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รายงานวิจัยฉบับสมบูรณ์

การใช้เทคนิค polyphasic approach เพื่อปรับปรุงการจัดกลุ่มของเชื้อราสกุล *Phyllosticta* ซึ่งเป็นเชื้อก่อโรคที่สำคัญ

A polyphasic approach to the revision of the important pathogenic genus *Phyllosticta*

โดย

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บทสรุปผู้บริหาร (EXECUTIVE SUMMARY)

1. ความสำคัญและที่มาของปัญหาในการการวิจัย (Rationale and review)

The genus *Phyllosticta* and its *Guignardia* sexual morph causes economically significant diseases of important crops and horticultural plants such as banana, citrus, grape, orchids and palms. Species concepts in *Phyllosticta* and *Guignardia* are however ambiguous as there are more than 3000 names (more than 100 accepted species in *Phyllosticta*) and very few characters to differentiate species. It is therefore important that species concepts are clarified so that plant pathologists can readily identify species, thus they can implement disease control management strategies. This project is important for *Phyllosticta* taxonomy and will study pathogenic species but also include saprobes, and endophytes. We will investigate the morphological and cultural characters as well as the phylogenetic relationships of *Phyllosticta* species on various hosts (e.g., on banana, citrus, grapes, orchids, palms) and attempt to link the taxa to their *Guignardia* sexual morph. Relationships will be elucidated using morphological and cultural characters and phylogenetic interpretation of gene sequences. This project will therefore provide a clear understanding of the taxonomy of *Phyllosticta* species in Thailand, and worldwide. We will also establish which species cause disease and reduce yield and quality of plant products.

2. วัตถุประสงค์ของโครงการวิจัย (Objective of the research)

i. To clarify the species of *Phyllosticta* associated with disease in a range of hosts in northern Thailand.
ii. To understand the relationship between morphology and phylogeny characters of *Phyllosticta* species and their relationships with hosts.
iii. To elucidate species concepts within the genus by linking molecular and morphological approaches.

3. ขอบเขตของโครงการวิจัย (Scope of the research)

The genus *Phyllosticta* contains more than 3000 names and presently there are more than 50 estimated species. *Phyllosticta* species are worldwide in distribution and cause major damage to cereals, vegetables, legumes, ornamental plants and fruit trees. The current naming of *Phyllosticta* species is largely based on a combination of morphological and cultural characteristics. These are however, limited numbers of morphological character-suites available in culture coupled with inherent phenotypic plasticity, precise identification of the species has always been difficult. Physiological specialization within species and overlapping host ranges mean that our current classification system is impracticable for users. This causes problems to systematists, plant pathologists, plant health practitioners, plant breeders and quarantine officers,

since they cannot name organisms confidently. In our proposal we will develop a practical phylogeny-based approach for identification of *Phyllosticta*, focusing on Thai species. Variations in the mitochondrial genome, ribosomal DNA, β-tubulin, TEF1α and other appropriate genes will be investigated; all are in wide use in other fungal genera to resolve problems in identification and taxonomy. The successful outcome of this project will have important practical implications to the plant pathology, plant breeding and quarantine communities and important publications. This is a hot topic and will result in several highly cited papers and bring Mae Fah University and Thailand as one of the world leaders in Plant Pathology research.

4. ระเบียบวิธีวิจัยและผลผลิตจากการวิจัย (Methodology and the research output)

Research Plan from October 2010 to September 2013

***Year 2*:** (start 1 October 2011 to 30 September 2012) Continue to collect diseased plants in Thailand and isolate taxa, and maintain cultures. Continue to carry out detailed study on the morphology and cultural characters of species. Investigate infraspecific variation within *Phyllosticta* using nuclear DNA and mtDNA. Publish first papers.

Probably the most significant finding of this study is the new species described from *Citrus maxima* (Pomelo) which causes tan spot on fruits. This finding will mean that Pomelo will no longer be subject to quarantine control in the European Union since Pomelo does not grow in these countries. *Phyllosticta* species are common on many hosts in Thailand and some species are host-specific. They are also endophytic in plants. In years 1 and 2 more than 200 collections of *Phyllosticta* were made in northern Thailand. There will be about 10 new species resulting from this study. The major finding of Year 2 is that *Phyllosticta capitalensis* is an endophyte and weak plant pathogen with a worldwide distribution presently known from 70 plant families. The fact that *P. capitalensis* is often isolated as an endophyte has important implications for studies in fungal biology and plant health. Recent studies based on molecular data have resolved some cryptic species and some novel taxa have been discovered. However, compared to the wide species diversity and taxonomic records, there is a lack of molecular studies to resolve current names in the genus. We have also sequenced the ITS, LSU, ACT, TEF and GPDH gene regions of most of our isolates and this will be continued in year three. In the final year this work will be completed. The study has resulted in five publications to date and another three publications are in preparation. We have also shown that *Phyllosticta* species have anti-microbial activity.

5. ประโยชน์ที่ได้รับ (Benefit)

We will develop a practical phylogeny-based approach for the identification of species of *Phyllosticta*, focusing on Thai species. Molecular and genetic identification will be used to define species. Analysis of ITS gene region and other gene regions will be used as genetic evidence to define species and resolve problems in identification and taxonomy. The successful outcome of this project have important practical implications to the plant pathology, plant breeding and quarantine communities and we will publish several important SCI papers resulting from this work which will be frequently cited. We have also shown antimicrobial activity in *Phyllosticta* spp. against gram positive and gram negative bacteria that can be applied for medicine and industry in the future.

บทคัดย่อ

เชื้อราสายพันธุ์ *Phyllosticta* ส่วนใหญ่เป็นเชื้อราสาเหตุโรคพืชซึ่งแพร่กระจายตัวไปทั่วโลก สามารถก่อให้เกิดโรคต่าง ๆมากมายรวมทั้งโรคใบจุดและใบจุดดำบนผลไม้ หลายสายพันธุ์ถูกรายงานว่าเป็นเชื้อราแซบโพรบและบางส่วน โดยเฉพาะอย่างยิ่ง *Phyllosticta capitalensis* เป็นเอนโดไฟท์ซึ่งแพร่กระจายไปทั่ว การศึกษาถึงลักษณะของเชื้อราชนิดนี้มีมาอย่างยาวนาน ทั้งทางด้านสัณฐานวิทยา ลักษณะของเชื้อบนอาหารเลี้ยงเชื้อ รวมถึงการอยู่ร่วมกับพืชอาศัย ถึงแม้ว่ามีการศึกษาทางด้านอนุกรมวิธานอย่างต่อเนื่องและมีการปรับปรุงแก้ไขการจัดหมวดหมู่และศึกษาจำนวนเชื้อราชนิดนี้หลายครั้ง แต่ก็ยังคงมีความสับสนมากในการกำหนดชื่อ *Phyllosticta* การวิเคราะห์ทางด้านชีวโมเลกุลจึงเข้ามามีบทบาทในการจัดจำแนกเชื้อราเช่น *Phyllosticta* โดยเบื้องต้น การใช้ส่วนของยีนITS สามารถใช้ในการจัดจำแนกได้ แต่ยังคงจำแนกในระดับสายพันธ์ได้ไม่ดีเท่าที่ควรในปัจจุบัน การเข้ามามีบทบาทของ multigene phylogenetic analysis โดยการวิเคราะห์เชื้อราในสกุลนี้จะใช้ 2 ยีน (ITS และ ACT) และ 5 ยีน (ITS, LSU, ACT, TEF และ GPDH) ในการแปลผลออกมาให้อยู่ในรูปของแผนผังวงศ์วานวิวัฒนาการควบคู่ไปกับการศึกษาด้านสัณฐานวิทยาและการศึกษาด้านความสามารถในการก่อโรค โดยในการศึกษาครั้งนี้ได้ทำการอธิบายรูปร่างลักษณะของเชื้อราสกุลนี้ประมาณ 10 ตัวอย่าง

ตั้งแต่เริ่มต้นการศึกษา (ตุลาคม 2010) มีเชื้อมากกว่า 10 ตัวอย่าง ได้ถูกยืนยันแล้วว่าเป็นเชื้อก่อโรค ซึ่งเป็นสายพันธุ์ที่พบในประเทศไทย ในปีแรกของงานวิจัย ได้มีการเก็บเชื้อได้มากกว่า 200 ตัวอย่างจากพืชและผลไม้ที่เป็นโรคในหลายพื้นที่ในประเทศไทยและมากกว่า 72 ตัวอย่างที่ถูกจำแนกให้อยู่ในสถานะ Endophyte ในปีที่สองได้ศึกษาถึง Phyllosticta capitalensis ซึ่งเป็นราที่อยู่ใน สถานะ Endophyte และ เชื้อก่อโรคที่ไม่รุนแรง โดยมีการกระจายตัวของโรคไปอย่างกว้างขวาง โดยพบเชื้อสายพันธ์นี้ในกว่า 70 สายพันธุ์ของพืชอาศัย รวมถึงทางคณะวิจัยได้ทำการศึกษาลำดับพันธุกรรมของยีน 5 ยีน ของเชื้อ 28 ตัวอย่างรวมทั้งการศึกษาทางด้านความสามารถในการก่อโรค ทางผู้วัจัยได้ทำการหาลำดับพันธุกรรมของเชื้อ 101 ตัวอย่าง จากคลังเก็บเชื้อหลายแห่งและยังคงทำการวิเคาระห์ดังกล่าวอย่างต่อเนื่อง จากข้อมูลเบื้องต้น ทางคณะวิจัยได้เชื้อพบเชื้อราสายพันธุ์ใหม่ 9 สายพันธุ์ เนื่องจากปัญกาด้านการจำแนกและระบุเชื้อเป็นปัญหาระดับโลก ทั้งนี้ทางผู้วิจัยมีความร่วมมือกับต่างประเทศทั้งคณะวิจัยจากประเทศจีนและ ความร่วมมือกับนักวิจัยจากประเทศแถบยุโรป เพื่อจัดการแก้ไขปัญหาดังกล่าวในเชิงลึกต่อไป ในการศึกษาวิจัยในปีที่ 2 ทางคณะผู้จัดทำ ได้ตีพิมพ์ผลงานจำนวน 5 เรื่องโดย 4 เรื่องถูกตีพิมพ์เผยแพร่ลงในวารสารทางวิทยาศาสตร์ที่ได้รับการยอมรับ (SCI) โดยมีตัววัดความถี่ของ

บทความในวารสารโดยเฉลี่ยที่ถูกนำไปอ้างอิง(impact facter) อยู่ที่ 5.03 และได้ถูกนำไปอ้างอิงถึง 19 ครั้งนับตั้งแต่บทความนั้นได้ถูกเผยแพร่ลงในวารสาร

คำสำคัญ: เอนโดไฟท์/ *Guignardia*/ ลักษณะทางสัณฐานวิทยา/ วงศ์วานวิวัฒนาการระดับโมเลกุล/ เชื้อราโรคพืช

ABSTRACT

Phyllosticta species are predominantly plant pathogens with a worldwide distribution. They are responsible for numerous diseases including leaf spots and black spots on fruits. Several species have been reported as saprobes and some, in particular *Phyllosticta capitalensis* as endophytes with a worldwide distribution. Species recognition in *Phyllosticta* has historically been based on morphology, culture characters and host-association. Although there have been several taxonomic revisions and enumerations of species, there is still considerable confusion in the determination of *Phyllosticta* species. Molecular sequence data analysis has become commonplace in classifying plant pathogenic genera like *Phyllosticta*. Initially ITS and morphology was used to characterize species, however, they could not resolve species well. Recent multigene phylogenetic analysis in the genus have involved multi-loci combined genes with two (ITS and ACT) and five genes (ITS, LSU, ACT, TEF and GPDH) trees, as well as morphology and pathogenicity testing, so at present there are about 10 described species in the genus

At the beginning of this study (October 2010) there more than ten confirmed "molecular" species in the genus causing plant diseases worldwide and only two were known from Thailand. We therefore initiated a survey of *Phyllosticta* species infecting plants in Thailand. In the first and second years of this study we collected more than 200 fresh specimens of various disease plants and fruits from different places in Thailand. From these we successfully isolated 72 strains from fresh diseased samples or as endophytes from asymptomatic samples. The major finding of year 2 is that *Phyllosticta capitalensis* is an endophyte and weak plant pathogen with a worldwide distribution presently known from 70 plant families. We also sequenced the five genes of 28 isolates and carried out morphological as well as pathogenicity studies. We also sequenced 101 strains of other species from various culture collections and are in the process of analysing this data. Initial results indicate we have nine potential new species. We have developed collaboration with China and Netherlands and are involved in developing a practical phylogeny and morphology based approach for the identification of *Phyllosticta* species. Our collaboration with Chinese and European colleagues will bring greater depth to the research and international agreement to the findings. During year two we published five papers of which four were SCI. One of these was a review paper on *Phyllosticta* and was published in an SCI journal of 5.03 and has been cited 19 times since being published.

