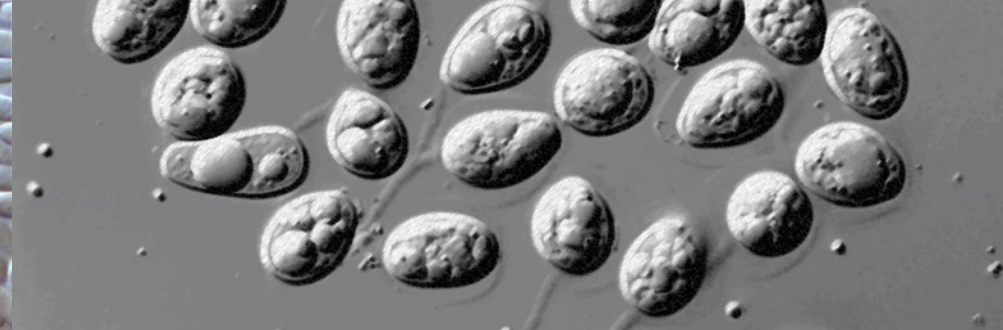
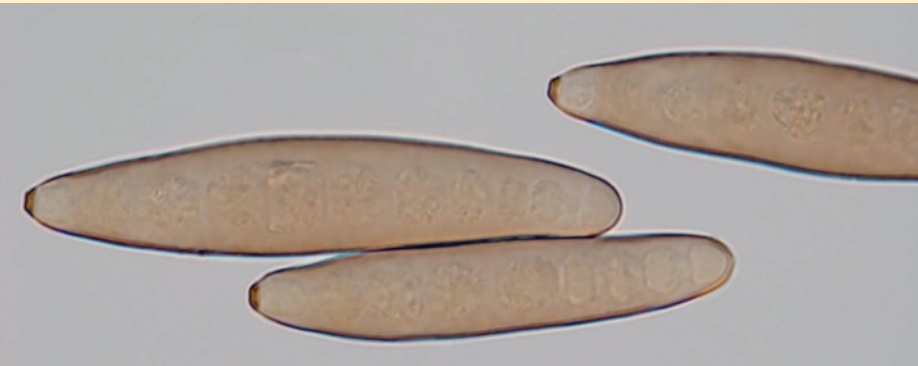


Diagnostics and surveys for plant pathogens in the ASEAN region

ASEAN Diagnosticians Forum, AANZFTA Economic Cooperation Work Program,
15-16 December 2015



Activities

Diagnostic workshops

- *Taxonomy of plant pathogenic fungi*, Mae Fah Luang University, Thailand 22-26 June 2015
- *Diagnosis of Ascomycetes: Bioinformatics and reporting*, Chiang Mai University, Thailand 5-9 October 2015
- *Identification of rust fungi*, Malaysia 2012

Surveys and diagnostic training

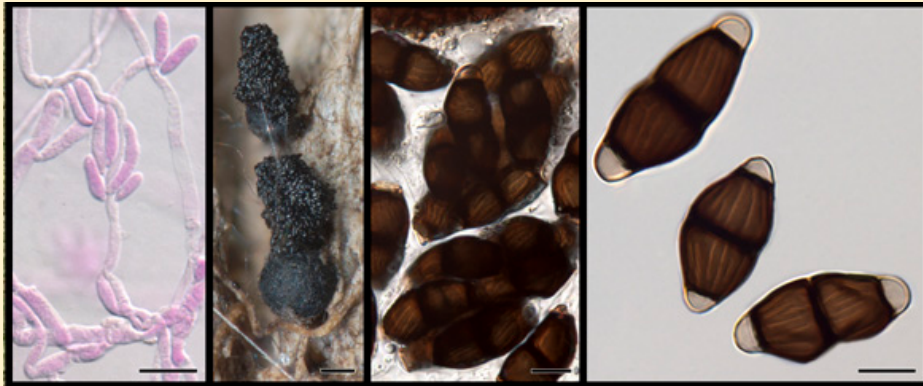
- Smut fungi in Thailand, 2006-2008
- Rust fungi in Vietnam, 2012
- Rust fungi in Philippines, 2012



