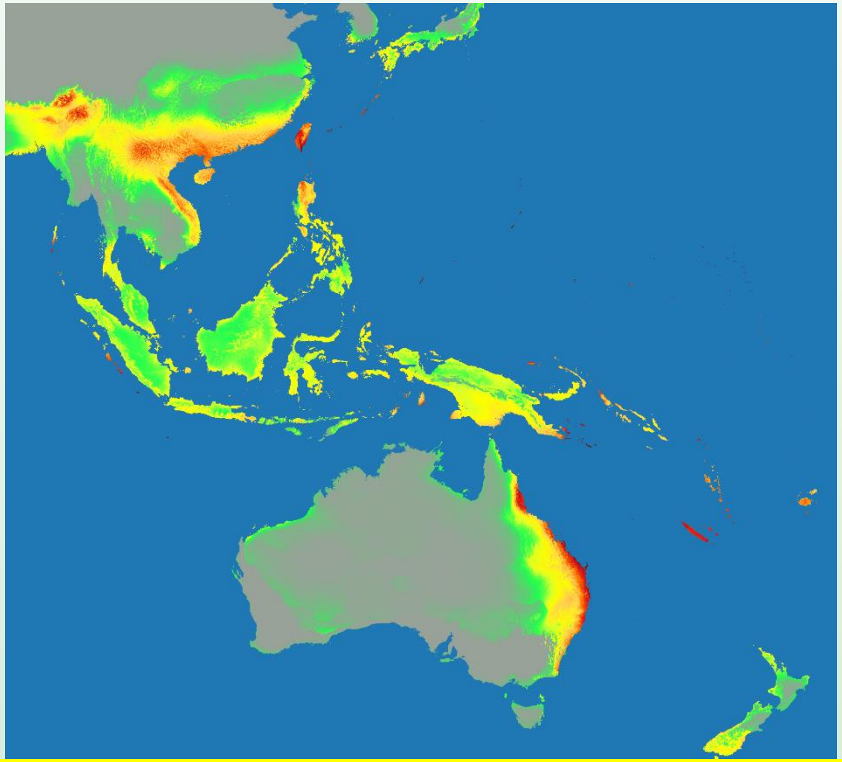
All genetic groups



Eastern Asia genetic group



Australia/Pacific island genetic group



Different genetic groups of *Phellinus noxius* pose distinct invasive threats (with unknown risks associated with hybridization)

Preliminary conclusions from ongoing genetic diversity study of *Phellinus noxius*

- **At least 3 distinct genetic groups of *P. noxius* are evident based on *tef1*:**
  - Eastern Asia (Hong Kong, Malaysia, Taiwan, Japan)
  - Austalia and Pacific islands (Guam, Saipan, Palau, Yap, Pohnpei, Kosrae, Japan, Taiwan)
  - American Samoa (separate from other population and least diverse)
- **Each genetic group of *P. noxius* poses a distinct invasive treat**
- **Some evidence for spread between geographic areas and potential hybridization**
- **Continued studies are needed to determine relationships of isolates from Central America and Africa**

Other DNA/RNA sequence-based genetic studies

- **Genome sequencing –** sequencing of the entire genome provides a wealth of information for interpreting biological relationships and ecological functions

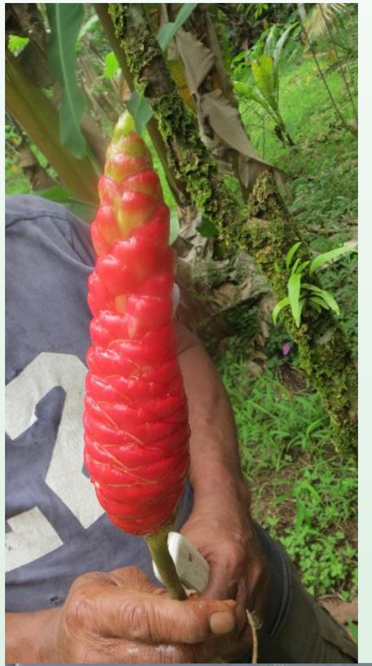
- **Transcriptome sequencing –** determines which genes are being expressed under a specific environment, and provides insights into pathogenicity mechanisms

