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*Pestalotiopsis* ในประเทศไทย

Biochemistry and Phylogeny of *Pestalotiopsis*

โดย

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## บทสรุปผู้บริหาร (EXECUTIVE SUMMARY)

### 1. ความสำคัญและที่มาของปัญหาในการการวิจัย (Rationale and review)

The species in the genus *Pestalotiopsis* have received much attention in recent years, not only because of their role as plant pathogens, but also as commonly isolated endophytes which have been shown to produce a wide range of chemically novel diverse metabolites. There are numerous reports in the literature that various species produce taxol, while others produce newly discovered compounds with medicinal potential and still others cause disease. The names assigned to these novel compound-producing taxa lack an accurate taxonomic basis, since the taxonomy of the genus is markedly confused. This confusion arises mainly due to the fact that *Pestalotiopsis* species are generally not host-specific, conidial characters vary and thereby species limits overlap and many of the sequences available in the GenBank for a species are not based on type. This calls for critical re-examination of the type material of the large number of species described under *Pestalotiopsis* using multi-gene analysis and establishment of epitypes, wherever necessary, with the living cultures. Only such combined molecular and morphological studies of the type would provide a reliable species base for taxonomic treatment of the genus *Pestalotiopsis*. So the current research mainly focuses on resolving the taxonomic confusion and harvesting natural chemical substances from economically important genus *Pestalotiopsis*.

### 2. วัตถุประสงค์ของโครงการวิจัย (Objective)

2.1 To document the diversity of *Pestalotiopsis* species mainly in northern Thailand and to isolate strains for conservation in the BIOTEC Culture Collection.

2.2 To construct phylogenetic trees to examine the inter-relationships of *Pestalotiopsis* species and to determine their relationships

2.3 To use a polyphasic approach and epitypification to stabilize the nomenclature of *Pestalotiopsis* species.

2.4 To establish chemical profiles for species and discover new bioactive compounds.

### 3. ขอบเขตของโครงการวิจัย (Scope of the research)

The genus *Pestalotiopsis* contains more than 230 names and presently there are no exact estimated species. The genus has received much attention from the scientific community. However, this not only because of its pathogenic nature, but rather because its species have been shown to produce many important secondary metabolites. *Pestalotiopsis* species are cosmopolitan in distribution and traditionally naming according to the host association. However, recent research showed that *Pestalotiopsis* are generally not host specific and may inhabit in range of hosts. Thus the actual number of species in *Pestalotiopsis* is likely to be much lower than presently recorded in the literature. Furthermore the species identification is challenging due to 1) conidial characters vary and species limits overlap and 2) species arrangements in literature are problematic.

In our study we will develop a polyphasic approach for identification of *Pestalotiopsis*, focusing on global species. The taxonomically important morphological characters will be identified and multi-locus DNA data will use to develop a strong taxonomic base to the genus. Re-examination of the type material of the large number of species described under *Pestalotiopsis* using multi gene analysis will be done with establishment of epitypes, with the living cultures. As a part of study species in Thailand will be documentation and at the later stages cultures will be used to screen novel secondary metabolites. The successful outcome of this project will have important practical implications to the plant pathology, plant breeding and quarantine communities and secondary metabolites profiling. One doctoral student will be train as an expert in the field. As an outcome several highly cited papers will be published and bring Mae Fah University and Thailand as one of the world leaders in mycological research.

### 4. ระเบียบวิธีวิจัยและผลผลิตจากการวิจัย (Methodology and the research output)

Research Plan from October 2011 to September 2014

**Year 1** (October 2011 to September 2012): This study will commence with the isolation of a number of *Pestalotiopsis* species from different substrates in Thailand and recovery of cultures from various culture collections such as CBS, CGMCC, ICMP and CABI. Isolated *Pestalotiopsis* spp. in different substrata around Thailand will be compared with *Pestalotiopsis* species available in different international culture collections. The morphology and cultural characters of species will be examined and phylogenetic study will be carried out including representatives of many species as possible, initially using rDNA ITS sequence data followed by beta-tubulin, TEF 1 $\alpha$  and other gene loci. The outcome of the research will undoubtedly be

useful to provide strong taxonomic foundation to this economically important genus *Pestalotiopsis* using polyphasic approach and epitypification. There is no doubt that many new species of the genus will emerge out in this molecular re-examination. Such a study will always aid in conservation of the biodiversity of *Pestalotiopsis* of Thailand and documentation of diversity.

## 5. ประโยชน์ที่ได้รับ (Benefit)

5.1 Addition of 50 distinct *Pestalotiopsis* strains per year to the BIOTEC and MFUCC Culture Collection.

5.2 Production of one scientific paper in an international refereed journal or oral papers at an international conference each year.

5.3 Training one researcher in taxonomy, plant pathology, molecular biology and metabolite profiling.

5.4 Production of new records for the checklist of Thai fungi.

5.5 Description of any new taxa collected during the research program.

5.6 Descriptions of new bioactive chemicals

## 6. แผนการถ่ายทอดเทคโนโลยีหรือผลการวิจัยสู่กลุ่มเป้าหมาย (A plan to transfer technology or research to target group)

The results will be published and be useful to systematists, plant pathologists, plant health practitioners, plant breeders, and quarantine officers. Any new chemical may have medicinal significance.