Keywords: endophytes / *Guignardia* /morphology / molecular phylogeny / plant pathogenic fungi

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ABBREVIATION AND SYMBOLS

ACT	=	Actin
%	=	Percent
cm	=	centimeter
DNA	=	Deoxyribonucleic acid
GPDH	=	Glyceraldehyde-3- phosphate dehydrogenase
ITS	=	Internal transcribed spacer
LSU	=	Large subunit (28S rDNA)
µm	=	micrometer
No.	=	Number
PDA	=	Potato Dextrose Agar
sp.	=	species
TEF1α	=	Translation elongation factor 1-alpha
USA	=	United States of America
USDA	=	United States Department Of Agriculture

CHAPTER 1

INTRODUCTION

The genus *Phyllosticta* contains more than 3,000 names and presently there are more than 50 estimated species. *Phyllosticta* species are worldwide in distribution and cause major damage to cereals, vegetables, legumes, ornamental plants and fruit trees. The current naming of *Phyllosticta* species is largely based on a combination of morphological and cultural characteristics. These are however, limited numbers of morphological character-suites available in culture coupled with inherent phenotypic plasticity, precise identification of the species has always been difficult. Physiological specialization within species and overlapping host ranges mean that our current classification system is impracticable for users. This causes problems to systematics, plant pathologists, plant health practitioners, plant breeders and quarantine officers, since they cannot name organisms confidently. In our proposal we will develop a practical phylogeny-based approach for identification of *Phyllosticta*, focusing on Thai species. Variations in the mitochondrial genome, ribosomal DNA, TEF1α and other appropriate genes will be investigated; all are in wide use in other fungal genera to resolve problems in identification and taxonomy. The successful outcome of this project will have important practical implications to the plant pathology, plant breeding and quarantine communities and important publications. This is a hot topic and will result in several highly cited papers and bring Mae Fah University and Thailand as one of the world leaders in Plant Pathology research.

CHAPTER 2

REVIEW OF RELATED LITERATURE

The genus *Phyllosticta* and its *Guignardia* sexual morph cause economically significant diseases of banana, citrus, coffee, grape, orchids, palms and mango (Van der Aa and Vaney, 2002; Wulanderi *et al.*, 2009). *Phyllosticta* species cause losses by damaging the fruits; or affecting leaves, thereby reducing yield and quality of plant products (Van der Aa and Vaney, 2002).

The diseases caused by *Phyllosticta* species are usually leaf spots which reduce the yield of the crop or make the leafy vegetables valueless. *Phyllosticta* species may cause black or tan spots on fruits such as orange or pomello; this makes the product both valueless, but also has important quarantine implications. For instance, in yam, *Phyllosticta dioscorae* appears as a leaf spot that spreads and develops rapidly and kills leaves, and sometimes entire yam plants. Citrus Black spot caused by *Phyllosticta citrocarpa* is a quarantine pest in Europe and the USA (Wulanderi *et al.*, 2009).

Many species of *Phyllosticta* are relatively unspecialized in their host range and disease symptoms (Van der Aa and Vaney, 2002), while other are thought to be specific in their host range. However, knowledge of host occurrence of most species is relatively poor and should be researched. The taxonomy of *Phyllosticta* species is complicated by the fact that there are few morphological characters to differentiate species and by the practices of some earlier mycologists, who defined new species based on fungus/host relationships with little or no consideration of morphology of previously described species (Van der Aa and Vaney, 2002).

Of the diseases caused by *Phyllosticta*, those on Citrus have been relatively well researched (Wulanderi *et al.*, 2009), however few other species have been well researched and our knowledge of the genus *Phyllosticta* in Thailand is poor. A few species of *Phyllosticta* causing leaf spot diseases have been reported in Thailand, such as *Phyllosticta* sp. on pear (Visarathanonth, http://www.actahort.org/members/showpdf?booknrarnr=279_67) and a *Phyllosticta* sp causing spots on Soybean leaves (Nachaiwiang *et al.*, 2001). *Phyllosticta* endophytes have been isolated from banana

and *Amomum* leaves (Photita et al. 2001; Bussaban et al. 2001) and a *Phyllosticta* sp. is known to cause post harvest disease of Durian (Poeltz, 2003). However a search on the topic reveals that very little is known concerning *Phyllosticta* species in Thailand and most taxa are named as *Phyllosticta* sp. There is obviously much work required to establish the diversity and importance of the genus in Thailand.

The clarification of species concepts in *Phyllosticta* is a matter of considerable practical importance for identifying taxa as well as establishing host range and geographic distribution data (Bailey *et al.*, 1992). This is essential for the work of quarantine and trade, and plant pathologists who need to diagnose and control diseases using appropriate disease management strategies. It is important that we develop new methods to identify *Phyllosticta* species easily using morphology or cultural data, but which can be confirmed by molecular data. This project thus will clarify the understanding of the taxonomy of *Phyllosticta* species, particularly for taxa which cause disease of a range of hosts using morphological characters and sequence data. It will also look for new methods to identify taxa.

Molecular approaches are being used to resolve problems in fungal taxonomy and fungal identification by many workers (Lee and Taylor 1990; Rollo et al. 1995; Ranghoo and Hyde 1998; Guo et al. 2000; Liew et al. 2002;). Because of the shortcomings of *Phyllosticta* systematics based on cultural characteristics and morphology there is need for a combined approach including the use of molecular data. The current classification of *Phyllosticta* species is broad and has a limited practical significance. It is well accepted that the systematic of the genus *Phyllosticta* awaits a detailed investigation and refinement.

CHAPTER 3
RESEARCH METHODOLOGY

Fungal isolates

(1) Collection of the samples

Phyllosticta isolates were collected from leaf spots and diseased fruits of various hosts, such as agaves, banana, coffee, palms, mango and Yams from the Provinces of Chiang Mai and Chiang Rai in northern Thailand.

(2) Morphological examination

Morphological characters of selected isolates collected, such as characters of culture colony, conidia, appressoria, setae and sclerotia were examined from pure culture.

(3) Phylogenetic study

Strains were grown on Malt Extract Agar at room temperature for 2-3 days, after which the mycelium was harvested. DNA were isolated using Ultraclean ™ Microbial DNA kit (Mo Bio, Calsbad, CA, USA) according to manufacturer's protocol. Transcribed spacer-polymerase chain reaction (ITS-PCR) was performed with primers V9G (5′-TTAAGTCCCTGCCCTTTGTA-3′; De Hoog & Gerrits van den Ende 1998) and ITS4 (5′ TCCTCCGCTTATTGATATGC-3′) as described by white et al 1990, the primer LROR (5′-GTACCCGCTGAACTTAAGC-3′) and LR5 (5′-TCCTACCACCAAGATCT-3′) were used for amplify part of 28S large subunit snRNA (LSU) were described by Vilgalys and Hester, 1990. Part of elongation factor 1-α gene (TEF-1) were amplified with forward primers EF1 (5′-ATGGGTAAGGA(A/G)GACAAGAC-3′) and reverse primer EF2 (5′-GGA(G/A)GTACCAGT(G/C)ATCATGTT-3′) (O'Donnell et al 1998). The primers ACT-512 F and ACT – 783R were used for amplify the part of actin gene (ACT) (Carbone and Kohn 1999). The partial glyceraldehyde-3- phosphate dehydrogenase (GPDH) were amplified by primers Gpd1-LM (5′-ATTGGCCGCATCGTCTTCCGCAA-3′) and Gpd2-LM (5′-

CCCACTCGTTGTCGTACCA-3′) for forward and reverse primers (Myllys *et al.* 2002). For *P. citricarpa* isolated were amplified by specific primer Gpd1 (Guerber et al. 2003) and GPDHR2 (5′- CTCRGMRGCRGCCTTGATGG-3′) was developed by Glienke et al. (2011). Cycle sequencing of PCR products was performed in PCR condition. PCR products were separated by gel electopholesis at 130 volt for 20 min in 1% agarose gel in 1x TAE running buffer and visualized under UV light by using a GeneGenius Gel Documentation and Analysis System (Syngene, Cambridge, UK). Purified PCR sequenced using both PCR primers with a BigDay Terminator Cycle Sequencing Kit V3.1 (Applied Biosystems, Foster City, CA, USA) containing AmpliTag DNA Polymerase. The amplify product were analyzed on an automatic DNA sequence (Perkin-Elmer, Norwalk, CN). Sequences generated were automatically aligned using MAFFT v.6 (http://mafft.cbrc.jp/alignment/server/). And the sequences were corrected manually aligned using MEGA v5.05 software (Tamura et al. 2011). Phylogenetic analyzing were executed by Phylogenetic analyses Using Parsimony; PAUP version 4.0b10 (Swafford 2003). For parsimony analysis, alignment gaps were treated as a fifth character state and all character were unordered and equal weight. *Botryosphaeria obtusa* was represented as outgroup for the phylogenetic tree. The confidence limit of the resulting tree was estimated by bootstrap analysis with 1000 replication (Hillis and Bull 1993). Tree length (TL), consistency (CI), retention index (RI) and rescaled consistency index (RC) were calculated and the resulting tree were done in Adobe Illustrator CS3. Novel and representative sequences were deposited in GenBank

CHAPTER 4

RESULTS AND DISCUSSION

Phyllosticta spp. were collected throughout Northern of Thailand from agricultural fields, waterfalls, national parks and house gardens. *Phyllosticta* species cause spots on living leaves and are also saprobes on dead leaves, but the pathogenic species are generally different from those on fallen leaves. Normally, pycnidia develop as black spots and black hyphae on leaf lesions. The *Guignardia* sexual and *Phyllosticta* asexual state are often found in the same leaf lesion. Fresh material of plant infected by *Phyllosticta* or *Guignardia* was isolated by endophyte technique, hyphal tip and single spore isolation. Conidia are typically small to medium sized, 5–10 µm in diam, hyaline, one–celled, have a thin and flexible sheath, are smooth-walled with an apical appendage. Often in dried specimens the appendage could not be observed. Characteristics and morphology have been examined in pure culture, where colonies form irregularly folded crusts and have dark mycelium. Some species produce white tendril of mycelium on the upper surface of the colony, which after 2 weeks on PDA is 2-3 cm in diam.

Life cycle

Concepts concerning life cycles of plant pathogens may have significant practical consequences for plant pathologists and taxonomists. Herein, we provide a schematic diagram of the life cycle of a typical species of *Phyllosticta* (*Phyllosticta ampelicida*, Fig. 1).

After infection by the *Phyllosticta* or *Guignardia* propagules, pycnidia and/or ascomata develop under the leaf tissue, and produce Leaf spot on the host. During the wet season conidia and ascospores, and sometimes the spermatial stage are present. Subsequently spores are released and ejected from the pycnidia and ascomata. The spores are carried by rain and wind to other leaves and young fruits. Germ tubes develop from spores and grow into leaves and develop within the plant tissue. The disease spreads by transmission by warm wind and rain during the wet season.

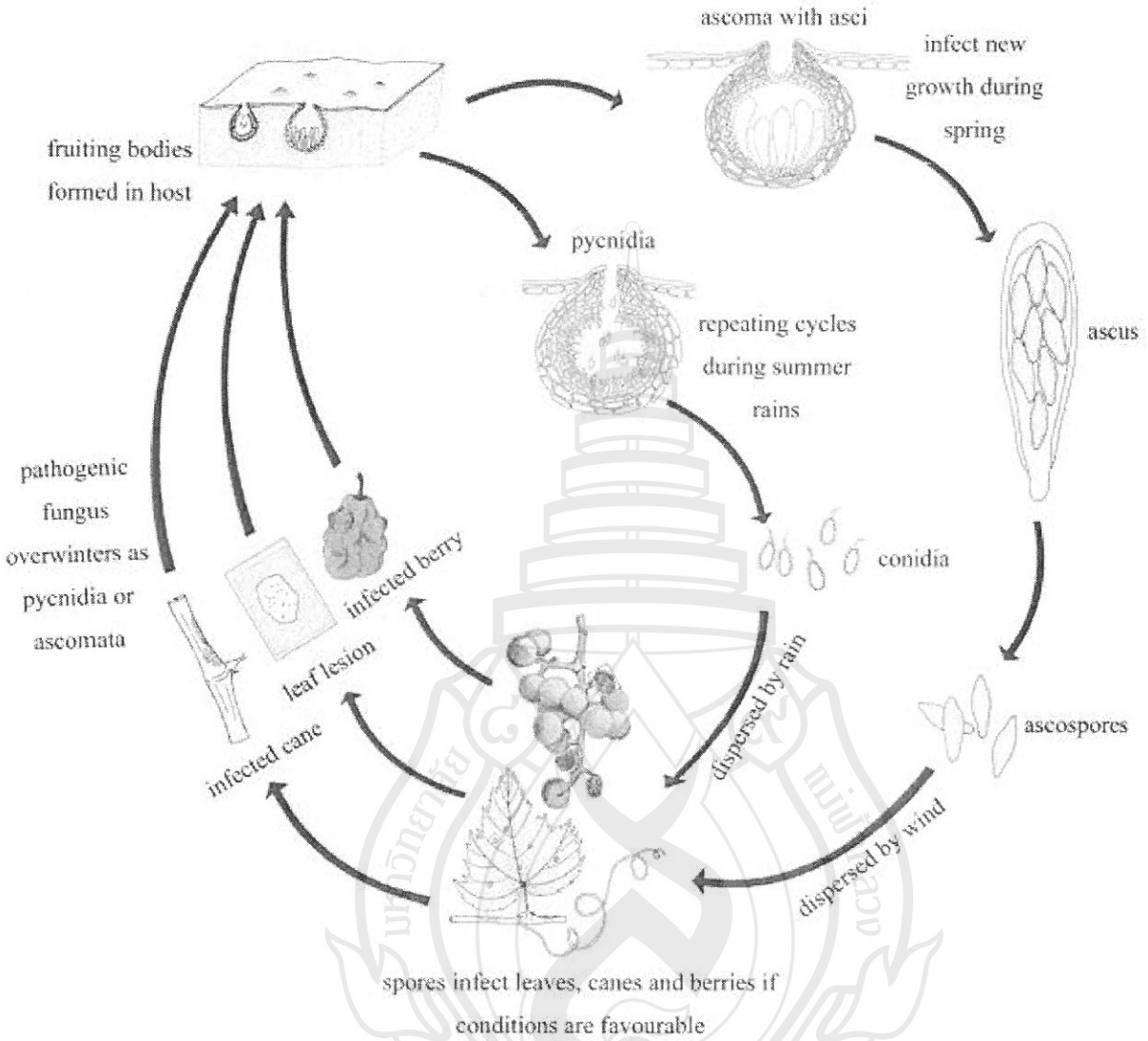

Figure 4-1 Schematic representation of the life cycle of *Phyllosticta* and its sexual morph redrawn from: http://www.oardc.ohio-state.edu/fruitpathology/organic/grape/All-Grapes.html

Host Range

Species in the genus *Phyllosticta* are mostly plant pathogens of a wide range of hosts and are responsible for diseases including Leaf spot and black spots on fruits (Baayen et al. 2001, Wulandari et al. 2009, Glienke et al. 2011, Wang et al. 2012). There are about 3200 names listed for the genus *Phyllosticta* in Index Fungorum (http://www.indexfungorum.org/; accessed February 2013) and 3340 names in MycoBank (http://www.mycobank.org/; accessed February 2013). The USDA Fungal Database lists 78 *Phyllosticta* records associated with plant hosts (http://nt.ars-grin.gov/fungaldatabases/; accessed February 2013).