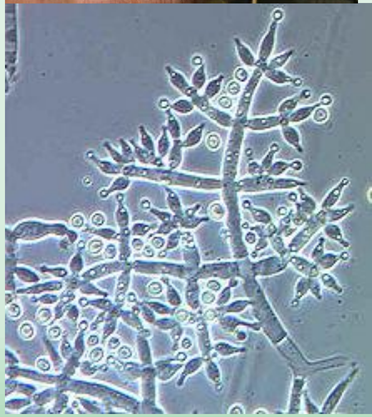
Genomic and transcriptomic sequencing provide basic research information toward understanding host-pathogen interactions that can contribute to the future development of management approaches.

Testing and developing management tools for brown root rot

Methods are needed to evaluate the efficacy of management methods, such as

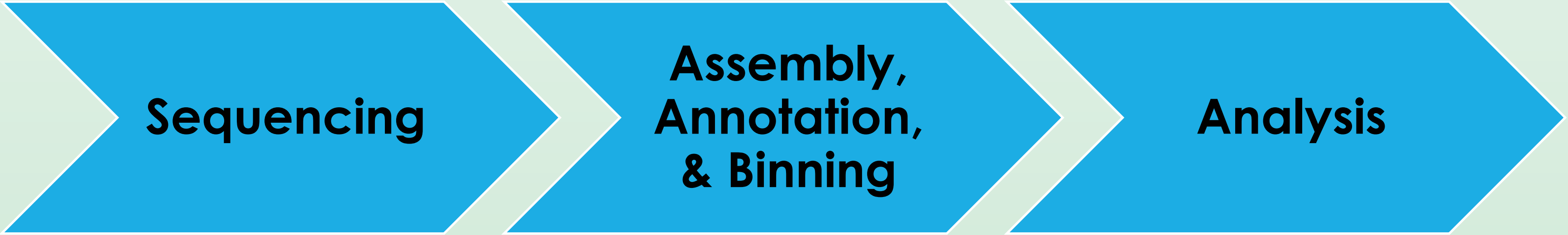
- **Minimizing inoculum (e.g., stump removal), and avoiding wounds;**
- **Fungicides or chemical treatments;**
- **Flooding;**
- **pH;**
- **Soil nutrients/fertilization;**
- **Understory cover crops;**
- **Biological control;**
- **Other manipulations of soil conditions to disfavor *P. noxius* and favor biological; control agents;**
- **etc.**





Metagenomics

- **“Environmental Genomics”** …study of genetic material recovered directly from environmental samples
- Marine microbial communities
  - Global Ocean Sampling expedition
- Medicine
  - Human Microbiome Project; http://commonfund.nih.gov/hmp/
- Biotechnology, Biofuel, Bioremediation
- Soil metagenomes
  - http://www.terragenome.org/



Sequencing

Assembly, Annotation, & Binning

Analysis

Utility of soil metagenomic tools for brown root rot management

- **Monitor the effect of diverse management practices on *Phellinus noxius* (e.g., stumping, urea, lime, flooding, cover crops, etc.)**

- **Investigate potential reasons that some planted species appear to be more susceptible to *P. noxius* than naturally regenerated native trees**

- **Develop novel management approaches by determining soil conditions that suppress *P. noxius* and/or enhance biocontrol agents (e.g., *Trichoderma*).**

General steps for soil metagenomic studies

- Devise sampling design
- Collect environmental data (date, temperature, moisture, plant coverage, host information, etc.)
- Collect soil samples (collect samples before treatment at post-treatment intervals)
- Place ~ 2g of representative soil sample in preservation solution for DNA/RNA (thoroughly mix soil samples or aggregate samples)
- Conduct soil analyses (e.g., pH, bulk density, organic matter, C, N, cations, texture, water-holding capacity, etc.)