## บทคัดย่อ

ในปัจจุบันนี้ เชื้อราสกุล *Pestalotiopsis* ได้รับความสนใจศึกษาอย่างแพร่หลาย โดยเชื้อราในสกุลนี้สามารถพบได้ทั้งลักษณะที่เป็นเอนโดไฟท์ และเชื้อสาเหตุก่อโรคในพืช นอกจากนี้เชื้อราในกลุ่มที่เป็นเอนโดไฟท์ยังมีความสามารถในการผลิตสารชีวภาพชนิดใหม่อย่างหลากหลาย ดังนั้น เหตุผลในการศึกษาเชื้อราสกุล *Pestalotiopsis* ได้แก่ 1) เป็นเชื้อราในกลุ่ม non-host specific ที่พบได้ทั่วไป 2) ลักษณะของโคนิเดียมีความหลากหลาย และในบางสปีชีส์มีข้อจำกัดของการใช้ลักษณะทางสัณฐานวิทยาในการจัดจำแนก 3) และในงานวิจัยของ Steyaert และ Guba ยังไม่สามารถแก้ปัญหาการจัดจำแนกในระดับสปีชีส์ได้อย่างชัดเจน รวมถึงจำนวนสปีชีส์ที่แท้จริงของเชื้อราในสกุล *Pestalotiopsis* มีจำนวนน้อยกว่าที่ถูกบันทึกไว้ในเอกสารตีพิมพ์ทางวิชาการในปัจจุบัน ในงานวิจัยนี้ การศึกษาตัวอย่างต้นแบบ (type material) และการจัดตั้ง epitype ด้วยผลของเชื้อที่แยกเพาะเลี้ยงได้ (living culture) นั้นเป็นสิ่งจำเป็นต่อความก้าวหน้าในการวิเคราะห์ข้อมูลระดับพันธุกรรมด้วยหลายยีน (multi gene analysis) ต่อการแยกความแตกต่างทางลักษณะทางสัณฐานวิทยาที่ชัดเจน และเป็นพื้นฐานการพัฒนาระบบอนุกรมวิธานระดับสปีชีส์ที่แข็งแกร่งของเชื้อราในสกุล *Pestalotiopsis* ดังนั้น โครงการวิจัยนี้จึงมุ่งเน้นความสำคัญของการแยกเพาะเลี้ยง (culture isolation) เชื้อราในสกุล *Pestalotiopsis* จากตัวอย่างพืชที่มีความแตกต่างกันในเขตพื้นที่ภาคเหนือของประเทศไทย โดยเฉพาะในกลุ่มที่เป็นเชื้อสาเหตุโรคพืชและกลุ่มเอนโดไฟท์ที่สามารถผลิตสารชีวภาพทุติยภูมิ (secondary metabolites) ที่มีคุณสมบัติทางเคมีได้ เชื้อที่แยกเพาะได้เลี้ยงได้ทั้งหมดจะฝากเก็บไว้ที่ MFLU และ BIOTEC Culture Collections ซึ่งไอโซเลทเหล่านี้ยังนำมาใช้ในการศึกษาความสัมพันธ์ในเชิงวิวัฒนาการด้วยการวิเคราะห์ข้อมูลทางชีวโมเลกุลและลักษณะทางสัณฐานวิทยา เพื่อช่วยในการตั้งชื่ออย่างถูกต้องและแก้ไขความสับสนในระดับอนุกรมวิธานของเชื้อราในสกุล *Pestalotiopsis* และอีกส่วนหนึ่งของไอโซเลทได้นำมาใช้หาสารชีวภาพทุติยภูมิชนิดใหม่ รวมถึงการตีพิมพ์ผลศึกษาลักษณะทางสัณฐานวิทยา และความสัมพันธ์วงศ์วานวิวัฒนาการเชิงโมเลกุล (molecular phylogeny) ของเชื้อราสกุล *Pestalotiopsis*