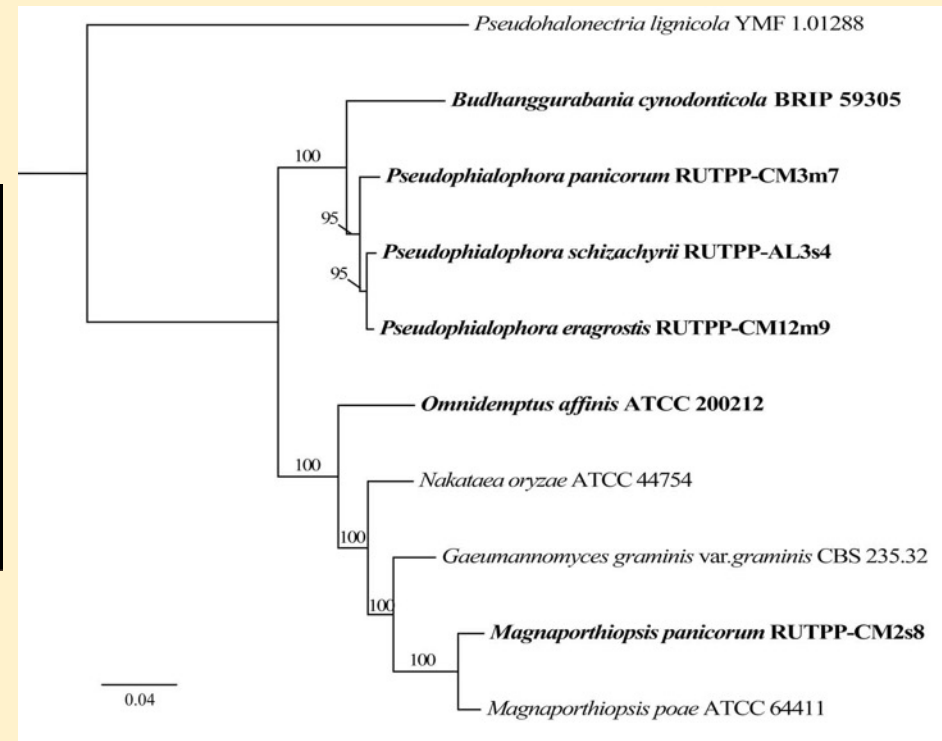
The standard methods for identifying plant pathogenic fungi has changed in the last 10 years.

The application of molecular phylogenetics based on DNA sequence data gains momentum

- classification must reflect phylogeny
- taxa must be monophyletic



Budhanggurabania cynodonticola



A consequence of the molecular revolution is that anamorphic and teleomorphic names have been replaced by **one name** for each fungus

The Amsterdam Declaration on Fungal Nomenclature

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Abstract: The Amsterdam Declaration on Fungal Nomenclature was agreed at an international symposium convened in Amsterdam on 19–20 April 2011 under the auspices of the International Commission on the Taxonomy of Fungi (ICTF). The purpose of the symposium was to address the issue of whether or how the current system of naming pleomorphic fungi should be maintained or changed now that molecular data are routinely available. The issue is urgent as mycologists currently follow different practices, and no consensus was achieved by a Special Committee appointed in 2005 by the International Botanical Congress to advise on the problem. The Declaration recognizes the need for an orderly transition to a single-name nomenclatural system for all fungi, and to provide mechanisms to protect names that otherwise then become endangered. That is, meaning that priority should be given to the first described name, except where that is a younger name in general use when the first author to select a name of a pleomorphic monophyletic genus is to be followed, and suggests controversial cases are referred to a body, such as the ICTF, which will report to the Committee for Fungi. If appropriate, the ICTF could be mandated to promote the implementation of the Declaration. In addition, but not forming part of the Declaration, are reports of discussions held during the symposium on the governance of the nomenclature of fungi, and the naming of fungi known only from an environmental nucleic acid sequence in particular. Possible amendments to the *Draft BioCode* (2011) to allow for the needs of mycologists are suggested for further consideration, and a possible example of how a fungus only known from the environment might be described is presented.

Key words:

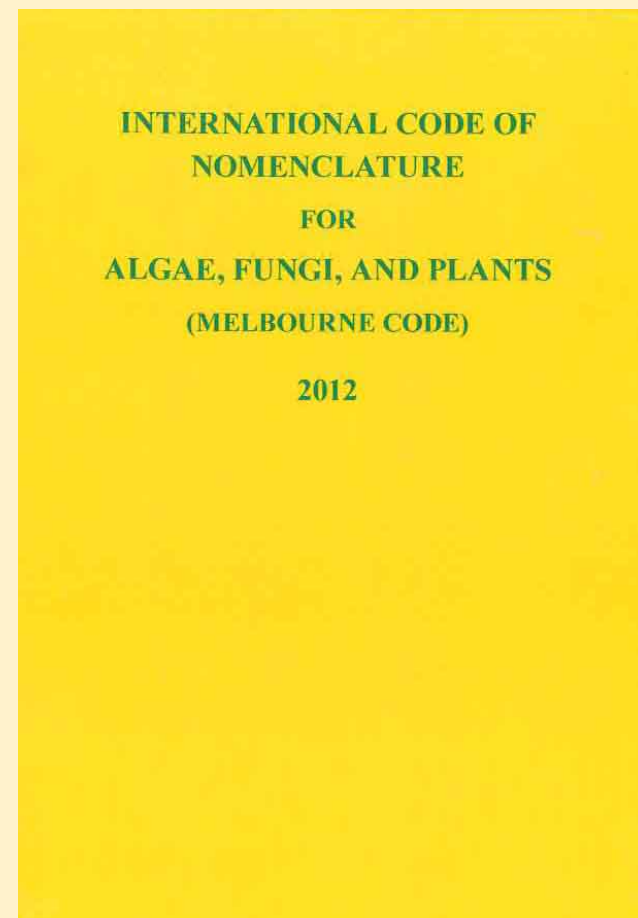
Anamorph
Article 59
BioCode
Candidate species
Environmental sequences
International Code of Botanical Nomenclature
MycCode
Pleomorphic fungi
Teleomorph

Article info: Submitted: 17 May 2011; Accepted: 31 May 2011; Published: 7 June 2011.

“..... a single-name nomenclatural system for all fungi....”

What else has changed in recent years?

- *International Code of Nomenclature for algae, fungi, and plants* (Melbourne Code) published in 2012
 - electronic publication
 - English descriptions
- All names must have a unique registration number e.g. MycoBank number (www.mycobank.org)
- Internal Transcribed Sequence (ITS) gene region selected as the official barcode gene for fungi



The challenge

How do we introduce molecular diagnostic methods in laboratories that do not have staff or facilities skilled in molecular methods?



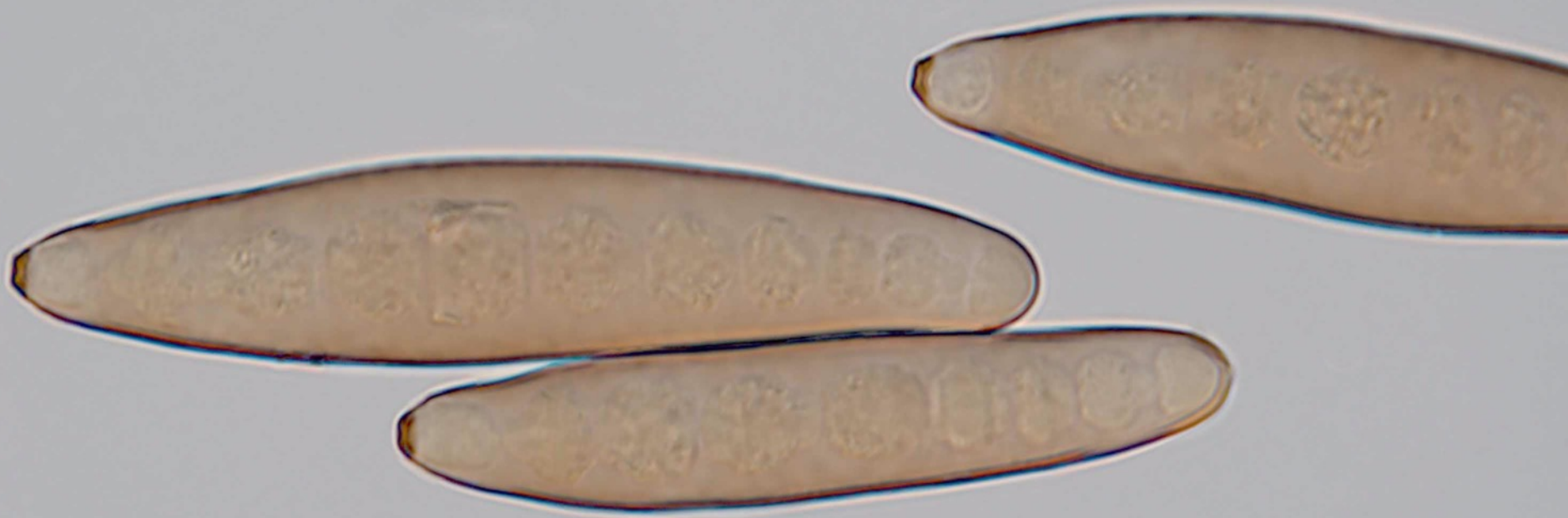
One solution



Hold two workshops that look at genera, which have many species that are difficult to identify by morphology.