Phyllosticta capitalensis has been repeatedly isolated worldwide from healthy plant tissues as an endophyte and rarely from leaf spot as a pathogen, and has been recorded from almost 70 plant families (Baayen et al. 2002, Okane et al. 2003, Motohashi et al. 2009). The fact that it is isolated so often as an endophyte has important implications to studies of fungal biology including plant pathology methodology, ecological results of endophyte studies and screening for novel compounds from endophytes.

Table 4-1: Hosts and countries from which *Phyllosticta capitalensis* has been isolated

Plant family	**Plant genus**	**Country**	**Reference**
Acanthaceae	*Mackaya*	South Africa	Carroll (1990)
Anacardiaceae	*Anacardium*	Brazil	Glienke et al. (2011)
	Comocladia	Puerto Rico	Carroll (1990)
	Loxostylis	South Africa	Carroll (1990)
	Mangifera	Brazil	Carroll (1990)
		Ghana	Baayen et al. (2002)
	Myracrodruon	Brazil	Glienke et al. (2011)
	Rhus	South Africa	Baayen et al. (2002)
	Sclerocarya	South Africa	Carroll (1990)
	Spondias	Brazil	Carroll (1990)
Annonaceae	*Monanathotaxis*	South Africa	Carroll (1990)
	Polyalthia	Thailand	Present study
Apocynaceae	*Aspidosperma*	Brazil	Glienke et al. (2011)
	Secamone	South Africa	Carroll (1990)
	Cerbera	Japan	Okane et al. (2003)
	Nerium	Japan	Motohashi et al. (2009)

Plant family	Plant genus	Country	Reference
Aquifoliaceae	*Ilex*	USA	Carroll (1990)
		Japan	Okane et al. (2003)
	Cerbera	Japan	Okane et al. (2003)
Araliaceae	*Cussonia*	South Africa	Carroll (1990)
	Hedera	South Africa	Carroll (1990)
	Polyscias	Puerto Rico	Carroll (1990)
	Schefflera	Costa Rica	Baayen et al. (2002)
	Polyscias	Thailand	Present study
Araceae	*Alocasia*	Thailand	Present study
	Anthurium	Thailand	Present study
	Dieffenbachia	Thailand	Present study
	Livistona	Thailand	Present study
	Spathiphyllum	Japan	Motohashi et al. (2009)
	Philodendron	Thailand	Present study
Asparagaceae	*Sansevieria*	Thailand	Present study
	*Ophiopogon (P)**	Thailand	Present study
Boraginaceae	*Cordia*	South Africa	Carroll (1990)
Calophyllaceae	*Calophyllum*	Thailand	Present study

Plant family	Plant genus	Country	Reference
Capparaceae	*Maerua*	South Africa	Carroll (1990)
Chrysobalanaceae	*Parinari*	South Africa	Carroll (1990)
Combretaceae	*Combretum*	South Africa	Carroll (1990)
Convolvulaceae	*Ipomoea*	Malaysia	Present study
Cornaceae (Nyssaceae)	*Curtisia*	South Africa	Baayen et al. (2002)
	Davidia	Japan	Motohashi et al (2009)
Celastraceae	*Putterlickia*	South Africa	Baayen et al. (2002)
Cercidiphyllaceae	*Cercidiphyllum*	Japan	Motohashi et al (2009)
Ebenaceae	*Diospyros*	South Africa	Carroll (1990)
	Euclea	South Africa	Carroll (1990)
Ericaceae	*Rhododendron*	Japan	Okane et al. (2003)
	Enkianthus	Japan	Okane et al. (2001)
	Vaccinium	New Zealand	Glienke et al. (2011)
Fabaceae	*Bowdichia*	Brazil	Glienke et al. (2011)
	Cercis	Japan	Motohashi et al (2009)
Fagaceae	*Lithocarpus*	Japan	Motohashi et al (2009)
Ginkgoaceae	*Ginkgo*	Japan	Motohashi et al (2009)
Lamiaceae	*Vitex*	Malaysia	Present study

Plant family	Plant genus	Country	Reference
Lauraceae	*Cinnamomum*	Japan	Okane et al. (2003)
	Ocotea	South Africa	Carroll (1990)
Lecythidaceae	*Barringtonia*	South Africa	Baayen et al. (2002)
Leguminosae	*Caesalpinia*	Japan	Okane et al. (2003)
Loganiaceae	*Stychnos*	South Africa	Carroll (1990)
	Anthocleista	South Africa	Carroll (1990)
Lythraceae	*Punica* (P)	Thailand	Present study
Malvaceae	*Hibiscus*	Thailand	Present study
Meliaceae	*Ekebergia*	South Africa	Carroll (1990)
	Trichilia	South Africa	Baayen et al. (2002)
Menispermaceae	*Cocculus*	USA	Carroll (1990)
Moraceae	*Artocarpus*	Thailand	Baayen et al. (2002)
	Ficus (P)	Thailand	Present study
	Morus	Thailand	Stringari et al. (2009)
Magnoliaceae	*Michelia*	Thailand	Present study
	Magnolia	Thailand	Glienke et al. (2011)
		USA	Carroll (1990)
Menispermaceae	*Tinospora*	Thailand	Present study

Plant family	Plant genus	Country	Reference
Euphorbiaceae	*Clutia*	South Africa	Baayen et al. (2002)
	Croton	South Africa	Carroll (1990)
	Codiaeum	Thailand	Present study
	Ctenomeria	South Africa	Carroll (1990)
	Euphorbia	Thailand	Present study
Flacourtiaceae	*Dovyalis*	South Africa	Carroll (1990)
Iteaceae	*Itea*	USA	Carroll (1990)
Lamiaceae	*Tectona*	Thailand	Present study
Musaceae	*Musa*	Thailand	Okane et al. (2003)
		Indonesia, USA	Glienke et al. (2011)
Myrtaceae	*Eucalyptus*	Brazil, South Africa	Carroll (1990), Glienke et al. (2011)
	Psidium	Brazil	Baayen et al. (2002)
Oleaceae	*Ligustrum*	Japan	Motohashi et al (2009)
	Schrebera	South Africa	Carroll (1990)
Ophioglossaceae	*Botrychium*	USA	Carroll (1990)
Orchidaceae	*Arundina*	Japan	Okane et al. (2003)
	Coelogyne	Thailand	Carroll (1990)
	Dendrobium	Thailand	Present study

Plant family	Plant genus	Country	Reference
	Paphiopedilium	Germany	Okane et al. (2001)
Orchidaceae	*Rhynchostylis* sp.	Malaysia	William & Liu (1976), Singh (1980)
	Stanhopea	Brazil	Glienke et al. (2011)
Pittosporaceae	*Pittosporum*	Hawaii	Baayen et al. (2002)
Poaceae	*Saccharum*	Thailand	Present study
Podocarpaceae	*Podocarpus*	South Africa	Carroll (1990)
Proteaceae	*Leucospermum*	Hawaii	Carroll (1990)
	Protea	Hawaii	Carroll (1990)
	Telopea	Australia	Carroll (1990)
Pittosporaceae	*Pittosporum*	Japan	Motohashi et al. (2009)
Pteridophta	**Pteridophytes**	Japan	Okane et al. (2003)
Rhamanaceae	*Scutia*	South Africa	Carroll (1990)
	Zizyphus	South Africa	Carroll (1990)
Rhizophoraceae	*Kandelia*	Japan	Okane et al. (2003)
Rosaceae	*Cliffortia*	South Africa	Carroll (1990)
	Rubus	Japan	Okane et al. (2003)
	Prunus	Japan	Okane et al. (2003)
	Eriobotrya	Japan	Motohashi et al. (2009)

Plant family	Plant genus	Country	Reference
Rubiaceae	*Canthium*	South Africa	Carroll (1990)
	Coprosma	Hawaii	Baayen et al. (2002)
	Gardenia	South Africa	Carroll (1990)
	Pavetta	South Africa	Carroll (1990)
	Rauvolfia	South Africa	Carroll (1990)
	Rothmannia	South Africa	Carroll (1990)
Rutaceae	*Zanthoxylum*	Japan	Okane et al. (2003)
	Citrus (P)	Argentina, Australia, Brazil, China, Hong Kong, New Zealand, South Africa, Taiwan, Thailand, USA	Glienke et al. (2011); Wang et al. (2012)
	Fortunella	USA	Carroll (1990)
	Vitex	South Africa	Carroll (1990)
	Zanthoxylum	Pueto Rico	Baayen et al. (2002)
Sapindaceae	*Allophylus*	South Africa	Carroll (1990)
	Dodonaea	Hawaii	Carroll (1990)
	Litchi	South Africa	Carroll (1990)

Plant family	Plant genus	Country	Reference
Sapindaceae	*Nephelium*	USA	Glienke et al. (2011)
Sapindaceae	*Paullinia cupana*	Brazil	Baayen et al. (2002)
Smilacaceae	*Smilax*	South Africa	Glienke et al. (2011)
Solanaceae	*Capsicum*	Dominican Republic	Glienke et al. (2011)
Stangeriaceae	*Stangeria*	South Africa	Baayen et al. (2002)
Sterculiaceae	*Sterculia*	Puerto Rico	Carroll (1990)
Theaceae	*Camellia*	USA	Baayen et al. (2002)
Tiliaceae	*Grewia*	South Africa	Carroll (1990)
Trimeniaceae	*Xymalos*	South Africa	Carroll (1990)
Ulmaceae	*Trema*	South Africa	Carroll (1990)
Veronicaceae	*Hebe (Veronica)*	South Africa	Carroll (1990)
Viscaceae	*Viscum*	South Africa	Stringari et al. (2009)
Vitaceae	*Ampelopsis*	USA	Baayen et al. (2002)
	Cryphostemma	South Africa	Carroll (1990)
	Rhoicissus	South Africa	Carroll (1990)
Zamiaceae	*Encephalartos*	South Africa	Carroll (1990)
Zingiberaceae	*Amomum*	Thailand	Okane et al. (2003)
	Zingiber	Thailand	Okane et al. (2003)

*(P) = Leaf spot

Molecular study

More than 70 isolates of *Phyllosticta* sp. were collected during the first and second year of the study and were characterised using conidial morphology, colony characters and growth rates. The species were collected from crops, fruit trees, ornamental trees and horticultural plants throughout northern Thailand from agricultural fields, waterfalls, national parks and house gardens. They were found on many hosts such as banana, *Baccaurea ramiflora*, *Caryota mitis*, *Cordyline fruticosa*, *Hibiscus syriacus*, Mangifera, *Ophiopogon japonicas*, Orchidaceae. 21 strains were sequenced using three genes. Strains were subject to phylogenetics analysis of multigene regions and compared with type species in GenBank. An example of a tree is given below (Fig 4-2).