คำสำคัญ: เอนโดไฟท์, โรคพืช, สารทุติยภูมิ, อนุกรมวิธาน

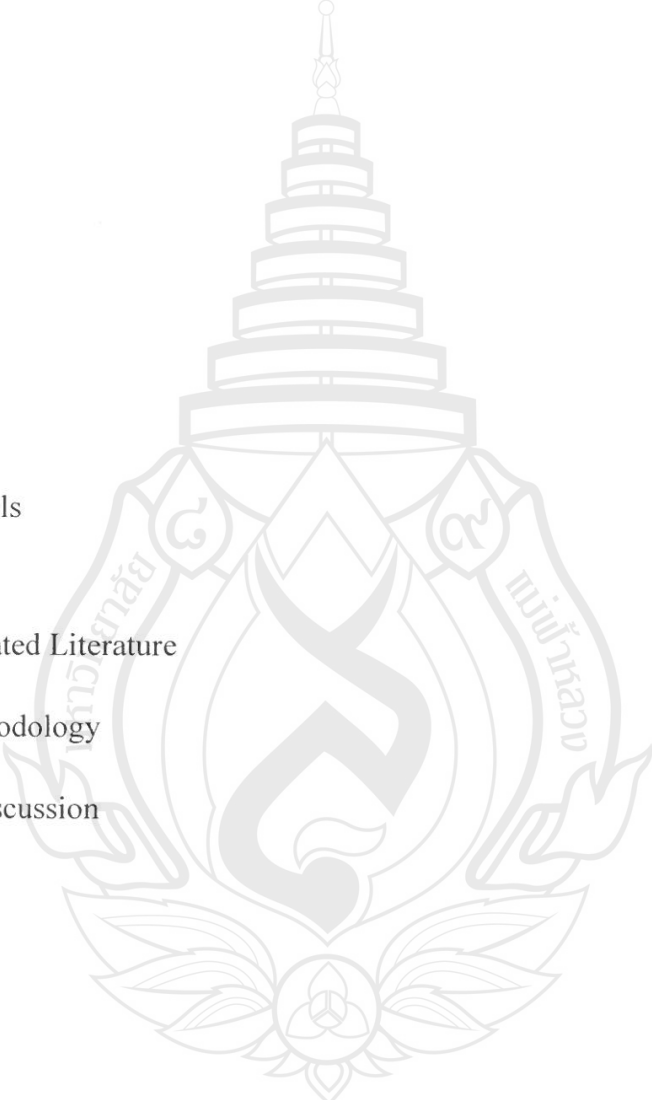
## ABSTRACT

The genus *Pestalotiopsis* has received much attention in recent years, not only because of its role as a plant pathogen, but also as a commonly isolated endophyte which has been shown to produce a wide range of chemically novel diverse metabolites. However due to the fact that 1) *Pestalotiopsis* are generally not host-specific 2) conidial characters vary and species limits overlap and 3) species arrangements in Steyaert and Guba are problematic, then the actual number of species in *Pestalotiopsis* is likely to be much lower than presently recorded in the literature. Re-examination of type materials and establishment of epitypes with living cultures is essential for progress and multi gene analysis with distinct morphological characters are needed to develop a strong species base taxonomic system for the genus *Pestalotiopsis*. The present study mainly focuses on the isolation of *Pestalotiopsis* found in different substrata around Northern Thailand especially important plant pathogens and chemically important endophytes. In the first year of this project we collected 200 specimens throughout Northern of Thailand and isolated 53 *Pestalotiopsis* strains. We also examined 300 isolates from CGMCC in China. These isolates were deposited in MFLU culture collection. We described a new species isolated from *Camellia sinensis* (tea) as species *Pestalotiopsis furcata*; one species was epitypified as a *P. theae*; and one species causing serious fruit rot disease of *Syzygium samarangense* fruit was described as a new species, *P. samarangensis*. Morphologically most *Pestalotiopsis* species clusters in three main clades and this is highly supported by analysis of ITS,  $\beta$ -tubulin and *tefl* sequence data . As a result of this study we have published four SCI papers including one important review paper and one large backbone tree for identifying species in the genus. At the later stages cultures will be used to screen novel secondary metabolites.

**Keywords:** Endophytes, Plant pathogen, Secondary metabolites, Taxonomy

**TABLE OF CONTENTS**

<b>Topic</b>	<b>Page</b>
Acknowledgements	I
Executive Summary	II
Abstract (Thai)	V
Abstract (English)	VI
Table contents	VII
List of Tables	VIII
List of Figures	IX
Abbreviations and symbols	X
Chapter 1 Introduction	1
Chapter 2 Review of Related Literature	2
Chapter 3 Research Methodology	5
Chapter 4 Results and Discussion	8
Chapter 5 Conclusion	18
References	19
Publications	22
Biography	30





## LIST OF TABLES

<b>Table</b>		<b>Page</b>
Table 4.1	Some <i>Pestalotiopsis</i> from northern Thailand	9
Table 4.2	Comparison of gene regions used in our study	16
Table 4.3	Comparison of gene regions tested but not used in the final phylogenetic studies	16



## LIST OF FIGURES

Figure		Page
Fig. 4.1	<i>Pestalotiopsis furcata</i> sp. nov., on leaf of <i>Camellia sinensis</i>	11
Fig. 4.2	<i>Pestalotiopsis theae</i> (epitype), on leaf of <i>Camellia sinensis</i>	13
Fig. 4.3	<i>Pestalotiopsis samarangensis</i> (holotype), on fruits of <i>Syzygium samarangense</i>	15
Fig. 4.3	Maximum parsimony phylogram generated from combine ITS, $\beta$ -tubulin and <i>tef1</i> analysis	18



## ABBREVIATION AND SYMBOLS

%	=	Percent
°C	=	Degree Celsius
µm	=	micrometer
µg	=	Microgram
cm	=	centimeter
g	=	gram
g/l	=	gram per liter
hr	=	hour
ml	=	milliliter
mm	=	millimeter
No.	=	Number
PDA	=	Potato Dextrose Agar
sp.	=	species
Temp.	=	Temperature
$\bar{x}$	=	Average
GPDH	=	Glyceraldehyde-3- phosphate dehydrogenase
ITS	=	Internal transcribed spacer
LSU	=	Large subunit (28S rDNA)
TEF1 $\alpha$	=	Translation elongation factor 1-alpha
USA	=	United States of America
USDA	=	United States Department of Agriculture

## CHAPTER 1

### INTRODUCTION

The genus *Pestalotiopsis* has received considerable attention in recent years, not only because of its role as a plant pathogen but also as a commonly isolated endophyte which has been shown to produce a wide range of chemically novel diverse metabolites. Classification in the genus has been previously based on morphology, with conidial characters being considered as important in distinguishing species and closely related genera. However it is concluded that the large number of described species has resulted from introductions based on host association. We suspect that many of these are probably not good biological species. Recent molecular data have shown that conidial characters can be used to distinguish taxa; however, host association and geographical location is less informative. The taxonomy of the genera complex remains confused. There are only a few type cultures and, therefore, it is impossible to use gene sequences in GenBank to clarify species names reliably. There are numerous reports in the literature that various species produce taxol, while others produce newly discovered compounds with medicinal potential and still others cause disease. The names assigned to these novel compound-producing taxa lack an accurate taxonomic basis, since the taxonomy of the genus is markedly confused. Until the important species have been epitypified with living strains that have been sequenced and deposited in public databases, researchers should refrain from providing the exact name of species. Species of *Pestalotiopsis* have been well-studied because of the diverse array of novel compounds that they have been shown to produce. As such, they are thought to be a rich source for bioprospecting when compared to those of other fungal genera. Moreover, species of *Pestalotiopsis* have been found to produce an enormous number of secondary metabolites that may have medicinal, agricultural and industrial applications.

## CHAPTER 2

### LITERATURE REVIEW

*Pestalotiopsis* Steyaert is an appendage-bearing conidial anamorphic form (coelomycetes) in the family Amphisphaeriaceae (Barr 1975), and molecular studies have shown that *Pestalotiopsis* is monophyletic (Jeewon et al. 2002, 2004). The genus has received much attention from the scientific community. However, this not because of its pathogenic nature (Rivera and Wright 2000; Yasuda et al. 2003), but rather because its species have been shown to produce many important secondary metabolites (Strobel et al. 2002; Aly et al. 2010; Xu et al. 2010). De Notaris (1839) introduced the genus *Pestalotia* De Not. based on the generic type *Pestalotia pezizoides* De Not.. However Steyaert (1949) revised *Pestalotia* and divided the genus into three main groups based on the conidial forms. Steyaert (1949) also introduced two new genera, *Truncatella* Steyaert for 4-celled conidial forms and *Pestalotiopsis* Steyaert for the 5-celled forms, while the 6-celled forms remained in *Pestalotia*. Until 1990, phylogenetic understanding of the taxonomy associated with *Pestalotiopsis* and allied genera was based mainly on conidial characters (Steyaert 1949; Guba 1961; Nag Rag 1993), conidiogenesis (Sutton 1980) and teleomorph association (Barr 1975, 1990; Metz et al. 2000; Zhu et al. 1991).