The utility of soil metagenomics depends on the collection of environmental metadata

Examples of environmental data collection:

Vegetation surveys

Tree health assessments

Tree canopy assessments

Installing soil temperature and moisture probes

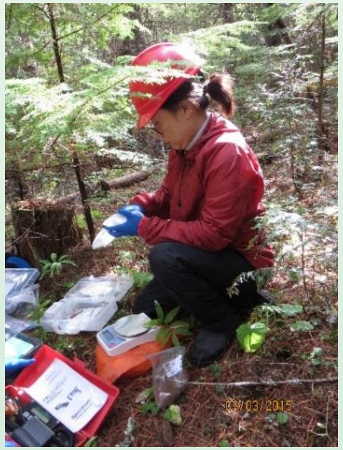
Collecting soil samples for soil analyses and metagenomic studies of the microbial populations

Collecting the soil core

Mixing soil sample

2 g soil subsample to
5 ml preservation buffer

Soil core



Weigh soil subsample

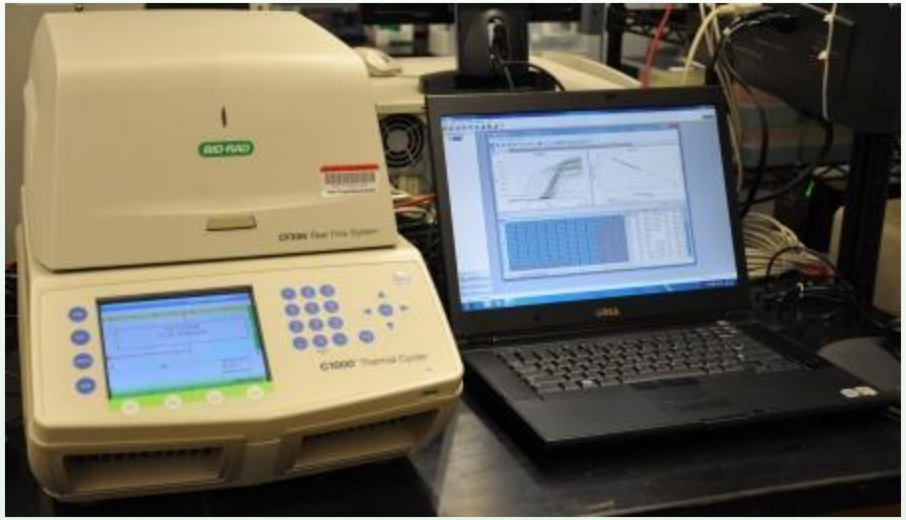
Soil subsample in tubes
with preservation solution

Soil analyses (environmental data)

Determine properties, e.g., pH, bulk density, organic matter, C, N, cations, texture, water-holding capacity