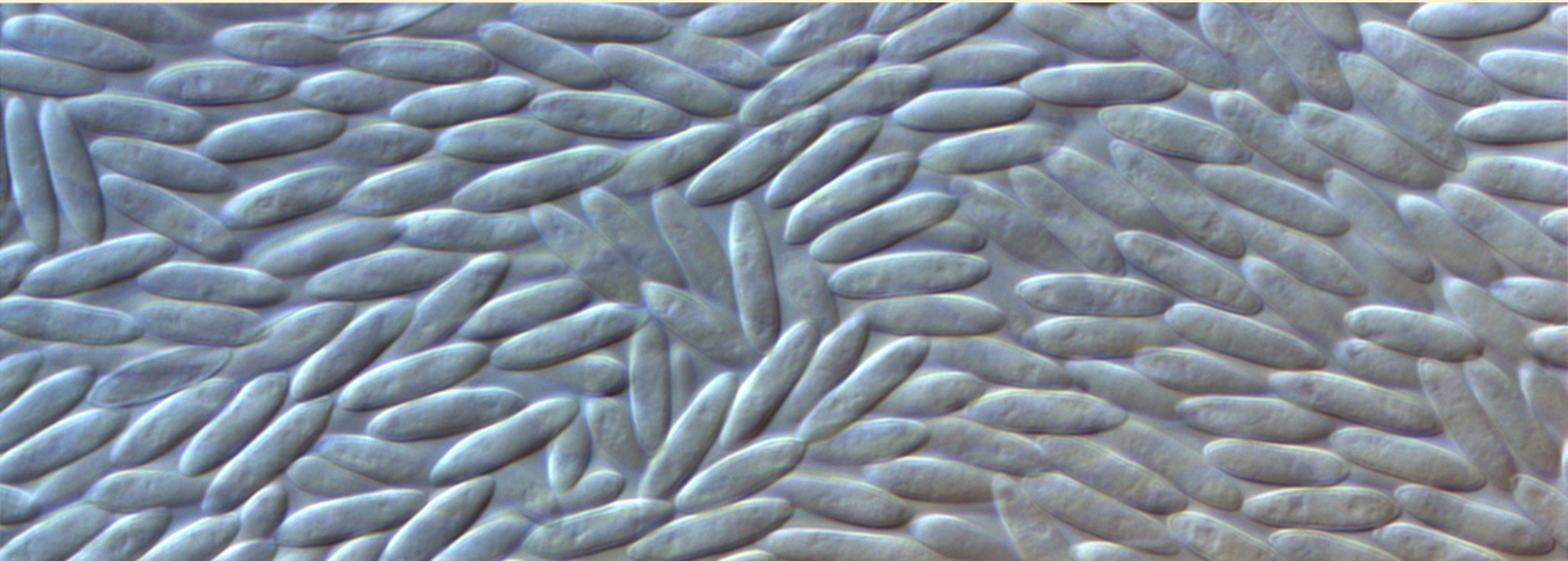
Introduction to *Bipolaris* and *Curvularia*