Morphological characters used to differentiate species of *Pestalotiopsis* and similar genera are limited (Hu et al. 2007); the morphological characters are plastid and morphological markers vary between host and environment (Egger 1995). Hu et al. (2007) showed that colony morphology (colour, growth rate and texture) is highly variable within single isolates of *Pestalotiopsis*; this phenomenon can be easily observed through repeated subculturing. Also within a single species, conidial morphology (shape and colour of the median cells), growth rate and fruiting structure, may vary (Jeewon et al. 2003). Jeewon et al. (2003) evaluated the morphological characters that could be used to differentiate species of *Pestalotiopsis*. He suggested that pigmentation of median cells has taxonomic value. Liu et al. (2010a) proposed that instead of using “concolorous” and “versicolor” as proposed by Steyaert (1949) and Guba (1961), “brown to olivaceous” and “umber to fuliginous” median cells can

be a key character in distinguishing species in *Pestalotiopsis*. Conidial morphology is the most widely used taxonomic character for the genus *Pestalotiopsis*.

Most species are divided into different groups based on the size of the conidia. Colour of the median cells is still a widely used character, and all species separate into three groups based on this- concolorous, versicolorous umber olivaceous and versicolorous fuliginous olivaceous. The length of the apical appendages and the number of the apical appendages are also widely used characters for species identification. Some species can also be identified by the presence of knobbed apical appendages. The apical appendages can arise from the top, middle, bottom or different positions in the apical hyaline cells and such characters are widely used in species identification. Furthermore the apical appendages can be divided into branches; in some species presence or absence of the basal appendages is another character for species diagnosis (Maharachchikumbura et al. 2011). Species of *Pestalotiopsis* commonly cause disease in a variety of plants (Hopkins and McQuilken 2000; Tagne and Mathur 2001), are commonly isolated as endophytes (Liu et al. 2006; Tejesvi et al. 2009; Watanabe et al. 2010) and some species likely have endophytic and pathogenic stages in their life cycle (Wei et al. 2007; Tejesvi et al. 2009). Species have also been recorded as saprobes (Agarwal and Chauhan 1988; Yanna et al. 2002) where they are recyclers of dead plant material (Osono and Takeda 1999; Tokumasu and Aoiki 2002) and even rarely cause disease in humans (Sutton 1999). According to Index Fungorum (<http://www.indexfungorum.org>, 2011) there are 235 *Pestalotiopsis* names, while in MycoBank ([www.mycobank.org](http://www.mycobank.org), 2011) there are 232 names. The reason for the large number of names is historical and may not reflect the actual number of species (Jeewon et al. 2004) and actual number of species may be fewer than 50 (Maharachchikumbura et al. 2011).

*Pestalotiopsis* are thought to be a rich source for bioprospecting when compared to those of other fungal genera (Aly et al. 2010; Xu et al. 2010). Strobel and Long (1998) described *Pestalotiopsis* as the 'E. coli of the temperate and tropical rainforest systems'. The majority of compounds have been discovered from endophytic strains of *Pestalotiopsis* (Lee et al. 1996; Li and Strobel 2001). Species of *Pestalotiopsis* have been shown to produce bioactive alkaloids, terpenoids, isocoumarin derivatives, coumarins, chromones, quinones, semiquinones, peptides,

xanthenes, xanthone derivatives, phenols, phenolic acids, and lactones with a range of antifungal, antimicrobial, and antitumor activities (Xu et al. 2010). Xu et al. (2010) reviewed 130 different compounds isolated from species of *Pestalotiopsis*.



## CHAPTER 3

### RESEARCH METHODOLOGY

#### (1) Collection of the samples

*Pestalotiopsis* isolates will be collected from the leaf spots and diseased fruits of various hosts, Provinces of Chiang Mai and Chiang Rai in northern Thailand. The cultures will be obtained from CGMCC, CBS and all other collaborating institutes. Most important species, type herbarium material will be loaned and re-examined.

#### (2) Morphological examination

The obtained pure colony will be transferred onto PDA medium. The plates will be incubated at room temperature (25°C) and to induce sporulation, sterilized Carnation leaves were put on to the medium. Morphology of fungal colonies will be recorded. Fungal mycelium and spores will be observed under light microscope and photographed.

#### (3) Phylogenetic study

DNA will be extracted from selected isolates and PCR will be carried out to amplify rDNA ITS region by using primers ITS 4 and ITS 5 and partial  $\beta$ -tubulin gene by using Bt2A and Bt2B or other gene loci (Calmodulin, Actin, RPB2 and *tef1*). Sequencing will be carried out for the respective region. Phylogenetic analysis of the sequences will be carried out by using PAUP \* and Mega4.

##### 3.1 Molecular analysis

##### DNA extraction

Total genomic DNA will be extracted from fresh cultures using a modified protocol of Doyle & Doyle (1987) and Lee & Taylor (1990). Fresh fungal mycelia (500 mg) will be scraped from the margin of the PDA plate incubated at 25°C for 7 to 10 days and transferred into a 1.5 ml centrifuge tube with 100 ml of preheated (60°C) 2X CTAB extraction buffer (2% (w/v) CTAB, 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, pH 8.0), and 200 mg sterilized quartz sand. Mycelia will be ground using a glass pestle for 5 min and add extra 500 ml preheated (60°C) 2X CTAB and,



incubated in a 65°C water bath for 30 min with occasional shaking. 500 ml of phenol:chloroform (1:1) will be added into each tube and shake thoroughly to form an emulsion. The mixture will be spun 11900 g for 15 min at 25°C in microcentrifuge and decants the supernatant phase into a fresh 1.5 ml tube. Supernatant containing DNA will be reextracted with phenol: chloroform (1:1) at 4°C until no interface was visible. 50 ml of 5M KOAc will be added into the supernatant followed by 400 ml of isopropanol and inverted gently to mix. The genomic DNA will be precipitated at 9200 g for 2 min at 4°C in a microcentrifuge. The DNA pellet will be washed with 70% ethanol twice and dried using SpeedVac® (AES 1010; Savant, Holbrook, NY, USA) until dry. The DNA pellet will be then resuspended in 100 ml TE buffer (10 mM Tris-HCl, 1 mM EDTA).