Phylogenetic analysis

Phylogenetic relationships were inferred using the internal transcribed spacer (ITS), 28s rRNA gene region (LSU), actin gene (ACT) translation elongation factor 1-α gene (TEF1) and glyceraldehyde-3- phosphate dehydrogenase (GPDH) sequences of 129 strains (including one outgroup) of *Phyllosticta* spp. were multiple aligned. The combined partial set of *Phyllosticta* comprised 2577 characters (including gaps), of which 1547 characters are constant, 296 characters are variable and parsimony-uninformative. Parsimony analysis generated 1000 trees, one of the trees was chosen by similar with bootstrap values (number of bootstrap replicated = 1000) and is shown in Fig. 4-3 (TL = 3173, CI = 0.517, RI = 0.906, RC = 0.468, HI = 0.483)

The phylogenetic tree using combined multi-locus genes shows 35 clades of *Phyllosticta* spp. Clade 1, consists of *Phyllosticta owaniana* isolated from *Brabejum stellatifolium*, South Africa. Clade 2 is composed of a single isolate of *P. pseudotsugae* isolated from *Pseudotsuga maziesii*, USA. Clade 3, *P. podocarpi* isolated from *Podocarpus lanceolata*, South Africa. Clade 4, one strain of *G. rhodorae* isolated from rhododendron, Netherlands. Clade 5, one isolates of *P. vaccinicola* from *Veccinium macrocarpum*, USA. Clade 6, one strain of *P. vaccinii* isolated from *Oxycoccus macrocarpus*, USA. Clade 7, *P. hypoglossi* isolated from *Rucus hypoglosum*, Italy. Clade 8, *P. cussonia* isolated from *Cussonia* sp., South Africa. Clade 9, a single strain of *P. spinarum* isolated from *Chamaecyparis pisifera*,

France. Clade 10, *P. hederaceae* isolated from *Hedera* sp., Spain and Italy. Clade 11, *P. citribraziliensis* represented a recently described species isolated from *Citrus* spp., Brazil. Clade 12, *P. bifrenariae* isolated from orchid, Brazil. Clade 13, *P. hymenocallidicola* isolated from *Hymenocallis littoralis*, Australia. Clade 14, *P. citrimaxima* isolated from *Citrus maxima*, Thailand. Clade 15, *P. citriasiana* isolated from *Citrus maxima*, Asia. Clade 16, *P. citricarpa* isolated from *Citrus* spp., worldwide. Clade 17, *G. philoprina* isolated from *Cryptomeria japonica*, USA and *Taxus baccata*, Netherlands. Clade 18, *P. macrophyllus* isolated from *Podocarpus maki*, New Zealand. Clade 19, contains one strain from Tasmania isolated from *Telopea speciosissima* as *P. telopeae*. Clade 20, single strain isolated from *Aesculus hippocastanum*, Germany as *G. aesculi*. Clade 21, *P. minima* isolated from *Acer rubrum,* USA. Clade 22, *P. pachystimae* isolated from *Paxistima myrsinites*, USA. Clade 23, *G. gaultheriae* isolated from *Gaulteria humifusa*, USA. Clade 24, one strain of *P. rubrum* isolated from *Acer rubrum*, USA. Clade 25, one strain of *P. grandicola* isolated from *Eucalyptus grandis*, Spain. Clade 26, two strains of *P. ilicicola* isolated from *Ilex aquifolium*, Europe. Clade 27 *P. mangiferaceae* isolated from *Mangifera indica*, Thailand. Clade 28 *P. brazillianae* isolated from *Mangifera indica*, Brazil. Clade 29 two strains of *P. mangifericola* isolated from *Mangifera indica*, Brazil. Clade 30, *P. eugeniae* isolated from *Eugenia aromatic*, Sumatra. Clade 31, five strains from *Aloe ferox*, South Africa. They were described as *P. aloeicola* Clade 32, one strain of *P. beaumarisii* isolated from *Muehlenbeckia adpressa*, Australia. Clade 33, *G. mangiferae* isolated from *Mangifera indica*, India. Clade 34, two strains of *P. cordylinophila* isolated from *Cordyline fruticosa*, Thailand. Clade 35, 52 isolates of *P. capitalensis* isolated from many host plants, worldwide.

Table 4-2 Isolates of *Guignardia* and *Phyllosticta* used in the phylogenetic study. Bold means type species

Strain	**Code**	**Host**	**Mode***	**Country**	**Gene and GenBank No.**		
					ITS	**TEF1**	**ACT**
G. bidwellii	CBS 111645	*Parthenocissus quinquefolia*	P	USA	JN692542	EU683653	JN692518
G. mangiferae	IMI 260576	*Mangifera indica*	E	India	JF261459	JF261501	JF343641
P. brazilianiae	LGMF 333	*Mangifera indica*	E	Brazil	JF343574	JF343595	JF343658
P. brazilianiae	LGMF 334	*Mangifera indica*	E	Brazil	JF343566	JF343587	JF343650
***P. brazilianiae* ex-type)**	**LGMF 330 CBS 126270**	***Mangifera indica***	**E**	**Brazil**	**JF343572**	**JF343593**	**JF343656**
P. capitalensis	CPC 20251	wild plant	P	Thailand	KC291333	KC342553	KC342530
P. capitalensis	CPC 20252	*Punica granatum*	P	Thailand	KC291334	KC342554	KC342531
P. capitalensis	CPC 20254	*Saccharum officinarum*	E	Thailand	KC291335	KC342555	KC342532
P. capitalensis	CPC 20255	*Arecaceae*	P	Thailand	KC291336	KC342556	KC342533
P. capitalensis	CPC 20256	***Ophiopogon japonicus***	P	Thailand	KC291337	KC342557	KC342534

Strain	Code	Host	Mode*	Country	Gene and GenBank No.		
					ITS	TEF1	ACT
P. capitalensis	CPC 20257	*Ficus benjamina*	P	Thailand	KC291338	KC342558	KC342535
P. capitalensis	CPC 20259	*Orchidaceae*	P	Thailand	KC291340	KC342560	KC342537
P. capitalensis	CPC 20263	*Magnoliaceae*	E	Thailand	KC291341	KC342561	KC342538
P. capitalensis	CPC 20266	*Polyscias* sp.	E	Thailand	KC291342	KC342562	KC342539
P. capitalensis	CPC 20268	*Hibiscus syriacus*	E	Thailand	KC291343	KC342563	KC342540
P. capitalensis	CPC 20269	*Ophiopogon japonicus*	E	Thailand	KC291344	KC342564	KC342541
P. capitalensis	CPC 20270	*Tectona grandis*	E	Thailand	KC291345	KC342565	KC342542
P. capitalensis	CPC 20272	*Orchidaceae*	P	Thailand	KC291346	KC342566	KC342543
P. capitalensis	CPC 20275	*Polyalthia longifolia*	E	Thailand	KC291347	KC342567	KC342544
P. capitalensis	CPC 20278	*Euphorbia milii*	E	Thailand	KC291348	KC342568	KC342545
P. capitalensis	CPC 20423	*Philodendron* 'Xanadu'	P	Thailand	KC291349	KC342569	KC342546

Strain	Code	Host	Mode*	Country	Gene and GenBank No.		
					ITS	TEF1	ACT
P. capitalensis	LC 0002	*Alocasia* sp.	E	Thailand	KC291350	KC342570	KC342547
P. capitalensis	LC 0008	*Anthurium* sp.	E	Thailand	KC291352	KC342572	KC342549
P. capitalensis	LC 0009	*Sansevieria hyacinthoides*	E	Thailand	KC291353	KC342573	KC342550
P. capitalensis	LC 0010	*Tinospora craspa*	E	Thailand	KC291354	KC342574	KC342551
P. capitalensis	LC 0025	*Calophyllum* sp.	E	Thailand	KC291355	KC342575	KC342552
P. capitalensis	CBS 100175	*Citrus* sp.	E	Brazil	FJ538320	FJ538378	FJ538436
P. capitalensis	CBS 114751	*Vaccinium* sp.	P	New Zealand	EU167584	FJ538407	FJ538465
P. capitalensis	CBS 115046	*Myracrodruon urundeuva*	E	Brazil	FJ538322	FJ538380	FJ538438
P. capitalensis	CBS 115047	*Aspidosperma polyneuron*	E	Brazil	FJ538323	FJ538381	FJ538439
P. capitalensis	CBS 115049	*Bowdichia nitida*	E	Brazil	FJ538324	FJ538382	FJ538440
P. capitalensis	CBS 123373	*Musa paradisiaca*	E	Thailand	FJ538341	FJ538399	FJ538457

Strain	Code	Host	Mode*	Country	Gene and GenBank No.		
					ITS	TEF1	ACT
P. capitalensis	CBS 123404	*Musa paradisiaca*	E	Thailand	FJ538333	FJ538391	FJ538449
P. capitalensis	LGMF 03	*Citrus lalifolia*	P	Brazil	JF261452	JF261494	JF343634
P. capitalensis	LGMF 181	*Citrus reticulata*	P	Brazil	JF261447	JF261489	JF343629
P. capitalensis	LGMF 219	*Citrus sinensis*	E	Brazil	JF261448	JF261490	JF343630
P. capitalensis	LGMF 240	*Citrus sinensis*	E	Brazil	JF261443	JF261485	JF343625
P. capitalensis	LGMF 222	*Citrus sinensis*	E	Brazil	JF261450	JF261492	JF343632
P. capitalensis	LGMF 220	*Citrus sinensis*	E	Brazil	JF261446	JF261488	JF343628
P. capitalensis	LGMF 358	*Mangifera indica*	E	Brazil	JF261449	JF261491	JF343631
P. capitalensis **(ex-epitype)**	**CPC18848**	***Stanhopea graveolens***	**P**	**Brazil**	**JF261465**	**JF261507**	**JF343647**
P. citriasiana **(ex-type)**	**CBS 120486**	***Citrus maxima***	**P**	**Thailand**	**FJ538360**	**FJ538418**	**FJ538476**
P. citriasiana	CBS 123370	*Citrus maxima*	P	Vietnam	FJ538355	FJ538413	FJ538471

Strain	Code	Host	Mode*	Country	Gene and GenBank No.		
					ITS	TEF1	ACT
P. citriasiana	CBS 123371	*Citrus maxima*	P	Vietnam	FJ538356	FJ538414	FJ538472
P. citriasiana	CBS 123372	*Citrus maxima*	P	Vietnam	FJ538357	FJ538415	FJ538473
P. citribraziliensis	LGMF09	*Citrus* sp.	H	Brazil	JF261436	JF261478	JF343618
P. citricarpa	CBS 102374	*Citrus aurantium*	P	Brazil	FJ538313	GU349053	FJ538429
P. citricarpa	CBS 120489	*Citrus sinensis*	P	Zimbabwe	FJ538315	FJ538373	FJ538431
***P. citricarpa* (ex-epitype)**	**CBS 127454**	***Citrus limon***	**P**	**Australia**	**JF343583**	**JF343604**	**JF343667**
P. citricarpa	CBS 127452	*Citrus reticulata*	P	Australia	JF343581	JF343602	JF343665
P. citricarpa	CBS 127455	*Citrus sinensis*	P	Australia	JF343584	JF343605	JF343668
P. citrichinaensis	**ZJUCC 200956**	***Citrus reticulata***	**P**	**China**	**JN791664**	**JN791515**	**JN791589**
P. citrichinaensis	ZJUCC 200964	*Citrus maxima*	P	China	JN791662	JN791514	JN791582
P. citrichinaensis	ZJUCC 2010150	*Citrus maxima*	P	China	JN791620	JN791459	JN791533

Strain	Code	Host	Mode*	Country	Gene and GenBank No.		
					ITS	TEF1	ACT
P. citrichinaensis	ZJUCC 2010152	*Citrus sinensis*	P	China	JN791611	JN791461	JN791535
P. hypoglossi	CBS 101.72	*Ruscus aculeatus*	P	Italy	FJ538365	FJ538423	FJ538481
P. hypoglossi	CBS 434.92	*Ruscus aculeatus*	P	Italy	FJ538367	FJ538425	FJ538483
P. hypoglossi	CBS 167.85	*Ruscus hypoglossum*	P	Italy	FJ538366	FJ538424	FJ538482
P. owaniana	CBS 776.97	*Brabejum stellatifolium*	P	South Africa	FJ538368	FJ538426	FJ538484
P. owaniana	CPC 14901	*Brabejum stellatifolium*	P	South Africa	JF261462	JF261504	JF343644
P. spinarum	CBS 292.90	*Chamaecyparis pisifera*	P	France	JF343585	JF343606	JF343669
P. podocarpi	CBS 111646	*Podocarpus falcatus*	P	South Africa	AF312013	KC357671	KC357670

*P= pathogen; E=endophyte

[1]CBS: CBS-KNAW Fungal Biodiverstiy Centre, Utrecht, The Netherlands; CPC: working collection of Pedro Crous housed at CBS; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, LC: culture collection of Nilam F. Wulandari, Chiangmai, Thailand. LGMF: culture collection of Laboratory of Genetics of Microorganisms, Federal University of Parana, Curitiba, Brazil, ZJUCC: Zhejiang University Culture Collection, Zhejiang, China.

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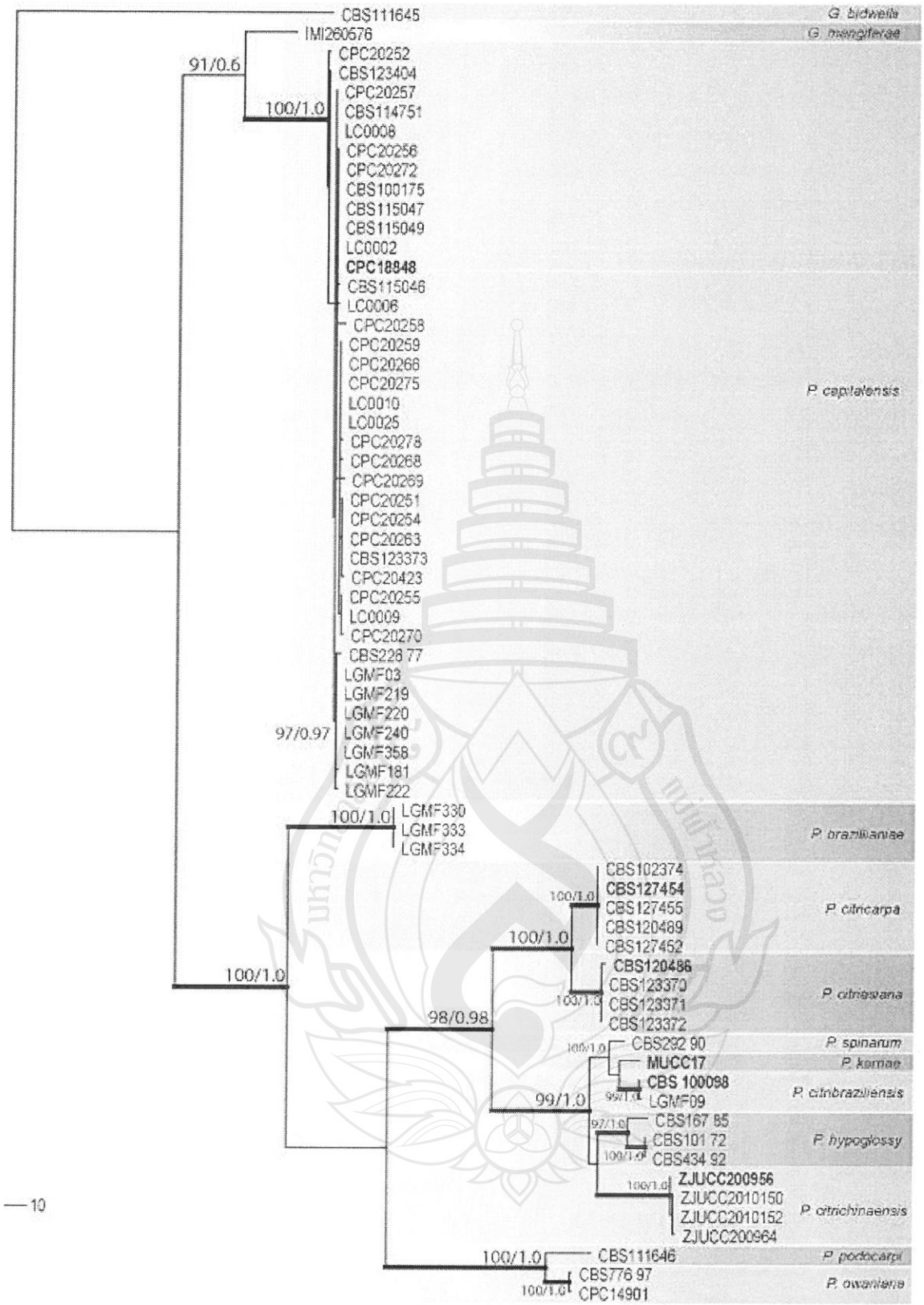