AANZFTA ECWP Diagnosis of Plant Diseases Workshops, 22-26 June 2015



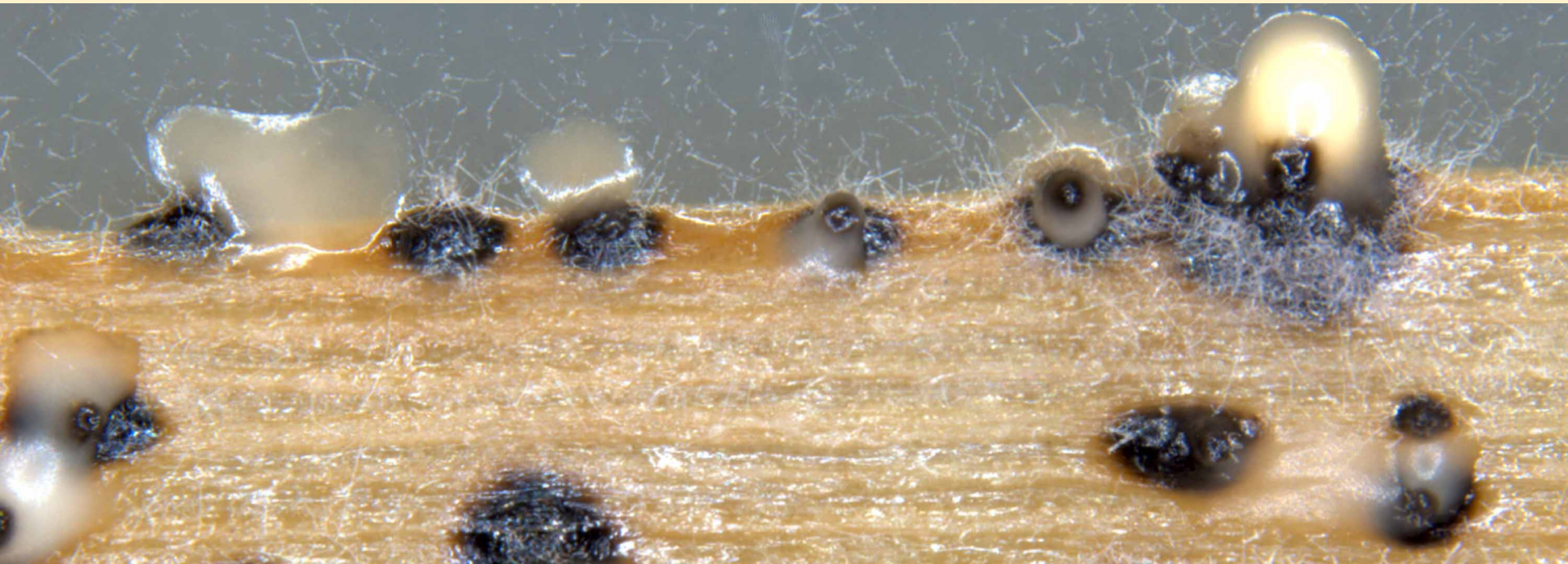
Introduction to *Colletotrichum*

AANZFTA ECWP Diagnosis of Plant Diseases Workshops, 22-26 June 2015



Introduction to *Diaporthe*

AANZFTA ECWP Diagnosis of Plant Diseases Workshops, 22-26 June 2015



Introduction to *Phyllosticta*

AANZFTA ECWP Diagnosis of Plant Diseases Workshops, 22-26 June 2015



The method

Send cultures directly to Macrogen, South Korea for DNA extraction, gene amplification and sequencing. This eliminates the need to have access to a molecular laboratory. Training is needed in phylogenetic analysis.



How successful was the first workshop?

After the first workshop participants were asked the following questions.

- Did you send fungal cultures to MacroGen for sequencing?
- How did you obtain your fungal cultures?
- How did you make sure the cultures were pure?
- How did you identify the isolates as one of the target taxa? Some of you sent isolates that were not target taxa. Explain why these fungi interested you.
- What were the host plants of your fungi?
- Did you have any difficulties sending the isolates?
- Do you have a suggestion that would make it easier for you to send isolates next time?
- How will you preserve your cultures?