### 3.2 PCR amplification

The ITS and 5.8S region of rDNA molecule will be amplified using primer pairs ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') (White et al 1990; Worapong et al 2002),  $\beta$ -tubulin gene region will be amplified with primer pairs BT2A (5'-GGTAACCAAATCGGTGCTGCTTTC-3') and BT2B (5'-ACCCTCAGTGTAGTGACCCTTGGC-3') (Glass and Donaldson, 1995; O'Donnell and Cigelnik, 1997) and *tefl* will be amplified using the primer pairs EF1-526F (5'-GTCGTYGTYATY GGHCAYGT-3') and EF1-1567R (5'-ACHGTRCCRATACCACCRATCTT-3'). All other regions will be amplification by their respective primers. The PCR will be performed with the 25  $\mu$ L reaction system consisting of 19.5  $\mu$ L of double distilled water, 2.5  $\mu$ L of 10 $\times$  Taq buffer with MgCl<sub>2</sub>, 0.5  $\mu$ L of dNTP (10 mM each), 0.5  $\mu$ L of each primer (10  $\mu$ M), 0.25  $\mu$ L Taq DNA polymerase (5 U/ $\mu$ L), 1.00  $\mu$ L of DNA template. The thermal cycling program will be as follows:

For ITS initial denaturing step of 95°C for 3 min, followed by 35 amplification cycles of 95°C for 30 sec, 52°C for 45 sec, and 72°C for 90 sec and a final extension step of 72°C for 10 min. For  $\beta$ -tubulin PCR conditions will be an initial step of 3 min at 95°C, 35 cycles of 1 min at 94°C, 50 s at 55°C, and 1 min at 72°C, followed by 10 min at 72°C. For *tefl*, initial step of 5 min at 94°C, 10 cycles of 30 s at 94°C, 55 s at 63°C or 66°C (decreasing 1°C per cycle), 90 s at 72°C, plus 36

cycles of 30 s at 94°C, 55 s at 53°C or 56°C, 90 s at 72°C, followed by 7 min at 72°C. The PCR products will be verified by staining with Goldview (Guangzhou Geneshun Biotech Ltd., China) on 1% agarose electrophoresis gels.

#### (4) Phylogenetic analysis

DNASar, SeqMan will be used to obtain consensus sequences from sequences generated from forward and reverse primers. Combination sequence obtained from three gene regions will be aligned using CLUSTALX (1.83) (Thompson et al., 1997). The sequence will be manually adjusted using BioEdit (Hall, 1999), to allow maximum alignment and maximum sequence similarity. A maximum parsimony analysis (MP) will be performed using PAUP \* (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford, 2002). Ambiguously aligned regions will be excluded and gaps were treated as missing data. Trees will be inferred using the heuristic search option with TBR branch swapping and 1,000 random sequence additions. Maxtrees will be unlimited, branches of zero length will be collapsed and all multiple parsimonious trees will be saved. Tree length [TL], consistency index [CI], retention index [RI], rescaled consistency index [RC], homoplasy index [HI], and log likelihood [-ln L] (HKY model) will be calculated for trees generated under different optimality criteria. The robustness of the most parsimonious trees will be evaluated by 1000 bootstrap replications resulting from maximum parsimony analysis, each with 10 replicates of random stemwise addition of taxa (Felsenstein 1985). The Kishino–Hasegawa tests (Kishino and Hasegawa 1989) will be performed in order to determine whether the trees inferred under different optimality criteria were significantly different. Trees will be viewed in Treeview (Page, 1996).

The techniques involved in the approach described are familiar with the investigators. Dr Hyde has extensive knowledge in collection, isolation, identification and growth of fungal cultures. Drs Ekachai has hand-on experience in the specific molecular and physiological evaluation techniques.

#### Secondary metabolite profiling

Secondary metabolite will be extraction from selected species of *Pestalotiopsis*, using methods describe by Li et al, 2007 and Xu et al. 2009.

## CHAPTER 4

### RESULTS AND DISCUSSION

In the first 10 months of this project 53 *Pestalotiopsis* isolates were collected throughout Northern of Thailand from agricultural fields, waterfalls, national parks and house gardens. Species of *Pestalotiopsis* cause a variety of disease in plants, including canker lesions, shoot dieback, leaf spots, needle blight, tip blight, grey blight, scabby canker, severe chlorosis, fruit rots and leaf spots. Fresh plant material infected by *Pestalotiopsis* was isolated by endophyte technique, hyphal tip and single spore isolation. Conidiomata in the genus as variable, ranging from acervuli to pycnidia. Conidiomata can be immersed to erumpent, unilocular to irregularly plurilocular with the locules occasionally incompletely divided and dehiscence by irregular splitting of the apical wall or overlying host tissue. Conidiophores partly or entirely develop inside the conidiomata, and they can be reduced to conidiogenesis cells which are discrete or integrated, cylindrical, smooth, colourless and invested in mucus. Pycnidia can mostly be seen with the unaided eye as a black or brown spore masses with copious conidia. Conidia fusiform to ellipsoid, straight to slightly curved, 4-septate, basal cell colourless or slightly colour; median cells 3, concolourous or versicolourous; apical cell colourless; apical appendages, tubular, 2 to 6 in number, arising from the upper portion of the apical cell, knob or knot. Basal appendages are usually present. Characteristics and morphology have been examined in pure culture, most species produce white colony, which after 1 weeks on PDA is 5-7 cm in diam.