Figure 4-2 Phylogenetic tree generated from 1000 replicates. Bootstrap values parsimony analysis/Bayesian analysis based on combined ITS rDNA, TEF1 and ACT sequence data. Bold represented type and ex-type. The tree is rooted with *Guignardia bidwellii* (CBS 111645)

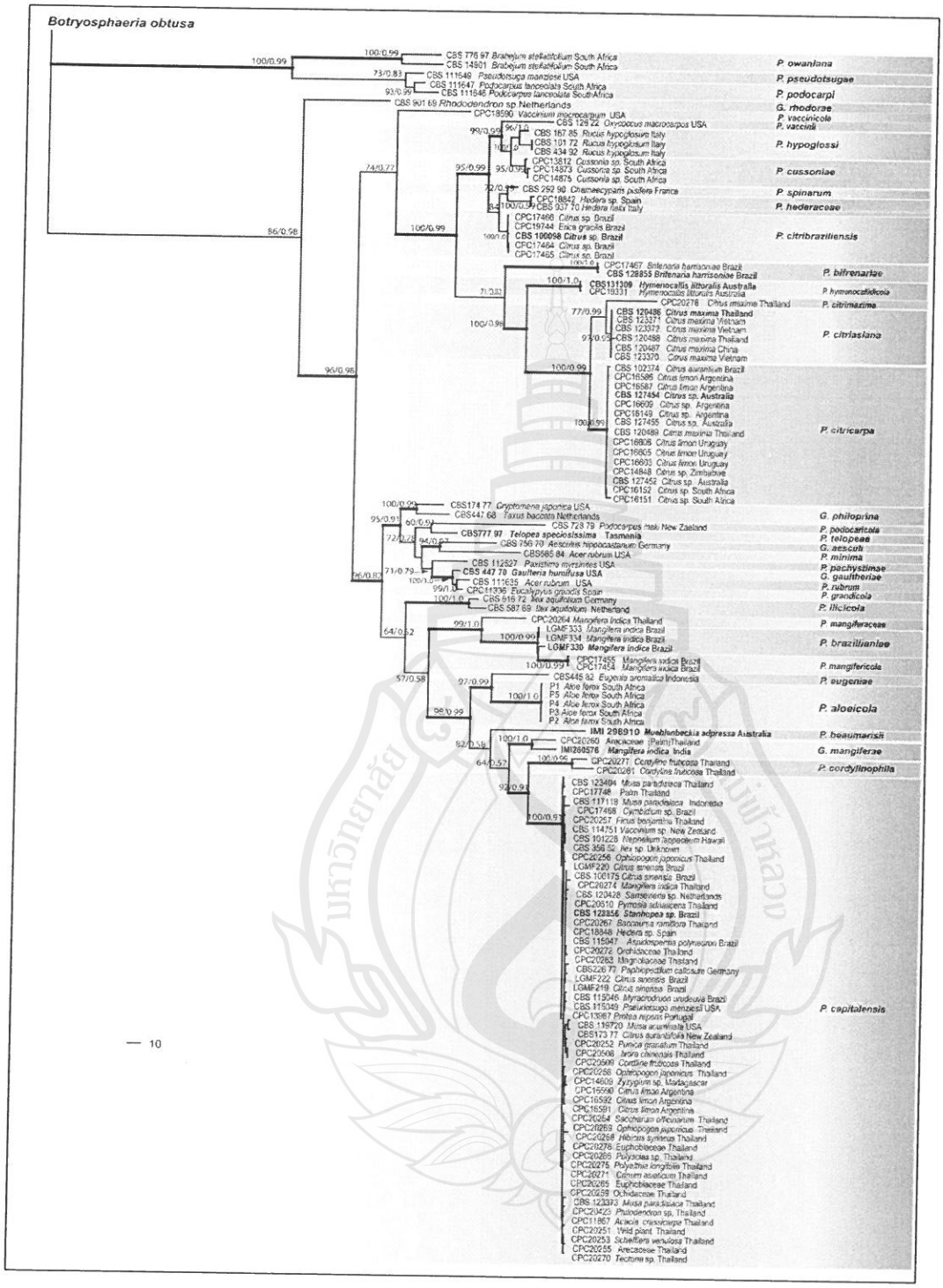

Figure 4-3 Phylogenetic tree of *Phyllosticta* generated from a maximum parsimony analysis based on the ITS, LSU, ACT, TEF and GPDH sequence alignment. Names in bold are represented types and ex-types. Values on the branches represent parsimony bootstrap support value (>50%). Thickened branches represent significant Bayesian analysis value (≥ 90%) and the scale bar indicates 10 changes. *Botryosphaeria obtusa* represent out group.

Taxonomy (new species)

The following nine strains are new species based on phylogenetic analysis and will be described in a paper to be published at the end of year three.

Phyllosticta aloeicola Wikee, P.W. Crous, S.J. Wijnand, K.D. Hyde & McKenzie sp. nov.

Host: *Aloe ferox*
Habitat: Living leaf
Collecting Site: South Africa
Specimens examined. South Africa, on living leaf of *Aloe ferox*, P.W. Crous and S.J. Wijnand

Phyllosticta citrimaxima Wikee, P.W. Crous, K.D. Hyde & McKenzie sp. nov.
Host: *Citrus maxima*
Habitat: Tan spot
Collecting Site: Thailand
Specimens examined: Specimens examined. Thailand, Chiangrai, Weing Khaen, on fruit peel of *Citrus maxima*, June 2011, Saowanee Wikee, MFUCC 13-0001 (holotype); ex-type culture CPC20276, culture MFLUCC10-0137.

Notes: *Phyllosticta citrimaxima* was isolated from *Citrus maxima*, which is commonly grown as an economic plant in Thailand and Asia. Recently, *P. citriasiana* Wulandari, Crous & Gruyter, *P. citribraziliensis* C. Glienke & Crous and *P. citrichinaensis* X.H. Wang, K.D. Hyde & H.Y. Li have been described from *Citrus maxima* in Vietnam and China (Wulandari et al. 2009, Glienke et al. 2011, Wang et al. 2012). The phylogenetic tree (Fig 10,11) showed that *P. citrimaxima* forms a distinct linage with strong bootstrap support. Wang et al. (2012) provided a table in which they compared the morphology of five *Phyllosticta* species associated with citrus: *P. citricarpa*, *P. citriasiana*, *P. capitalensis*, *P. citribraziliensis*, *P. citrichinaensis*. *P. citrimaxima* produces smaller conidia (5–8 × 3–7 µm) than *P. citricarpa* (11–12 × 6–8 µm), *P. citriasiana* (12–14 × 6–7 µm), *P. capitalensis*(11–12

× 6–7 µm), *P. citribraziliensis* (10–12 × 6–7 µm) and *P. citrichinaensis* (8–12 × 6–9 µm) but longer apical appendage (2–16 µm) than any of these four species except for *P. citrichinaensis* (14–26 µm).

Phyllosticta grandicola Wikee, P.W. Crous, M.J. Wingfield, K.D. Hyde & McKenzie sp. nov.

Host: *Eucalyptus grandis*
Habitat: Living leaf
Collecting Site: Spain
Specimens examined. Spain, on living leaf of *Eucalyptus grandis*., M.J. Wingfield, ex-type culture CPC11336

Phyllosticta hederaceae Wikee, P.W. Crous, U. Damm, K.D. Hyde & McKenzie sp. nov.

Host: *Hedera* sp.
Habitat: Living leaf
Collecting Site: Spain, Italy
Specimens examined Spain, on living leaf of Hedera sp. ex-epitype culture CPC18842

Phyllosticta macrophyllus Wikee, P.W. Crous, K.D. Hyde & McKenzie sp. nov.

Host: *Podocarpus maki*
Habitat: Seed
Collecting Site: New Zealand
Specimens examined. New Zealand, on seed of *Podocarpus maki* (intercepted from USA, Florida), September 1979, G. Laundon, Levin, CBS H-13109 (holotype); ex-type culture CBS 728.7
Note: *P. macrophyllus* had been identified by CBS as *G. philoprina* (Berk. & M.A. Curtis) Aa (http://www.cbs.knaw.nl/Collections/BioloMICS.aspx ; accessed March 2013). Phylogenetic linage has shown that it is unique from *G. philoprina*, which is known from *Rhododendron*, *Ilex* and *Taxus*.

Phyllosticta mangiferaceae Wikee, P.W. Crous, K.D. Hyde & McKenzie sp. nov.

Host: *Mangifera indica*

Habitat: Healthy leaf

Collecting Site: Thailand

Specimens examined: Thailand, Chiangrai, Nang Lae, on healthy leaf of *Mangifera indica*, June 2011, Saowanee Wikee; ex-type culture CPC20276, culture MFLUCC10-0029.

Note: *P. mangiferaceae* was isolated as an endophyte from healthy leaf of *Mangifera indica*. Several species have been reported as pathogens on *M. indiga* including *G. mangiferae* and *P. brazilianiae* (Glienk et al. 2011). *P. mangiferaceae* produced many conidia on oat meal agar and formed appressoria within 2 days. Morphology, it is distinct from *P. capitalensis* (8–11 × 5–6 µm) in producing larger conidia (6–13 × 4–6) and forms a distinct linage with 99% bootstrap support. *P. mangiferaceae* appears most closely related to *P. brazilianiae.*

Phyllosticta mangifericola Wikee, P.W. Crous, C. Glienke, K.D. Hyde & McKenzie sp. nov.

Host: *Mangifera indica*

Habitat: Leaf spot

Collecting Site: Brazil

Specimens examined. Brazil, on living leaf of *Mangifera indica*, Glienke and Stringari,ex-type culture CPC17454

Phyllosticta rubrum Wikee, P.W. Crous, K.D. Hyde & McKenzie sp. nov.

Host: *Acer rubrum*

Habitat: Living leaf

Collecting Site: USA

Specimens examined. USA, Missouri, on *Acer rubrum*, July 1999, G. Carroll, ACRU 1-1 (holotype); ex-type culture CBS 111635

Note: *Phyllosticta rubrum* has been identified as

P. minima (Berk. & M.A. Curtis) Underw. & Earle

= *P. acericola* Cooke & Ellis, Grevillea, 8(45):11, 1879

= *P. arida* Earle, Bulletin of the Torrey Botanical Club, 25:367, 1898

≡ *S. minima* Berk. & M.A. Curtis, Grevillea, 3(25):2, 1874

≡ *P. minima* (Berk. & M.A. Curtis) B. Sutton, 1897

≡ *Phoma minima* (Berk. & M.A. Curtis) Sacc., Sylloge Fungorum, 3:115, 1884

Phyllosticta vaccinicola Wikee, P.W. Crous, K.D. Hyde & McKenzie sp. nov.

Host: *Vaccinium macrocarpum*

Habitat: Living leaf

Collecting Site: USA

Specimens examined. USA, on living leaf of *Vaccinium macrocarpum*, Mariusz Tadych; ex-type culture CPC18590

In this study intron dominated sequence (ITS, ACT, TEF) and highly conserved gene coding region (LSU, GPDH) were used where ITS region are more conserved than other protein coding genes (ACT and TEF). However, the result from 5 gene tree and 2 gene tree (sequence data are limited in some fungi) tended to be the same, suggesting that for *Phyllosticta* phylogeny study ITS and ACT regions that are sufficiently distinct, but tree is more stable using more high conserved gene coding regions.

Epitypification is necessary for studying of taxonomy fungi. The taxonomy based on morphological characteristics could be done by study of holotype (material that represents a type species or genus or family). But some materials are lost, in poor condition or no cultures. Phylogenetic analysis can not be done without molecular data from type cultures. Therefore, epitypification can solve this problem (Hyde and Zhang 2008). Epitypes have been designated based on a collection from the same geographical area where fungi were first collected. Recently, epitypification and phylogeny are widely used features in taxonomy and identification of fungi such as *Colletotrichum* (Shenoy et al. 2007, Than et al. 2008, Su et al. 2011), *Ophiostoma* (Zhou et al. 2004), and *Pseudocercospora* (Avila et al. 2005).

Furthermore, a better understanding of species relationship of *Phyllosticta* and *Guignardia* knowledge about pathogenic form or non pathogenic form will improve the methods to control these fungi in the economically and ecologically safe.