5 steps from culture to identification

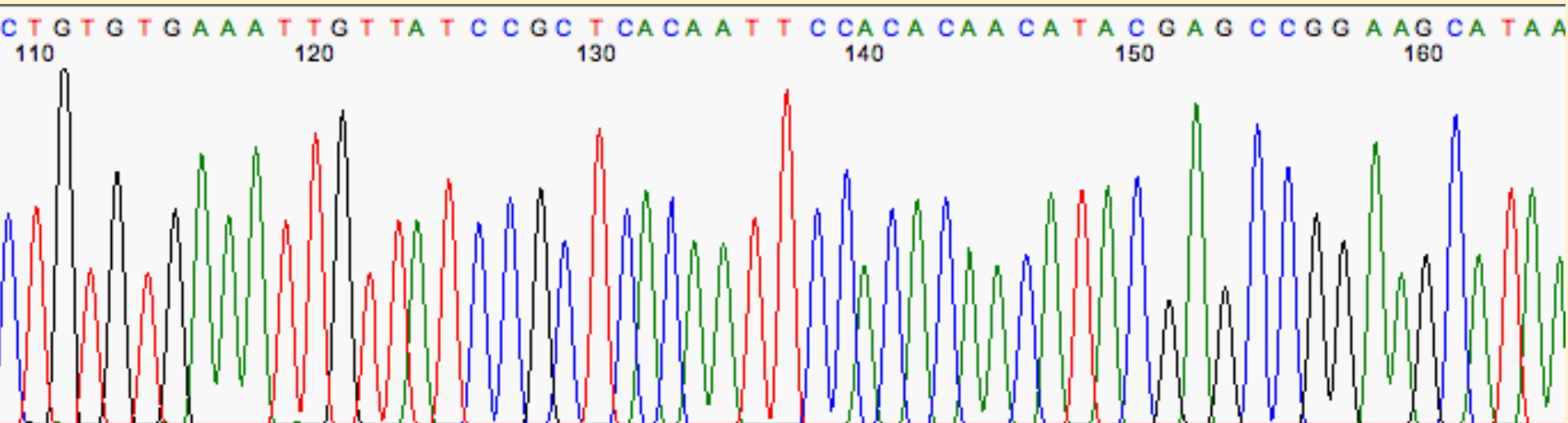
Step 1 Choice of genes

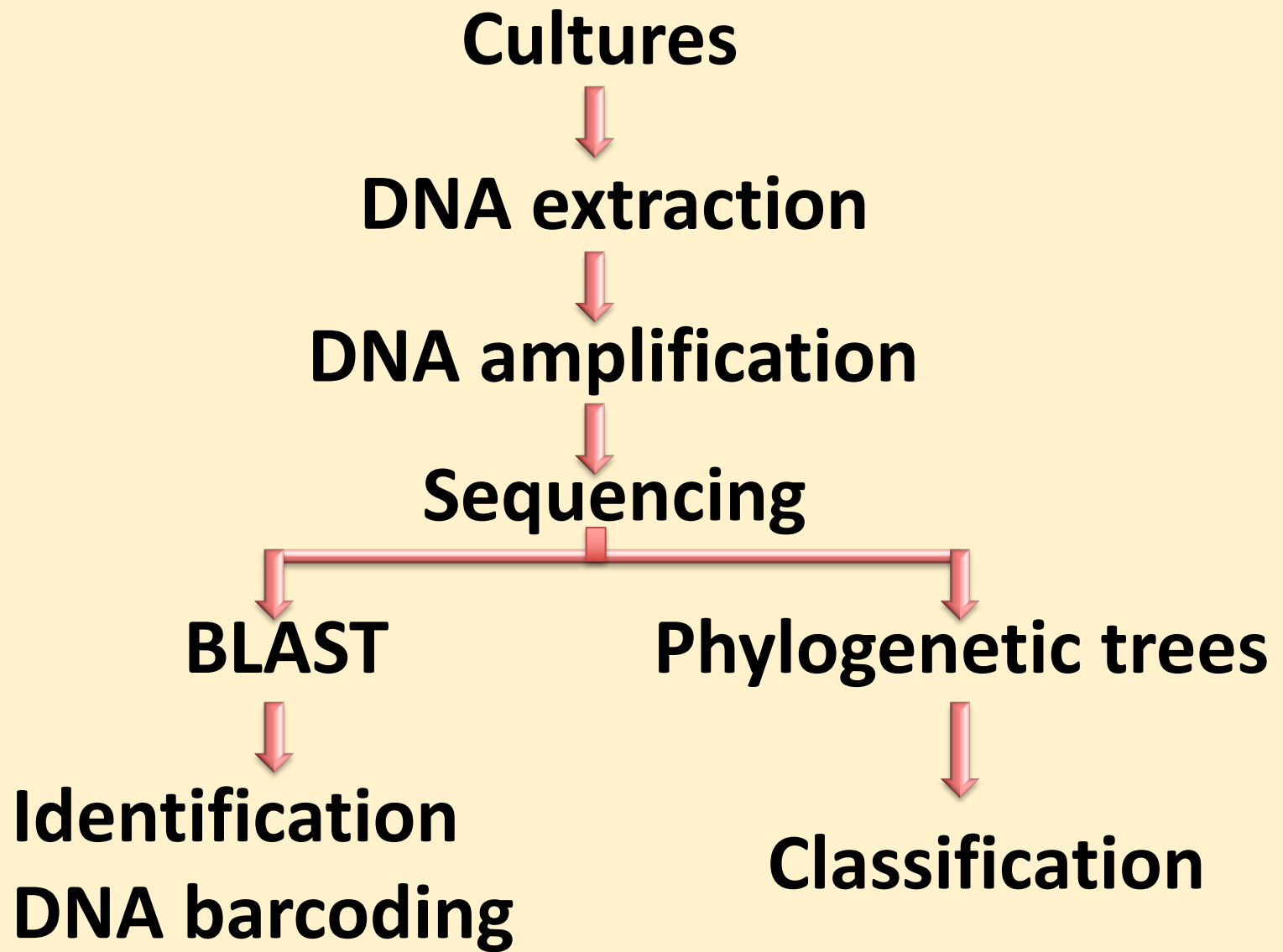
Step 2 Chromatograms