**Table 4.1** Some *Pestalotiopsis* from northern Thailand

ORIGINAL Code	SPECIES Name	HOST Name	SUBSTRATE	COLLECTION SITE
SAJ-0008	<i>Pestalotiopsis adusta</i>	<i>Syzygium</i> sp.	Leaf	Chiang Rai, Thailand
SAJ-0009	<i>Pestalotiopsis</i> sp.	<i>Phyllanthus emblica</i>	fruit	Market in front of Mae Fah Luang University, Chiang Rai, Thailand
SAJ-0010	<i>Pestalotiopsis samarangensis</i>	<i>Syzygium samarangense</i>	fruit	Market in front of Mae Fah Luang University, Chiang Rai, Thailand
SAJ-0011	<i>Pestalotiopsis</i> sp.	<i>Dracontomelon mangifera</i>	Leaf	Nam Tak Huey Mesak Forest Park, Chiang Rai, Thailand
SAJ-0012	<i>Pestalotiopsis</i> sp.	<i>Rhododendron</i> sp.	Leaf	Mae Fah Luang University, Chiang Rai, Thailand
SAJ-0013	<i>Pestalotiopsis</i> sp.	<i>Chisocheton siamensis</i>	Leaf	Huay Mesak Waterfall, Tool Kwan, Chiang Rai, Thailand
SAJ-0014	<i>Pestalotiopsis</i> sp.	Unknown sp.	Leaf	Nursery orchids, Ching Mai province, Thailand
SAJ-0015	<i>Pestalotiopsis</i> sp.	<i>Artocarpus heterophyllus</i>	Decaying leaf	Mushroom Research Centre (MRC), Bahn Pha Deng, Chiang Mai Province, Thailand
SAJ-0016	<i>Pestalotiopsis</i> sp.	<i>Musa paradisiaca</i>	Leaf	Rachana Dormitory, Mae Fah Luang University, Ching Rai, Thailand
SAJ-0017	<i>Pestalotiopsis furcata</i>	<i>Camellia sinensis</i>	Leaf	Mushroom Research Centre (MRC), Bahn Pha Deng, Chiang Mai Province, Thailand
SAJ-0018	<i>Pestalotiopsis</i> sp.	Unknown sp.	Leaf	Mushroom Research Centre (MRC), Bahn Pha Deng, Chiang Mai Province, Thailand
SAJ-0019	<i>Pestalotiopsis</i> sp.	<i>Areca</i> sp.	Leaf	Rachana Dormitory, Mae Fah Luang University, Ching Rai, Thailand
SAJ-0020	<i>Pestalotiopsis</i> sp.	<i>Saccharum</i> sp.	Leaf	Mushroom Research Centre (MRC), Bahn Pha Deng, Chiang Mai Province, Thailand
SAJ-0021	<i>Pestalotiopsis</i> sp.	Unidentified sp.	Leaf	Vientiane Capital, Dove Makkhai Village, Laos
JKC-0001	<i>Pestalotiopsis</i> sp.	Palm	Leaf	Mushroom Research Centre (MRC), Bahn Pha Deng, Chiang Mai Province, Thailand
JKC-0006	<i>Pestalotiopsis</i> sp.	Palm	Rachis	Khow waterfall, Chian grai, Thailand
SAJ027	<i>Pestalotiopsis theae</i>	<i>Camellia sinensis</i>	Leaf	Mushroom Research Centre (MRC), Bahn Pha Deng, Chiang Mai Province, Thailand

#### 4.1 New species/epitype of *Pestalotiopsis* collected during this study

##### 1. *Pestalotiopsis furcata* Maharachchikumbura & K.D. Hyde, sp. nov. Fig. 4.1

MycoBank: MB564563

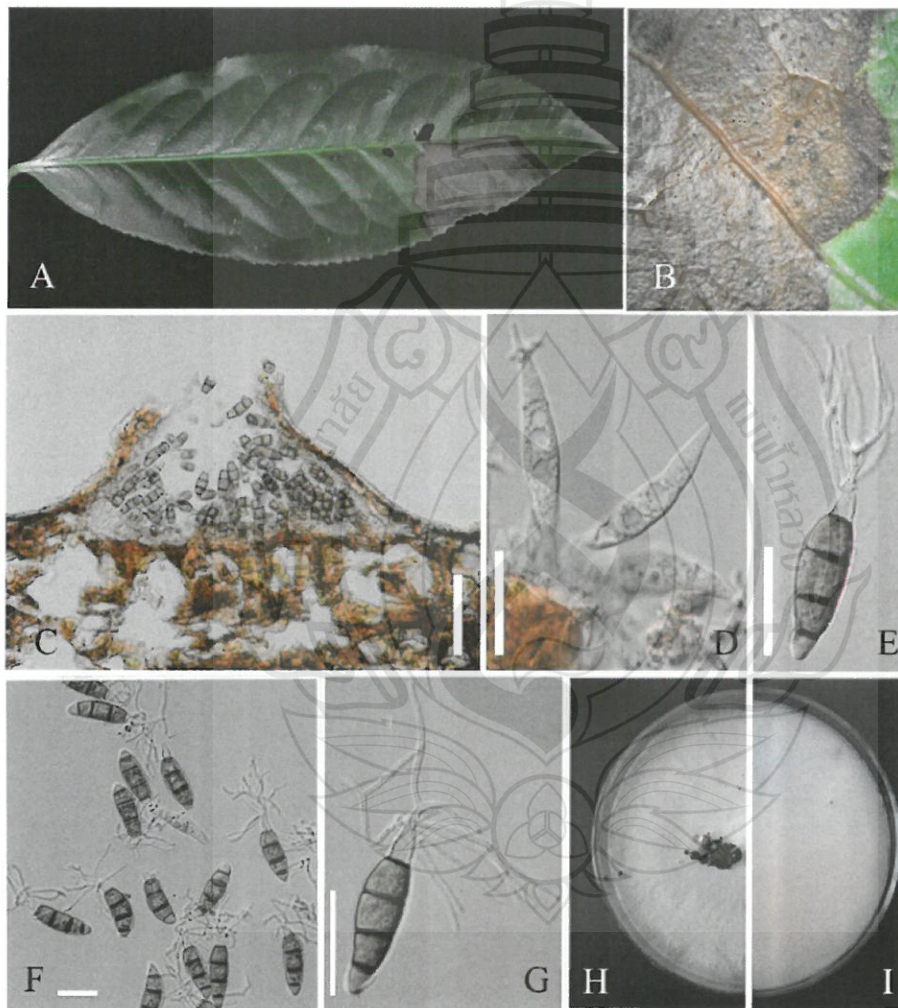
*Etymology*: The specific epithet is based on the branching nature of the apical appendages of the species.