Antimicrobial activity

Four *Phyllosticta* crude extracts showed an inhibitory effect against at least one of the test microbes with clear zones ranging from 7 to 11.5 mm diam (Table 3). Interestingly, the extracts derived from *P. capitalensis* not only exhibited highest antibacterial activity but they also showed a wide range of inhibitory activity against both Gram-positive and Gram-negative bacteria. The extracts produced from different strains of *P. capitalensis* (MFLUCC 10-0138 and MFLUCC 12-0015) caused different profiles of antibacterial activity. However, none of these fungal extracts could inhibit the growth of *Aspergillus niger*. These fungal extracts will be subject to further analysis as it has been reported that several *Phyllosticta* species can produce a wide range of secondary metabolites with diverse biological activities (Ezra et al. 2004, Gangadevi and Muthumary 2008, Le Calvé et al. 2011, Mangunwardoyo et al. 2012, Peláez et al. 1998, Radu et al. 2002, Srinivasan et al. 2010, Strobel et al. 2004, Wijeratne et al. 2008).

Screening endophytes for novel compounds

It has been common practice to isolate endophytes from medicinal plants using the premise that strains will be isolated that can produce bioactive compounds similar to those produced by the plant (Krohn et al. 2007, Huang et al. 2008, Kumaran et al. 2008, Xu et al. 2010, Zhao et al. 2010). The fungi are thought to have obtained the mechanisms of production of natural products from the plant by so called horizontal gene transfer (Strobel 2004). It is clear in the case of *Phyllosticta* that *P. capitalensis* will probably be the only endophyte species isolated. Therefore, we recommend that researchers screening for novel compounds should study the saprobes and pathogens as well as the endophytes. This will give a higher fungal diversity and higher likelihood of isolating rare and unusual species, and thus a higher likelihood of discovering greater chemical diversity.

Table 4-3 Antimicrobial activity of *Phyllosticta* spp. against gram positive and gram negative bacteria.

Strains (MFLUCC)	Inhibition zone mm (diam. mean ± SD)					
	Bacillus cereus	*Staphylococcus aureus*	*Micrococcus luteus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella typhimurium*
P. citriasiana (10-0137)	7.5±0.7	-	-	-	-	-
P. capitalensis (10-0138)	11.5±0.7	7.0±0.0	10.5±0.7	9.5±0.7	8.0±0.0	9.0±1.4
P. cordylinophila (12-0014)	7.0±0.0	-	-	-	7.0±0.0	-
P. capitalensis (12-0015)	8.0±1.4	7.0±0.0	8.0±1.4	8.5±0.7	7.0±0.0	7.0±0.0

(-) = no inhibition activity

CHAPTER 5

CONCLUSION

Phyllosticta species are predominantly plant pathogens with a worldwide distribution. They are responsible for numerous diseases including leaf spots and black spots on fruits. Several species have been reported as saprobes and some, in particular *P. capitalensis* are endophytes with a worldwide distribution. Species recognition in *Phyllosticta* has historically been based on morphology, culture characters and host-association. Accuracy in identifying species is important in identifying plant disease, in understanding disease epidemiology, in developing protocols import and export of crops and in developing disease resistant plants. Although there have been several taxonomic revisions and enumerations of *Phyllosticta* species, there is still considerable confusion in the determination of species. Molecular sequence data analysis has become commonplace in classifying plant pathogenic genera such as *Phyllosticta*. Initially ITS and morphology was used to characterize species, however, the ITS gene cannot resolve species well. Recent multigene phylogenetic analysis in the genus have involved multi-loci combined genes with two (ITS and ACT) and five genes (ITS, LSU, ACT, TEF and GPDH) trees, as well as morphology and pathogenicity testing, so at present there are about 10 described species in the genus

At the beginning of this study (October 2010) there more than ten confirmed "molecular" species in the genus causing plant diseases worldwide and only two were known from Thailand. We therefore initiated a survey of *Phyllosticta* species infecting plants in Thailand. In the first and second years of this study we collected more than 200 fresh specimens of various disease plants and fruits from different places in Thailand. From these we successfully isolated 72 strains from fresh diseased samples or as endophytes from asymptomatic samples. We also started to sequence these isolates and carry out morphological as well as pathogenicity studies. We identified several new potential species. We are involved in developing a practical phylogeny and morphology based approach for the identification of *Phyllosticta* species, focusing on Thai species. However, since this is a global problem we have

also chose to collaborate with Chinese and European colleagues in order to bring greater depth to the research and international agreement to the findings. In year two we will continue to isolate more strains of *Phyllosticta* from Thailand, sequence multigenes and carry out multigene analysis. We will also study worldwide strains of the genus and develop a practical phylogeny and morphology based approach for the identification of *Phyllosticta* species. Our first publications from the grant appeared in year two, which included a review paper on the problems in identification of the genus *Phyllosticta* and their biology, use in biological control and novel compound discovery from the genus.

The major finding of Year 2 is that *Phyllosticta capitalensis* is an endophyte and weak plant pathogen with a worldwide distribution presently known about 70 plant families. We isolated *P. capitalensis* from different host plants in northern Thailand, and in the process establish their different life modes. Twenty-one strains of *P. capitalensis* isolated as endophytes from 20 hosts, were subjected to phylogenetic analysis. An additional 14 strains of *P. capitalensis* from other hosts and geographic locations were also obtained from established culture collections. In all cases there was no infection of the healthy plant leaves, suggesting that this endophyte does not cause disease on healthy, unstressed host plants. The fact that *P. capitalensis* is often isolated as an endophyte has important implications for studies in fungal biology and plant health. Due to its endophytic nature, *P. capitalensis* is commonly found associated with lesions of plants, and frequently incorrectly identified as a species of serious quarantine importance, which again has serious implications for trade in agricultural and forestry produce. We have also multigene sequence data which has been analyzed for 129 isolates and resulted in 35 taxa of which nine are new species. The study has resulted in five publications up to the end of year two. Future work will investigate more species at the molecular level and result in a comprehensive understanding of the genus. *Phyllosticta* species also have the ability in antimicrobial activity. The crude extracts of four species of *Phyllosticta* inhibited growth of *Escherichia coli*, *Bacillus cereus* and *Pseudomonas aeruginosa* but were not active against *Aspergillus niger*.

Table 5-1 List of publications resulting from the grant

Years	Publications
2011	Wulandari N, To-Anun C, McKenzie E, Hyde KD (2011) ***Guignardia bispora* and *G. ellipsoidea* spp. nov. and other *Guignardia* species from palms (Arecaceae).** Mycosphere 2(2):115–128
2011	Ko Ko TW, McKenzie EHC, Bahkali AH, To-anun C , Chukeatirote E, Promputtha I, Abd-Elsalam KA, Soytong K, Wulandari NF, Sanoamuang N, Jonglaekha N, Rampai Kodsueb R, Cheewangkoon R, Wikee S, Chamyuang S, Hyde KD (2011) **The need for re-inventory of Thai phytopathogens.** Chiang Mai J. Sci. 2011; 38(4) : 625-637
2011	Wikee S, Udayanga D, Crous PW, Chukeatirote E, McKenzie EHC, Bahkali AH, Dai DQ, Hyde KD (2011) ***Phyllosticta*—an overview of current status of species recognition.** Fungal Divers 51:43–61
2011	Wikee S, Wulandari NF, McKenzie EHC, Hyde KD (2011b) ***Phyllosticta ophiopogonis* sp. nov. from *Ophiopogon japonicas* (Liliaceae).** Saudi J Biol Sci 19(2):13–16
2012	Wang X, Chen G, Huang F, Zhang J, Hyde KD, Li H (2012) Phyllosticta species associated with citrus diseases in China. Fungal Divers 52:209–224

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BIOGRAPHY AND PUBLICATIONS

ประวัติคณะผู้วิจัย (CV of Investigator)

Principal Investigator(PI)

1.) Name:

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ภาษาอังกฤษ: Dr. Kevin D Hyde

Date of Birth : May 5, 1955

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5.) Education Background

- **Doctor of Science**, University of Wales, 2001
  DISSERTATION: *Biodiversity and Biology of Tropical Microfungi*
- **Doctor of Philosophy,** University of Portsmouth, UK, 1987
  DISSERTATION: *Marine Mycology*
- **Master of Science,** University of Portsmouth, UK, 1981
  DISSERTATION: *Biodeterioration*
- **Postgraduate Certificate of Education**, Bristol University, UK, 1980

- **Bacholer of Science**, University of Wales, Cardiff, 1979 (Zoology)

6.) Field of Specialization Skill

Mycology, Plant Pathology

7.) ประสบการณ์ที่เกี่ยวข้องกับการบริหารงานวิจัยทั้งภายในและภายนอกประเทศโดยระบุสถานภาพในการทำงานวิจัยกว่าเป็นผู้อำนวยการแผนงานวิจัย หัวหน้าโครงการวิจัย หรือผู้ร่วมวิจัยในผลงานวิจัย

1.) Project Title: Diversity, molecular taxonomy of nematophagous fungi and characterisation of

microbial enzymes associated with nematode infection (awarded ****)

Principal Investigator: Dr. KD Hyde, Principal investigator.

Investigator: Dr. R Jeewon, Co-Investigator

Project Status: Completed

Project Objective: The main objectives of this project are:

1) To survey nematophagous fungi in Hong Kong.

2) To investigate the genetic and evolutionary links between virulent and avirulent NF based on rDNA and protein sequences.

3) To extract and purify extracellular enzymes from virulent microbes involved in nematode infection.

4) To test the ability of the expressed proteins to break down the cuticle of nematodes.

5) Evaluate the expressed proteins in nematode biocontrol.

Resulted in 3 SCI publications

2.) Project Title: Evolutionary relationships of loculoascomycetes (fungi) based on a phylogenetic

Approach (awarded *****)

Principal Investigator: Dr. KD Hyde.

Co-Investigator: Dr. R Jeewon., Dr. A. Aptroot., Dr. J.M. Moncalvo.

Project Status: Completed

Project Objective: 1. To verify whether Luttrell's hypothesis that loculoascomycetes are phylogentically distinct from unitunicate ascomycetes based on several genes.

2. To assess the usefulness of different genes used in fungal molecular phylogenetics.

3. To test the hypothesis that certain named anamorphic fungi are part of the life cycle of their associated telemorphs.

4. To validate (or refute) the various current terms for morphological structures of fruiting bodies and interascal filaments.

Resulted in 5 SCI publications

3.) Project Title: Molecular evolution of genes for phylogenetic analysis of the class Sordariomycetes

(Mycota) – (awarded ****

Principal Investigator: Dr. KD Hyde.

Co-Investigator: Dr. ECY Liew., Dr. J.M. Moncalvo ,Dr. D.S. Hibbett.,

Project Status: Completed.

Project Objective:

This resulted in 3 SCI publications

ผลงานวิจัยตีพิมพ์บางส่วน

1. Phengsintham P, **Hyde KD,** Braun U (2009) *Cercospora* and allied genera from Laos. 1. Notes on *Zasmidium* (Stenella sen l.). Cryptogamie Mycologie 30: 243-262.
2. Pinnoi A, Phongpaichit P, **Hyde KD**, Jones EBG (2009) Biodiversity of fungi on *Calamus* (Palmae) in Thailand . Cryptogamie Mycologie 30: 181-190.
3. Prihastuti H, Cai L, Chen H, McKenzie EHC, **Hyde KD** (2009) Characterization of *Colletotrichum* species associated with coffee berries in northern Thailand. Fungal Diversity 39: 89-109.
4. Schoch CL, Crous PW, Groenewald JZ, Boehm EWA, Burgess TI, de Gruyter J, de Hoog GS, Dixon LJ, Grube M, Gueidan C, Harada Y, Hatakeyama S, Hirayama K, Hosoya T, Huhndorf SM, **Hyde KD,** Jones EBG, Kohlmeyer J, Kruys A, Li YM, Lucking R, Lumbsch HT, Marvanova L, Mbatchou JS, Mcvay AH, Miller AN, Mugambi GK, Muggia L, Nelsen MP, Nelson P, Owensby CA, Phillips AJL, Phongpaichit S, Pointing SB, Pujade-Renaud V, Raja HA, Plata ER, Robbertse B, Ruibal C, Sakayaroj J, Sano T, Selbmann L, Shearer CA, Shirouzu T, Slippers B, Suetrong S, Tanaka K, Volkmann-Kohlmeyer B, Wingfield MJ, Wood AR, Woudenberg JHC, Yonezawa H, Zhang Y, Spatafora JW (2009). A class-wide phylogenetic assessment of Dothideomycetes. Studies in Mycology 1: 1-15.
5. Shearer CA, Raja HA, Miller AN, Nelson P, Tanaka K, Hirayama K, Marvanova L, **Hyde KD**, Zhang Y (2009) The molecular phylogeny of freshwater Dothideomycetes. Studies in Mycology 64: 145-153.
6. Swe A, Jeewon R, Pointing SB, **Hyde KD**, (2009). Diversity and abundance of nematode-trapping fungi from decaying litter in terrestrial, freshwater and mangrove habitats. Biodiversity and Conservation 18: 1695-1714.
7. Tang AMC, Jeewon R, **Hyde KD** (2009) A re-evaluation of the evolutionary relationships within the Xylariaceae based on ribosomal and protein-coding gene sequences. Fungal Diversity 34: 127-155.
8. Thongkantha S, Jeewon R, Vijaykrishna D, Lumyong S, McKenzie EHC, **Hyde KD** (2009) Molecular phylogeny of Magnaporthaceae (Sordariomycetes) with a

new species *Ophioceras chiangdaoense* from Dracaena loureiroi in Thailand. Fungal Diversity 34: 157-173.