Step 3 Sequence assembly and alignment

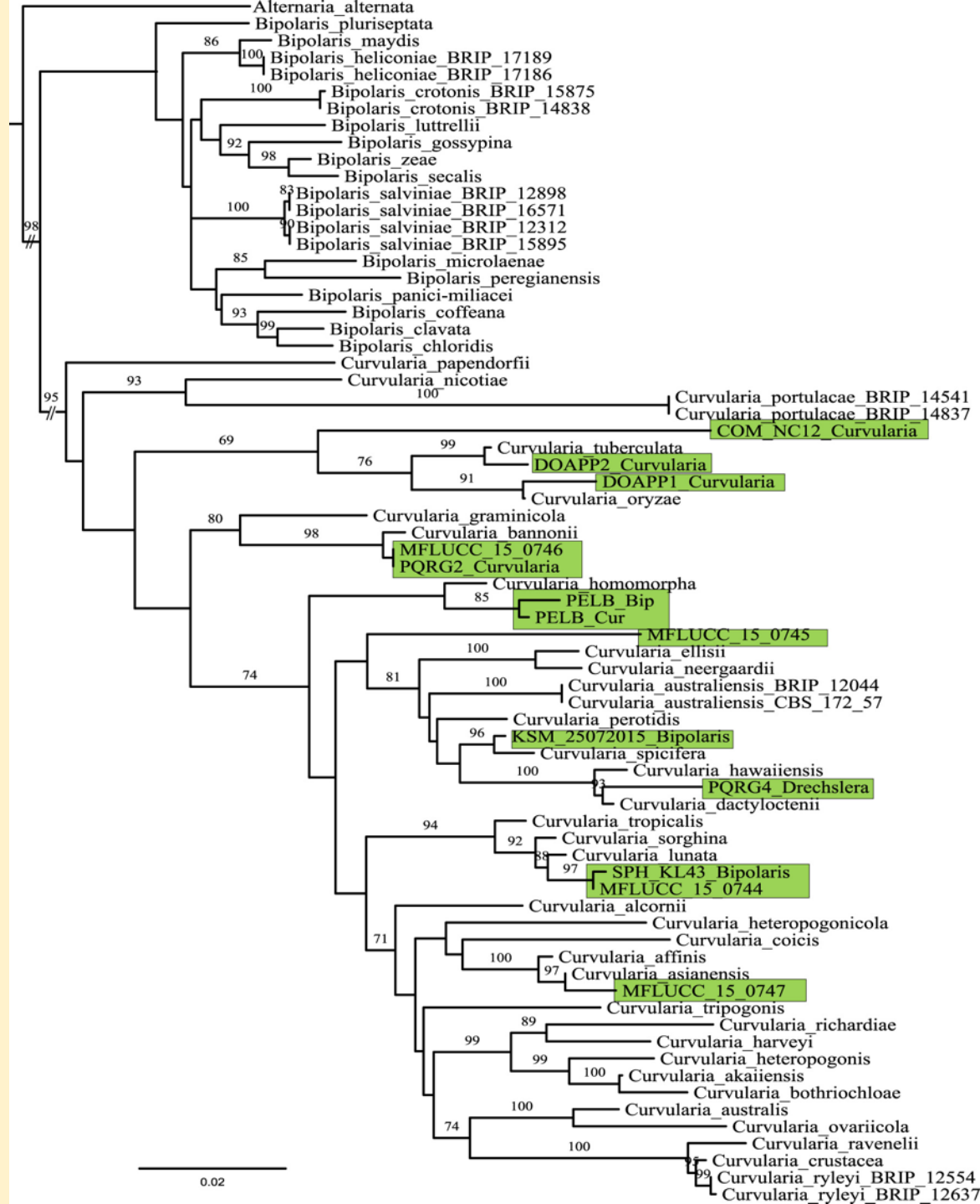
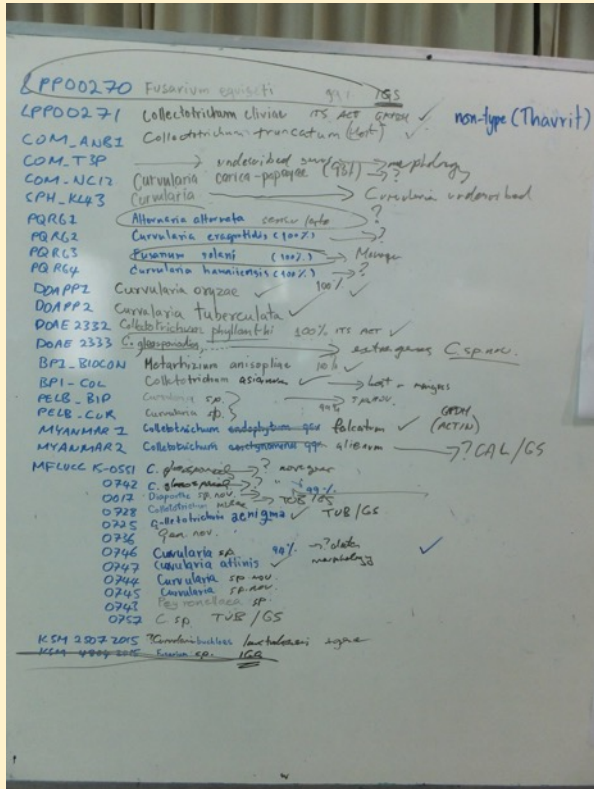
Step 4 BLAST <http://blast.ncbi.nlm.nih.gov/Blast.cgi>