Associated with grey blight on leaves of *Camellia sinensis*, small, rounded, yellow-green spots on the leaves become brown to grey, with concentric rings bearing black, scattered conidiomata. *Conidiomata* acervuli scattered or gregarious, rarely confluent, subepidermal in origin, erumpent when mature, round to oval in outline, conical to oval in longitudinal section, 180–300  $\mu\text{m}$  wide, 70–160  $\mu\text{m}$  high, unilocular, glabrous; wall tissue (stroma and parietal cells) only a few cells thick (14–22  $\mu\text{m}$ ), forming a *textura angularis*, cell walls thick, outermost layer hyaline, inner layers pale brown to brown, encrusted. *Conidiophores* reduced to conidiogenous cells lining the inner wall of the conidiomatal cavity. *Conidiogenous* cells discrete, lageniform, smooth, thin-walled, hyaline, with 2–3 proliferations. *Conidia* fusoid to ellipsoid, straight to slightly curved, 4-septate, 29–39  $\times$  8.5–10.5  $\mu\text{m}$  ( $\bar{x}$  = 35.5  $\times$  9.7  $\mu\text{m}$ ), basal cell obconic, hyaline or slightly olivaceous, thin- and smooth-walled, 4.9–6.4  $\mu\text{m}$  long ( $\bar{x}$  = 5.8  $\mu\text{m}$ ), with 3 median cells, doliiform to subcylindrical, with thick verruculose walls, constricted at the septa, concolorous, olivaceous, septa and periclinal walls darker than the rest of the cell, wall rugose, together 20.7–25  $\mu\text{m}$  long ( $\bar{x}$  = 23.4  $\mu\text{m}$ ) (second cell from base 7–9  $\mu\text{m}$  ( $\bar{x}$  = 7.9  $\mu\text{m}$ ); third cell 7.5–9.1  $\mu\text{m}$  ( $\bar{x}$  = 8.2  $\mu\text{m}$ ); fourth cell 7.2–9.2  $\mu\text{m}$  ( $\bar{x}$  = 8.0  $\mu\text{m}$ ); apical cell hyaline, conic to cylindrical 6.3–8.44  $\mu\text{m}$  long ( $\bar{x}$  = 7.48  $\mu\text{m}$ ); 5–9 tubular apical appendages, some appendages branched, arising from the upper portion of the apical cell, 20–35  $\mu\text{m}$  long ( $\bar{x}$  = 27.7  $\mu\text{m}$ ), unequal; basal appendages absent.

Colonies on PDA reaching 7 cm after 7 days at 25°C, edge entire, whitish, with dense, aerial mycelium on surface, fruiting bodies black, gregarious; reverse of culture white.

*Habitat/Distribution*: Known to inhabit living leaves of *Camellia sinensis*, Thailand.

*Material examined:* THAILAND, Chiang Mai Prov., Mae Taeng Distr., Ban Pha Deng, Mushroom Research Centre, N 19°17.123' E 98°44. 009', elevation 900 m, rainforest, on living leaves of *Camellia sinensis*, January 20, 2010, S.S.N. Maharachchikumbura S200110 (MFLU12-0112, holotype) - ex-type culture MFLUCC 12-0054; *ibid.*, July 10, 2010, S.C. Karunarathna S100710 (MFLU12-0113); *ibid.*, September 9, 2011, S.S.N. Maharachchikumbura S110911 (MFLU12-0114); *ibid.*, December 9, 2011, S.S.N. Maharachchikumbura S91211 (MFLU12-0115).