DNA/RNA analysis from soil subsample

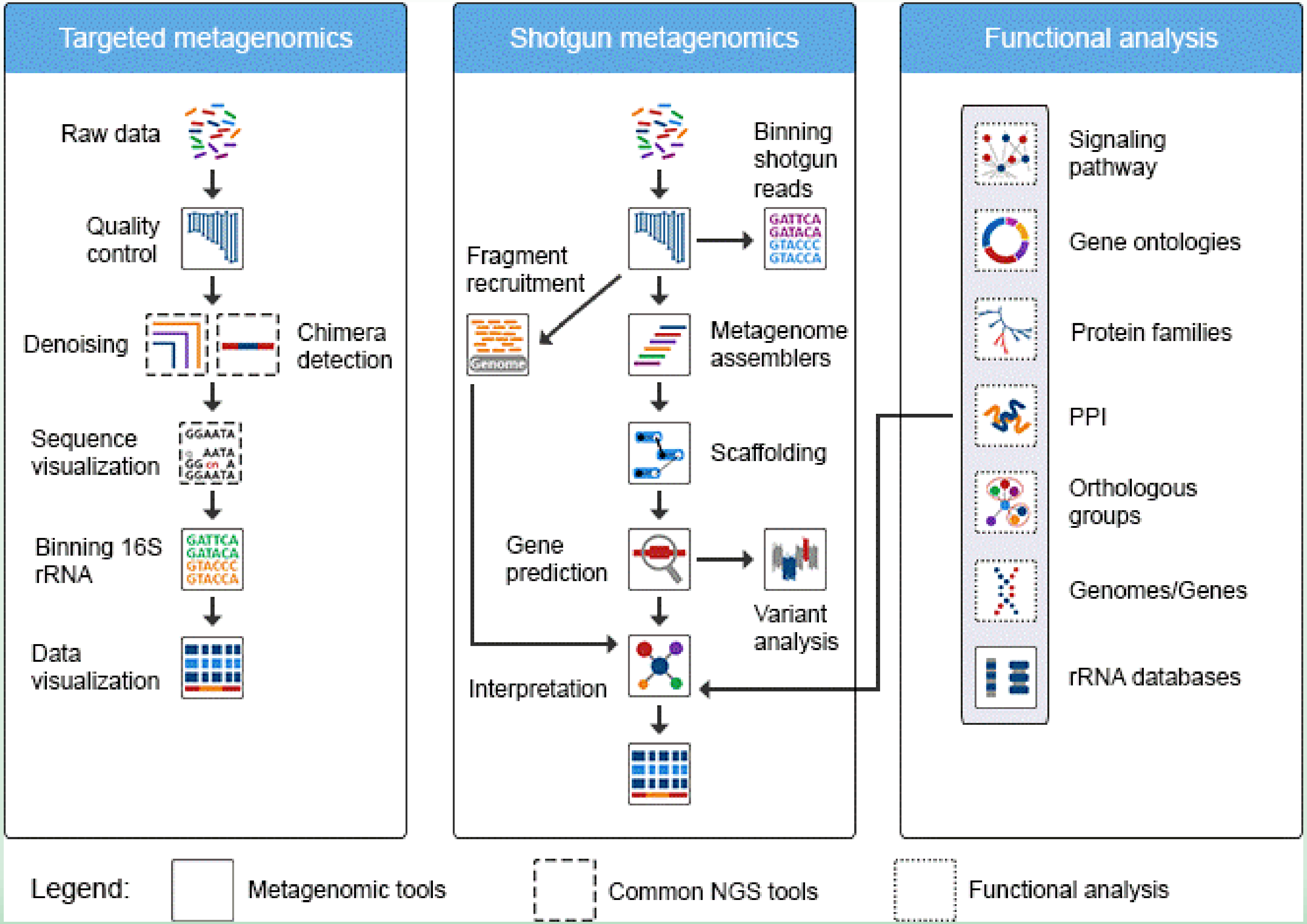
DNA/RNA extraction



Primers, barcoding, and PCR to amplify labelled DNA segments

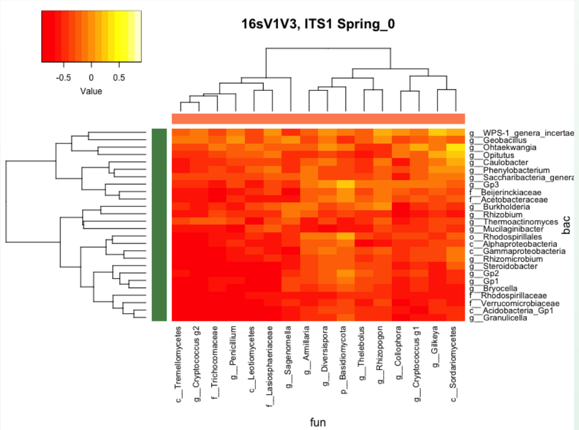
Next generation sequencing

Bioinformatics



From: OMICtools

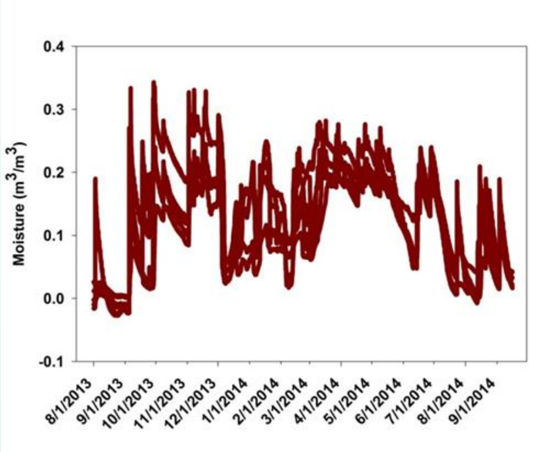
Output from metagenomics (barcoding) and bioinformatics:



1) **Identify:**
   - 10,000s bacterial taxa,
   - ~1, 000s fungal taxa, and
   - other taxa (e.g, insects, nematodes) within each sample.

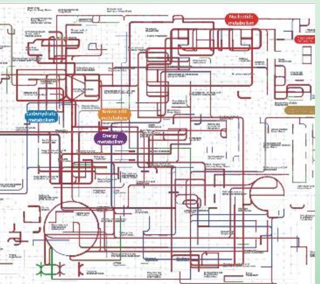
2) **Compare relative abundances of taxa among soil samples:**

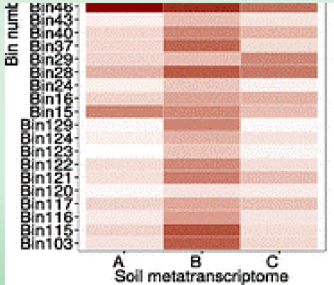
3) **Allows analyses of environmental factors that influence populations of specific microbes.**

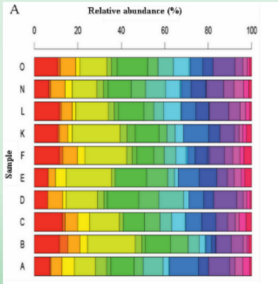