Step 5 Tree building





How successful was the second workshop?



Curvularia tree

What are the costs?

	Cost (USD) / sample / locus			
	Culture		gDNA	
	Package services	Non-package services	Package services	Non-package services
Forward and reverse direct sequencing	40	10	40	10
Purification		2		2
PCR		5		5
gDNA preparation		10		-
Total cost	40	27	40	17
Discount price	36	24.30	36	15.30
<ul style="list-style-type: none"> - Service packages are available for some loci e.g. LSU, ITS, SSU. - Optimisation of PCR conditions cost 100 USD for each gene region. - Shipping fee is free of charge if each order is over 100 USD. 				

What is the role of ARDN if cultures or DNA samples are sent directly to an international sequencing laboratory?

Diagnostic Specialist (ASEAN)

Clearing House (ARDN)

Diagnostic Specialist (outside ASEAN region)

Provincial laboratory

National Diagnostic Network (NDN) Coordinator (NPPO)

Sequencing laboratory

Surveys for rust and smut fungi

- <http://collections.daff.qld.gov.au/web/key/smutfungi>
- <http://collections.daff.qld.gov.au/web/key/rustfungi>
- <http://collections.daff.qld.gov.au/web/key/ThaiSmutfungi>
- <http://collections.daff.qld.gov.au/web/key/ASEANrust/Media/Html/index.html>

Development of these tools has provided training/opportunities in

- Surveillance
- Specimen preservation
- Herbarium management
- Diagnostics and identification
- Taxonomy and research
- Further education



Regional Publications

- Doungsa-ard C, McTaggart AR, Geering ADW, Dalisay TU, Ray J, Shivas RG (2015) *Uromycladium falcatarium* sp. nov., the cause of gall rust on *Paraserianthes falcataria* in south-east Asia. *Australasian Plant Pathology* 44: 25–30.
- Deng H, Tan YP, Shivas RG, Niu YC (2015) *Curvularia tsudae* comb. nov. et nom. nov., formerly *Pseudocochliobolus australiensis*, and a revised synonymy for *Curvularia australiensis*. *Mycoscience* 56: 24–28.
- Dhileepan K, Balu A, Murugesan S, Senthilkumar P, Shivas RG (2013). Survey and prioritisation of potential biological control agents for prickly acacia (*Acacia nilotica* subsp. *indica*) in southern India. *Biocontrol Science and Technology* 23: 646–664.
- Seemadua S, Bhasabutra T, Beasley DR, Tan YP, Shivas RG (2012). A new host genus and species (*Afzelia xylocarpa*) for *Phakopsora pachyrhizi* found in Thailand. *Australasian Plant Disease Notes* 7:125–126.
- Bhasabutra T, Seemadua S, Shivas RG (2012). First record of *Prospodium appendiculatum* on *Tecoma stans* in Thailand. *Australasian Plant Disease Notes* 7: 123–124.

The Future

The identification of plant pathogens is reliant on molecular methods, even for obligate fungi (rusts and fungi).

Reliable pest lists (regional and national) depend on

Plant pest surveys

Specimen collection

Specimen preservation in recognised collections

Reliable identification (by molecular means)

Maintenance of these collections

Publication (e.g. Lucid keys, scientific literature)