9. Wannathes N, Desjardin DE, **Hyde KD**, Perry BA, Lumyong S (2009) A monograph of *Marasmius* (Basidiomycota) from Northern Thailand based on morphological and molecular (ITS sequences) Fungal Diversity 37: 209-306.
10. Wongsawas M, Wang HK, **Hyde KD**, Lin FC (2009) *Dictyosporium zhejiangense* sp nov., a new freshwater anamorphic fungus from China. Cryptogamie Mycologie 30(4): 355-362.
11. Wongsawas M, Wang HK, **Hyde KD**, Lin FC (2009) Two new hyphomycetes from submerged wood collected in China. Sydowia 61: 345-351.
12. Wulandari NF, To-Anun C, **Hyde KD**, Duong LM, de Gruyter J, Meffert JP, Groenewald JZ and Crous PW (2009) *Phyllosticta citriasiana* sp nov., the cause of Citrus tan spot of *Citrus maxima* in Asia. Fungal Diversity 34: 23-39.
13. Yang YL, Liu ZY, Cai L, **Hyde KD,** Yu ZN and McKenzie EHC (2009) *Colletotrichum anthracnose* of Amaryllidaceae. Fungal Diversity 39: 123-146.
14. Zhang Y, Fournier J, Crous PW, Pointing SB, **Hyde KD** (2009) Phylogenetic and morphological assessment of two new species of *Amniculicola* and their allies (Pleosporales). Persoonia 3: 48-54.
15. Zhang Y, **Hyde KD** (2009) Transfer of *Pseudoparodia pseudopeziza* to Patellariaceae (Patellariales). Nova Hedwigia 88: 211-215.
16. Zhang Y, Schoch CL, Fournier J, Crous PW, de Gruyter J, Woudenberg JHC, Hirayama K, Tanaka K, Pointing SB, Spatafora JW, **Hyde KD** (2009) Multi-locus phylogeny of Pleosporales: a taxonomic, ecological and evolutionary re-evaluation. Studies in Mycology 64: 85-102.
17. Zhang Y, Wang HK, Fournier J, Crous PW, Jeewon R, Pointing SB, **Hyde KD** (2009) Towards a phylogenetic clarification of *Lophiostoma / Massarina* and morphologically similar genera in the Pleosporales. Fungal Diversity 38: 225-251.
18. Zhang Y, Zhou DQ, Zhao Q, Zhou TX, **Hyde KD** (2010) Diversity and ecological distribution of macrofungi in the Laojun Mountain region, Southwestern China. Biodiversity and Conservation 19(12): 3545-3563.
19. Zhao RL, Desjarden DE, Soytong K, Perry BA, **Hyde KD** (2010) A monograph of *Micropsalliota* in Northern Thailand based on morphological and molecular data.

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21. Liu JK, Phookamsak R, Jones EBG, Zhang Y, Ko-Ko TW, Hu HL, Boonmee S, Doilom M, Chukeatirote E, Bahkali AH, Wang Y, **Hyde KD** (2011) Astrosphaeriella is polyphyletic, with species in Fissuroma gen. nov., and Neoastrosphaeriella gen. nov. Fungal Diversity 51: 135–154 DOI 10.1007/s13225-011-0142-9.
22. Manamgoda DS, **Hyde KD,** Bahkali AH, Cai L (2011) *Cochliobolus*: an overview and the current status of species. Fungal Diversity 51: 3-42 DOI 10.1007/s13225-011-0139-4.
23. Mungai P, **Hyde KD**, Cai L, Njogu J, Chukeatirote E (2011) Coprophilous ascomycetes of northern Thailand. Current Research in Environmental & Applied Mycology 1(2): 135–159 DOI 10.5943/cream/1/2/2.
24. Udayanga D, Liu XZ, McKenzie EHC, Chukeatorate E, Bahkali H.A, **Hyde KD** (2011) The genus *Phomopsis*: biology, species concepts, future and names of important phytopathogens. Fungal Diversity 50(1): 189-225 DOI 10.1007/s13225-011-0126-9.
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26. Vitoria NS, Cavalcanti MAQ, **Hyde KD**, Bezerra JL (2011) *Arecomyces* new to Brazil, including *A. attaleae* sp nov. Cryptogamie Mycologie 32: 103-108.
27. Wikee S, Cai L, Pairin N, McKenzie EHC, Su YY, Chukeatirote E, Thi HN, Bahkali AH, Moslem MA, Abdelsalam K, **Hyde KD** (2011) *Colletotrichum* species from Jasmine (Jasminum sambac). Fungal Diversity 46: 171-182 DOI 10.1007/s13225-010-0049-x.
28. Wikee S, Udayanga D, Crous PW, Chukeatirote E, Eric HC McKenzie, Bahkali H, **Hyde KD** (2011) *Phyllosticta* – An overview of current status of species recognition. Fungal Diversity 51: 43–61 DOI 10.1007/s13225-011-0146-5.

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32. Cheewangkoon R, Groenewald JZ, **Hyde KD**, To-anun C, Crous PW (2012) Chocolate spot disease of Eucalyptus. Mycological Progress 11(1): 61-69 DOI 10.1007/s11557-010-0728-8.

33. Chomnunti P, Bhat DJ, Jones EBG, Chukeatirote E, Bahkali AH and **Hyde KD** (2012) Trichomeriaceae, a new sooty mould family of Chaetothyriales Fungal Diversity 56 (1): 63-76 DOI 10.1007/s13225-012-0197-2.

34. Chen J, Zhao RL, Karunarathna SC, Callac P, Raspé O, Bahkal AH, **Hyde KD** (2012) Agaricus megalosporus: A new species in section Minores. Cryptogamie, Mycologie 33 (2): 145-155.

35. Chomnunti P, Ko Ko TW, Chukeatirote E, **Hyde KD**, Cai L, Jones EBG, Kodsueb R, Hassan BA and Chen H (2012) Phylogeny of Chaetothyriaceae in northern Thailand including three new species. Mycologia 104 (2): 382-395 DOI 10.3852/11-066.

36. Chukeatirote E, Maharachchikumbura SSN, Wongkham S, Sysouphanthong P, Phookamsak R, **Hyde KD** (2012) Cloning and sequence analysis of the cellobiohydrolase i genes from some basidiomycetes. Mycobiology 40(2): 107-110.

37. Dai DQ, Bhat DJ, Liu JK, Chukeatirote E, Zhao RL, **Hyde KD** (2012) *Bambusicola*, a new genus from bamboo with asexual and sexual morphs. Cryptogamie Mycologie 33(3): 363-379.

38. De Silva DD, Rapior S, Fons F, Bahkali AH, **Hyde KD** (2012) Medicinal mushrooms in supportive cancer therapies: An approach to anti-cancer effects and putative mechanisms of action. Fungal Diversity 55(1): 1-35 DOI 10.1007/s13225-012-0151-3.

39. De Silva DD, Rapior S, **Hyde KD,** Bahkali AH (2012) Medicinal mushrooms in prevention and control of diabetes mellitus. Fungal Diversity 56 (1): 1-29 DOI 10.1007/s13225-012-0187-4.

40. Guzmán G, Guillén FR, **Hyde KD**, Karunarathna SC (2012) Psilocybe s.s. in Thailand: Four new species and a review of previously recorded species *Mycotaxon* 119: 65-81 DOI 10.5248/119.65.

41. Hu DM, Cai L, **Hyde KD** (2012) Three new ascomycetes from freshwater in China. Mycologia 104: 1478-1489 DOI 10.3852/11-430.

42. Hu DM, Cai L, Bahkali AH, **Hyde KD** (2012) Two new freshwater species of annulatascaceae from China. Mycotaxon 120: 81-88 DOI 10.5248/120.81.

43. Hu DM, Chen H, Cai L, Bahkali AH, **Hyde KD** (2012) Aquapeziza: A new genus from freshwater and its morphological and phylogenetic relationships to Pezizaceae. Mycologia, 104(2): 540-546 DOI 10.3852/11-123.

44. Manamgoda DS, Cai L, McKenzie EHC, Crous PW, Madrid H, Chukeatirote E, Shivas RG, Tan YP, **Hyde KD** (2012) A phylogenetic and taxonomic re-evaluation of the *Bipolaris* - *Cochliobolus* - Curvularia Complex. Fungal Diversity 56(1): 31-144 DOI 10.1007/s13225-012-0189-2.

45. Mortimer PE, Karunarathna SC, Li Q, Gui H, Yang X, Yang X, He J, Ye L, Guo J, Li H, Sysouphanthong P, Zhou D, Xu J, **Hyde KD** (2012) Prized edible Asian mushrooms: Ecology, conservation and sustainability Fungal Diversity 56(1): 31-47 DOI 10.1007/s13225-012-0196-3.

46. Mungai PG, Chukeatirote E, Njogu JG, **Hyde KD** (2012) Coprophilous ascomycetes in Kenya: *Saccobolus* species from wildlife dung. Mycosphere 3(2): 111-129 DOI 10.5943/mycosphere/3/2/2.

47. Mungai PG, Njogu JG, Chukeatirote E, **Hyde KD** (2012) Studies of coprophilous ascomycetes in Kenya – *Ascobolus* species from wildlife dung. Current Research in Environmental & Applied Mycology 2(1): 1-16 DOI 10.5943/cream/2/1/1.

48. Mungai PG, Chukeatirote E, Njogu JG, **Hyde KD** (2012) Studies of coprophilous ascomycetes in Kenya: Sordariales from wildlife dung. Mycosphere 3(4): 437–448 DOI 10.5943 /mycosphere/3/4/7.

49. Mungai PG, Chukeatirote E, Njogu JG, **Hyde KD** (2012) Studies of coprophilous ascomycetes in Kenya. *Podospora* species from wildlife dung. Mycosphere 3(6): 978–995 DOI 10.5943 /mycosphere/3/6/12.

50. Mungai PG, Njogu JG, Chukeatirote E, **Hyde KD** (2012) Studies of coprophilous ascomycetes in Kenya. Coprophilous *Schizothecium* from wildlife dung. Current Research in Environmental & Applied Mycology 2(2): 84–97 DOI 10.5943/cream/2/2/1.

51. Mungai PG, Chukeatirote E, Njogu JG, **Hyde KD** (2012) Coprophilous ascomycetes in Kenya: *Chaetomium* species from wildlife dung. Current Research in Environmental & Applied Mycology 2(2): 113–128 DOI 10.5943/cream/2/2/3.

52. Mungai PG, Njogu JG, Chukeatirote E, **Hyde KD** (2012) Coprophilous ascomycetes in Kenya: *Sporormiella* from wildlife dung. Mycology 3(4): 234-251.

53. Noireung P, Phoulivong S, Liu F, Cai L, Mckenzie EHC, Chukeatirote E, Jones EBG, Bahkali AH, **Hyde KD** (2012) Novel species of *Colletotrichum* revealed by morphology and molecular analysis. *Cryptogamie, Mycologie* 33(3): 347-362.

54. Peng LJ, Yang YL, **Hyde KD,** Bahkali AH, Liu ZY (2012) *Colletotrichum* species on Citrus leaves in Guizhou and Yunnan provinces, China. Cryptogamie Mycologie 33(3): 267-283.

55. Phengsintham P, Chukeatirote E, McKenzie EHC, Moslem MA, **Hyde KD**, Braun U (2012) Fourteen new records of *Cercosporoids* from Thailand Maejo International Journal of Science and Technology 6(1): 47-61.

56. Phengsintham P, Chukeatirote E, McKenzie EHC, **Hyde KD**, Braun U (2012) *Cercospora senecionis-walkeri* – a new leaf-spotting hyphomycete from Laos and Thailand. Plant Pathology & Quarantine 2(1): 70–73 DOI 10.5943/ppq/2/1/10.

57. Phengsintham P, Chukeatirote E, McKenzie EHC, **Hyde KD**, Braun U (2012) Tropical phytopathogens 2: *Pseudocercospora fuligena*. Plant Pathology & Quarantine 2(1): 57-62 DOI 10.5943/ppq/2/1/8.
58. Phoulivong S, McKenzie EHC, **Hyde KD** (2012) Cross infection of *Colletotrichum* species; a case study with tropical fruits. Current Research in Environmental & Applied Mycology 2(2): 99–111 DOI 10.5943/cream/2/2/2.
59. Rao S, Chan Y, Lacap DC, **Hyde KD**, Pointing SB, Farrell RL (2012) Low-diversity fungal assemblage in an Antarctic Dry Valleys soil. Polar Biology 35(4): 567-574 DOI 10.1007/s00300-011-1102-2.
60. Udayanga D, Liu X, Crous PW, McKenzie EHC, Chukeatirote E, **Hyde KD** (2012) A multi-locus phylogenetic evaluation of Diaporthe (Phomopsis). Fungal Diversity 56 (1): 157-171 DOI 10.1007/s13225-012-0190-9.