Output from soil metatranscriptomics:

1) **Identify 10,000s of expressed genes from diverse microbial taxa;**
2) **Expressed genes can be associated with ecological function** (e.g., pathogenicity, decomposition, symbiosis, antibiosis, nutrient cycling, etc.);
3) **Relative expression of genes can be compared across soil samples.**







Brown root rot disease is the result of complex ecological interactions that make management difficult

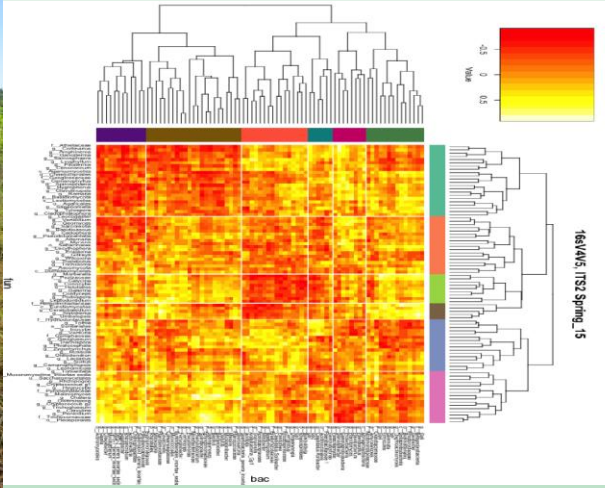
- *Phellinus noxius* genetics, biology, ecology
- Host tree genetics, biology, ecology
- Soil microbial communities (and other biotic environment)
- Neighboring vegetation that influences soil microbes
- Abiotic environment (e.g., soil temperature, moisture, pH, organic matter, N, C, cations, texture, water-holding capacity, etc.)

A combination of soil metagenomics/metatranscriptomics with environmental metadata offers a powerful approach to understand the complex ecological interactions associated with brown root rot disease, which will help develop effective disease management strategies.

Application of soil metagenomics for brown root rot disease

- Allows timely and precise testing of management treatments on *Phellinus noxius* and other soil microbes
- Discover key relationships among biotic/abiotic environment, *Phellinus noxius*, and biocontrol agents to develop novel approaches for disease management.

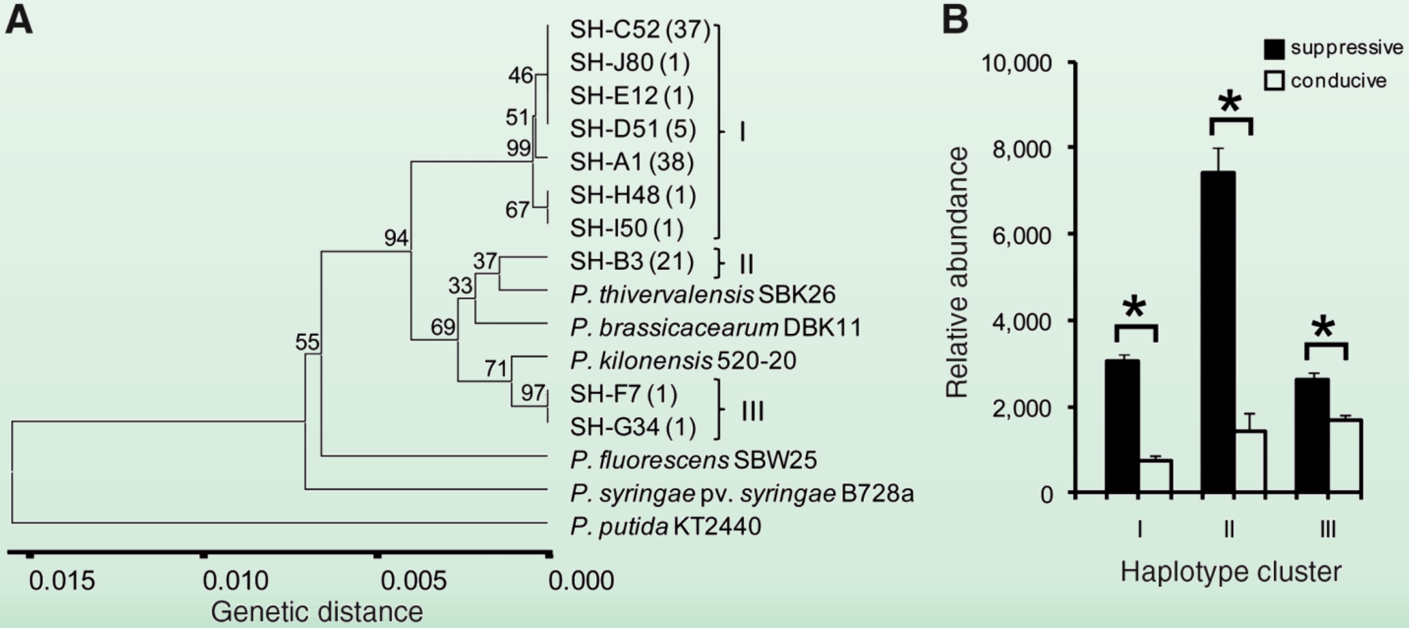


Disease-Suppressive Soils

- Crop plants suffer less from specific soil-borne pathogens than expected owing to the activities of other soil microorganisms

- Metagenomics and culture-dependent functional analyses **identified key taxa and genes** involved in suppression of fungal root pathogens (Mendes et al. 2011. Science)



Acknowledgments

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谢谢

Thank you!