Co-investigator 1

Ekachai Chukeatirote

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Current position: Lecturer

Academic qualifications:

1996 – 1999	PhD in Biochemistry, Research School of Biosciences, University of Kent at Canterbury, UK; Project title "Evolution of CUG codon reassignment in *Candida* species" with Prof. Mick Tuite

1995 – 1996 MSc in Biotechnology, University of Kent, UK; Project title "Cloning of Ser-tRNA^{CAG} genes from various *Candida* species and expression in *Saccharomyces cerevisiae*" with Prof. Mick Tuite

1990 – 1994 BSc (First Class Hons.) in Biology, Department of Biology, Faculty of Science, Chiang Mai Univeristy, Thailand; Project title "Lactic acid production by starch-utilising lactic acid bacteria" with Assoc. Prof. Dr. Saisamorn Lumyong

Awards and Scholarships:

1995 – 1999 Postgraduate studentship sponsored by the DPST project to pursue MSc/PhD study aboard

1998 Travel grant from the Genetics Society of America (GSA), Bethesda, MD, USA (for Yeast Genetics and Molecular Biology Meeting); from the organising committee, University of Crete, Heraklion, Greece (for Evolutionary Biology Meeting); from Department of Biosciences, University of Kent, UK (for Translation UK)

1994 Prof. Dr. Dhab Nelanithi Foundation Award

1990 – 1994 Studentship under the DPST project, Chiang Mai University

Work experiences:

2000 – present Lecturer, Mae Fah Luang University

Academic committee for Undergraduate and Postgraduate Programme in Biotechnology, Mae Fah Luang University

2001 – 2002 Postdoctoral Fellow, Department of Applied Chemistry, Faculty of Engineering, Oita Univeristy, Japan

1995 – 1999 Teaching Assistance in the following undergraduate practical:

Nucleic Acids and Proteins, Enzyme Kinetics, Gene Cloning, Yeast Mutagenesis, Immunology, Microbiology, UKC, UK

Membership of Learning Societies:

- Editorial Board, Research Journal of Microbiology (2005 – present)
- Member of Thai Society of Biotechnology (2004 – present)
- Member of the Science Advisory Board (2004 – present)
- Member of CRN Microbiology (2004 – present)
- Member of Society of General Microbiology (1995 – 1999)
- Member of Researcher Panel of the IRPUS Project, Thailand (2005 – present)
- Member of the Thai-UK Alumni and Professional Network (2004 – present)

Selected publications:

- Dajanta K, **Chukeatirote E** and Apichartsrangkoon A. 2008. Effect of lactoperoxidase system on keeping quality of raw cow's milk in Thailand. *Int J Dairy Sci* 3: 112-116.
- Onto S, Laosat N, Suksawat W, Popluechai S, Eungwanichayapant PD and **Chukeatirote E**. 2008. Phylogenetic analysis of *Cucumis sativus* using RAPD molecular markers. *J Plant Sci* 3: 105-110.
- Sakai K, Fujii N and **Chukeatirote E** (2007) Racemisation of L-lactic acid in pH-swing open fermentation of kitchen refuse by selective proliferation of *Lactobacillus plantarum*. *J Biosci Bioeng* **102**: 227-232.
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Co-investigator 2

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Education Background:

Ph.D. (Biology), Faculty of Science, Chiang Mai University, Chiang Mai, Thailand, October, 2006

B.Sc. (Medical Technology) Hons, Faculty of Associated Medical Science, Chiang Mai University, Chiang Mai, Thailand, May, 2001

Scholarship The Royal Golden Jubilee Ph.D. Program (2001-2006)

Scientific Interests/Expertise:

- Field research including fungal succession, plot and collecting endophytic and,
- saprobic fungi
- Fungal taxonomy, fungal isolation, identification, fungal cultures
- Molecular biology including technique of DNA extraction from mycelium and
- fruitbody, PCR techniques, DNA sequencing, DNA bar coding
- Use of computer software to analyze genetic data
- Enzymatic study from endophytic and saprobic fungi
- Digital imaging of fungi
- Produce online interactive key for ascomycete fungi for www.discoverlife.org
- Immunology techniques, ImmunoSorbent Assay (ELISA), Western blot

Publications:

- **Promputtha I.** and Miller A.M. 2009. Three new species of *Acanthostigma* (Tubeufiaceae, Pleosporales) from the Great Smoky Mountains National Park. Mycologia (Accepted)
- **Promputtha I.**, Hyde K.D., McKenzie E.H.C., Peberdy J.F., Lumyong P. and Lumyong S. 2010. Do degrading enzymes affecting the process of endophytic fungi becoming saprobe? Fungal Diversity (In press).
- **Promputtha I.,** Lumyong S., Vijaykrishna D., McKenzie E.H.C., Hyde K.D. and Jeewon R. 2007. A phylogenetic evaluation of whether endophytes become saprotrophs at host senescence. Microbial Ecology 53: 579–590.
- **Promputtha I**., Jeewon R., Lumyong S., McKenzie E.H.C. and Hyde K.D. 2005.Ribosomal DNA fingerprinting in the identification of non sporulating endophytes from Magnolia liliifera (Magnoliaceae). Fungal Diversity 20: 167–186.

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- **Promputtha I.**; Hyde K.D.; Lumyong P.; McKenzie E.H.C. and Lumyong S. 2005. Fungi on Magnolia liliifera: Cheiromyces magnoliae sp. nov. from dead branches. Nova Hedwigia 80: 527–532.
- **Promputtha I.**, Lumyong S., Lumyong P., McKenzie E.H.C. and Hyde K.D. 2004. Fungal saprobes on dead leaves of Magnolia liliifera (Magnoliaceae) in Thailand. Cryptogamie Mycologie 25: 315–321.
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Coinvestigator 3

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3.) Position: Lecturer (15 October 2007-present)

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5.) Education Background

- B.Sc. in Agriculture (Plant Pathology) Hons. Faculty of Agriculture
  Chiang Mai University, Chiang Mai, Thailand. March, 2001

- Ph.D. in Biodiversity and Ethnobiology, Faculty of Science
  Chiang Mai University, Chiang Mai, Thailand. October, 2007

6.) Field of Specialization Skill

Mycology, Plant Pathology

Scholarships

Centre for Research in Fungal Diversity (2002–2005)

The Commission on Higher Education (2005–2007)

Awards

Aventis CropScience Award 2000- The 3rd Place Winning Team of "Innovative Rice Production Technology" Essay Contest.

The Excellence Dissertation Award Academic Year 2007 on Thesis Entitled "Biodiversity of Saprobic Fungi on Woody Litter" from Chiang Mai University Graduate School.

7.) ประสบการณ์ที่เกี่ยวข้องกับการบริหารงานวิจัยทั้งภายในและภายนอกประเทศโดยระบุสถานภาพในการทำงานวิจัยว่าเป็นผู้อำนวยการแผนงานวิจัย หัวหน้าโครงการวิจัย หรือผู้ร่วมวิจัยในผลงานวิจัย

ผลงานวิจัยที่ทำเสร็จแล้วและตีพิมพ์

1. **Kodsueb, R.**, McKenzie, E.H.C., Lumyong, S. and Hyde, K.D. (2008). Diversity of saprobic fungi on *Magnoliaceae*. *Fungal Diversity* 30: 37-53.
2. **Kodsueb, R.**, McKenzie, E.H.C., Lumyong, S. and Hyde, K.D. (2008). Fungal succession on woody litter of *Magnolia liliifera* (*Magnoliaceae*). Fungal Diversity 30: 55-72.
3. **Kodsueb R.,** Jeewon R., Hyde K.D., McKenzie E.H.C., Ho W.H. and Lumyong S. (2007). Molecular phylogeny of new synnematous hyphomycete taxon from Thailand and its teleomorphic affinities to Massarinaceae (Pleosporales). *Botanical Journal of the Linnean Society* 155: 283–296.
4. **Kodsueb R.**, McKenzie, E.H.C., Ho, W.H., Hyde K.D., Lumyong P. and Lumyong S. (2007). New anamorphic fungi from decaying woody litter of. *Michelia baillonii* (Magnoliaceae) in northern Thailand. *Cryptogamie Mycologie* 28: 237–245.
5. **Kodsueb, R.**, Jeewon, R., Vijaykrishna, D., McKenzie, E.H.C., Lumyong, P., Lumyong, S. and Hyde, K.D. (2006). Systematic revision of *Tubeufiaceae* based on morphological and molecular data. *Fungal Diversity* 21: 105–130.
6. **Kodsueb R.,** Lumyong S., Hyde K.D., Lumyong P. and McKenzie E.H.C. (2006). *Acrodictys micheliae* and *Dictyosporium manglietiae*, two new anamorphic fungi from woody litter of Magnoliaceae in northern Thailand. *Cryptogamie Mycologie* 27: 111–119.
7. **Kodsueb R.,** Jeewon R., Lumyong S., Vijaykrishna D., Aptroot A., McKenzie E.H.C. and Hyde K.D. (2006). The family Pleosporaceae: intergeneric relationships and phylogenetic perspectives based on sequence analyses of partial 28S rDNA. *Mycologia* 98: 571–583.
8. **Kodsueb**, **R.**, Lumyong S. and Hyde K.D. (2004). Terrestrial Lignicolous Microfungi. In *Thai Fungal Diversity* (eds. E.B.G. Jones, M. Tanticharoen and K.D. Hyde). BIOTEC, Thailand: 155–161.
9. **Kodsueb R.,** Lumyong S., Lumyong S., McKenzie E.H.C., Ho W.H. and Hyde K.D. (2004). *Acanthostigma* and *Tubeufia* species, including *T.*

claspisphaeria sp. nov., from submerged wood in Hong Kong. *Mycologia* 96: 667–674

10. Hyde K.D., Jeewon R, Bahl J., Bhilabut B., Bussaban B., Cai L., Damodar S.B., **Kodsueb R.**, Lam C.W.H., Lam D.M., Photita W., Promputtha I., Tang A.M.C., Thongkantha S., Vijaykrishna D., Yeung S.Y. (2004). Fungal studies at the University of Hong Kong. Annual Meeting of Mycological Society of America, Asheville, North Carolina, USA, 2004.

Conference Papers/ Abstracts/Oral presentations

1. **Kodsueb R.**, Lumyong S., McKenzie E.H.C. and Hyde K.D. (2008). Study of fungi on wood in Doi Suthep-Pui National Park, Chiang Mai, Thailand. 25th Annual Conference of the Microscopy Society of Thailand (MST25). 9–11 January 2008. Amarin Lagoon Hotel, Phitsanulok, Thailand (p. 30–31).
2. **Kodsueb R.**, McKenzie E.H.C., Lumyong S. and Hyde K.D. (2007). Diversity of saprobic fungi on Magnoliaceae. The Asian Mycology Congress 2007 and the 10th International Marine and Freshwater Mycological Symposium. 2–6 December 2007. Park Royal Hotel, Penang, Malaysia. (p. 168).
3. **Kodsueb R.**, Jeewon R., McKenzie E.H.C., Lumyong S., Aptroot A., Vijaykrishna D. and Hyde K.D. (2006). The family Pleosporaceae: intergeneric relationships and phylogenetic perspectives based on sequence analyses of partial 28S rDNA. 8th International Mycological Congress (IMC8 2006). 21–25 August 2006. Cairns Convention Centre, Queensland, Australia. (p. 18).
4. **Kodsueb R.**, Jeewon R., Lumyong P., Hyde K.D. and Lumyong S. (2005). A revision of Tubeufiaceae based on morphological and molecular data (LSU rDNA). British Mycological Society Annual Meeting 2005: Exploitation of Fungi. 5–8 September 2005. Hulme Hall, University of Manchester, Manchester, United Kingdom (p. 83).
5. **Kodsueb R.**, Lumyong S., McKenzie E.H.C., Lumyong P. and Hyde K.D. (2004). Biodiversity and fungal community on terrestrial and submerged *Magnolia liliifera* wood in Doi Suthep-Pui National Park, Thailand. The 10th

International Congress for Culture Collections (ICCC10)—Innovative Roles of Biological Resource Centers. 10–15 October 2004. Tsukuba International Congress Center (Epochal Tsukuba), Tsukuba, Japan (p. 557).

6. **Kodsueb R.**, Lumyong S., Lumyong P., McKenzie E.H.C. and Hyde K.D. (2004). The relationship between fungi on wood from riparian vegetation and the freshwater habitats. The IV Asia-Pacific Mycological Congress & the IX International Marine and Freshwater Mycology Symposium 2004. 14–19 November 2004. Lotus Pangsuankaew Hotel, Chiang Mai, Thailand (p. 250).
7. **Kodsueb R.**, Lumyong S., Lumyong P., Ho W.H. and Hyde K.D. (2002). *Acanthostigma* and *Tubeufia* species from submerged wood in Hong Kong, including *T. claspisphaeria* sp. nov. The 3rd Asia-Pacific Mycological Congress on Biodiversity and Biotechnology (AMC2002). 4–8 November 2002. Yunnan University, Kunming, Yunnan, China (p. 135).

Workshops in field specialization:

1. Workshop on Gene Discovery of Uncultured Microbes Using Metagenomic Approach, 13-16 May 2008. BIOTEC Auditorium Room, BIOTEC Building, Thailand Science Park, Pathumthani, Thailand, organized by BIOTEC, NSTDA and Thammasart University.
2. Workshop on Unculturable Microbes: Molecular Techniques and Biotechnology Application, 9–10 January 2006. BIOTEC Auditorium Room, BIOTEC Building, Thailand Science Park, Pathumthani, Thailand, organized by BIOTEC, NSTDA and MOST (Thailand).
3. Workshop on Microbial Commercialisation and Entrepreneurship, 20–24 June 2005. Organised by Department of Biology, Faculty of Science, Chiang Mai University.
4. Workshop on Principle Techniques in Plant Pathology and Applied Mycology, 24–25 March 2005. Hosted by the Department of Plant Pathology, Faculty of Agriculture, Chiang Mai University. Higher Education Link: Mycological Network between Chiang Mai University and Liverpool John Moores University.

5. GBIF & EASIANET Proposed Collection/Names/Images digitisation workshop, 14–19 March 2005. Hosted by Centre for Research in Fungal Diversity, Department of Ecology and Biodiversity, The University of Hong Kong, Pokfulam Road, Hong Kong.
6. Molecular Phylogenetics Workshop, 17–22 March 2004. Organised by Department of Ecology and Biodiversity, The University of Hong Kong, Pokfulam Road, Hong Kong.
7. Workshop on Mycology Taxonomy, Molecular Systematics and Using Key, Isolation and Preservation of Fungi. 7–27 July 2003. Mushroom Research Centre, Mae Taeng, Chiang Mai, Thailand.