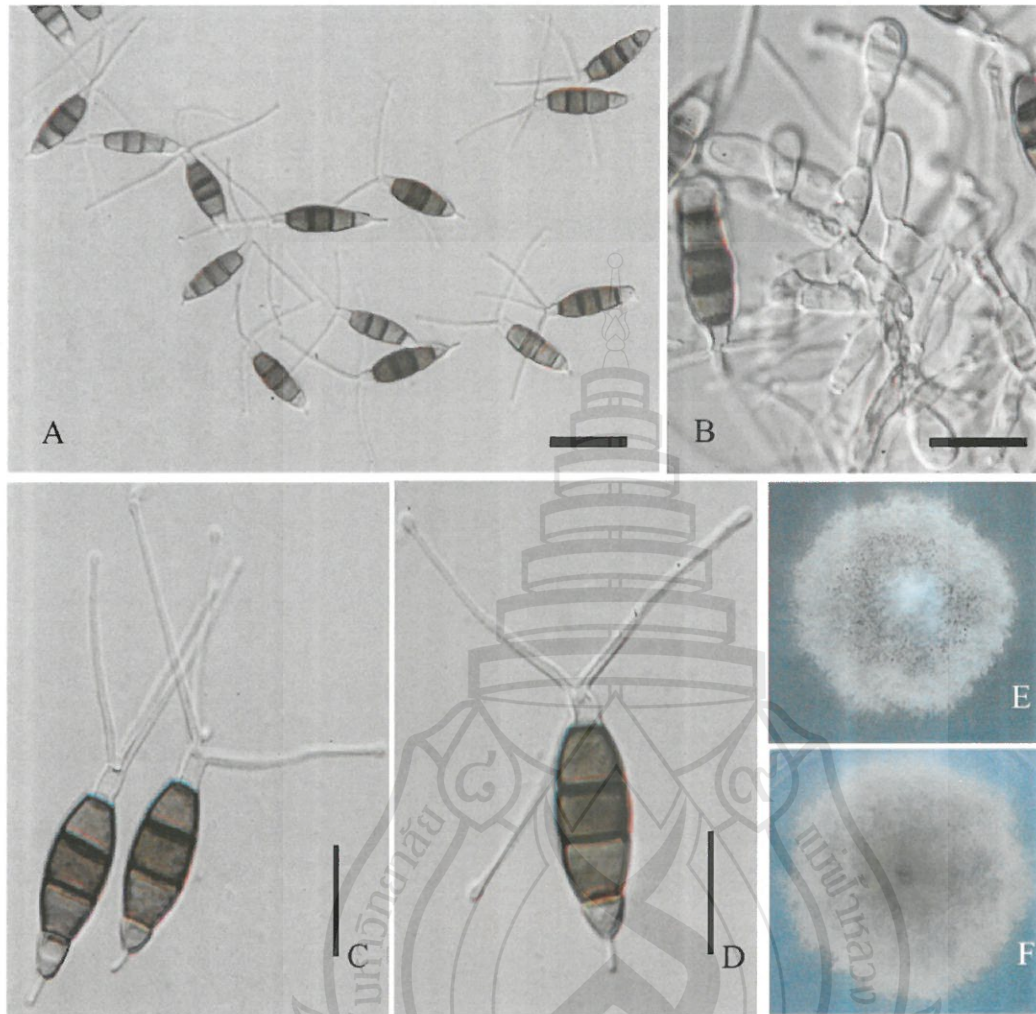
**Figure 4.1** *Pestalotiopsis furcata* sp. nov. (holotype). A. Blight on leaf of *Camellia sinensis*. B. Conidiomata, split irregularly. C. Section of conidiomata. D. Conidiophores/ conidiogenous cells. E–G. Conidia with branched appendages. H, I. Colony on PDA, H from above, I from below. Scale Bars: C = 50  $\mu$ m, D– G= 20  $\mu$ m.

## 2. *Pestalotiopsis theae* (Sawada) Steyaert (EPITYPE)

**Fig. 4.2**

*Conidiophores* growing in clusters, simple, short, filiform, fugacious, smooth, thin-walled, hyaline,  $4-8 \times 1-2 \mu\text{m}$  ( $\bar{x} = 6 \times 1.5 \mu\text{m}$ ). *Conidia* fusiform to ellipsoid, straight to slightly curved, 4-septate  $22.5-28 \times 6.7-8.2 \mu\text{m}$  ( $\bar{x} = 25.5 \times 7.6 \mu\text{m}$ ), basal cell conic or obconic, hyaline, thin and smooth walled,  $3.9-5.3 \mu\text{m}$  long ( $\bar{x} = 4.55 \mu\text{m}$ ), with 3 median cells, thick verruculose walls, constricted at the septa, concolorous, dark brown, septa and periclinal walls darker than the rest of the cell, together  $14.5-18.5 \mu\text{m}$  long ( $\bar{x} = 16.7 \mu\text{m}$ ) (second cell from base  $5-7.2 \mu\text{m}$  ( $\bar{x} = 6.3 \mu\text{m}$ ); third cell  $4.8-6 \mu\text{m}$  ( $\bar{x} = 5.4 \mu\text{m}$ ); fourth cell  $5-6.8 \mu\text{m}$  ( $\bar{x} = 5.7 \mu\text{m}$ )); apical cell hyaline, cylindrical  $4.2-5.9 \mu\text{m}$  long ( $\bar{x} = 5.2 \mu\text{m}$ ); 3-4 apical appendages, tubular, arising from the upper portion of the apical cell,  $22.5-31 \mu\text{m}$  long ( $\bar{x} = 26.5 \mu\text{m}$ ), slightly swollen at the apex; basal appendages, filiform,  $4-7 \mu\text{m}$ . Colonies growing relatively fast on PDA, reaching 7 cm after 5 days at  $25^{\circ}\text{C}$ , fimbriate, whitish, dense, aerial mycelium on surface, fruiting bodies black; reverse of the culture yellowish white.

*Material examined:* TAIWAN, Taipei, on living leaves of *Camellia sinensis*, 13 July 1908, Y. Fujikuro, determined by K. Sawada (BPI 406804, ex-holotype); THAILAND, Chiang Mai PROV., Mae Taeng Distr., Ban Pha Deng, Mushroom Research Centre, N  $19^{\circ}17.123'$  E  $98^{\circ}44.009'$ , elevation 900 m, rainforest, on living leaves of *Camellia sinensis*, January 20, 2010, S.S.N. Maharachchikumbura St200110 (MFLU12-0116, epitype designated here) – ex-type culture MFLUCC 12-0055



**Figure 4.2** *Pestalotiopsis theae* (epitype) A. Conidia in culture. B. Conidiogenous cells. C, D. Conidia. E, F. Colony in culture, E. from above, F. from below. Scale Bars: A-B = 20  $\mu$ m, C-D=15  $\mu$ m.

**3. *Pestalotiopsis samarangensis* Maharachchikumbura & K.D. Hyde, sp. nov. Fig. 4.3**

MycoBank: MB 800178

*Etymology*: The specific epithet is based on the host species, from which the fungus was isolated.

*Conidiomata* acervuli, in concentric bands, confluent, erumpent when mature, rounded to oval in outline, epidermal to superficial in origin, basal stroma and lateral wall 2–4 cells thick; cells hyaline to pale brown, textura angularis 100–350  $\mu$ m wide,

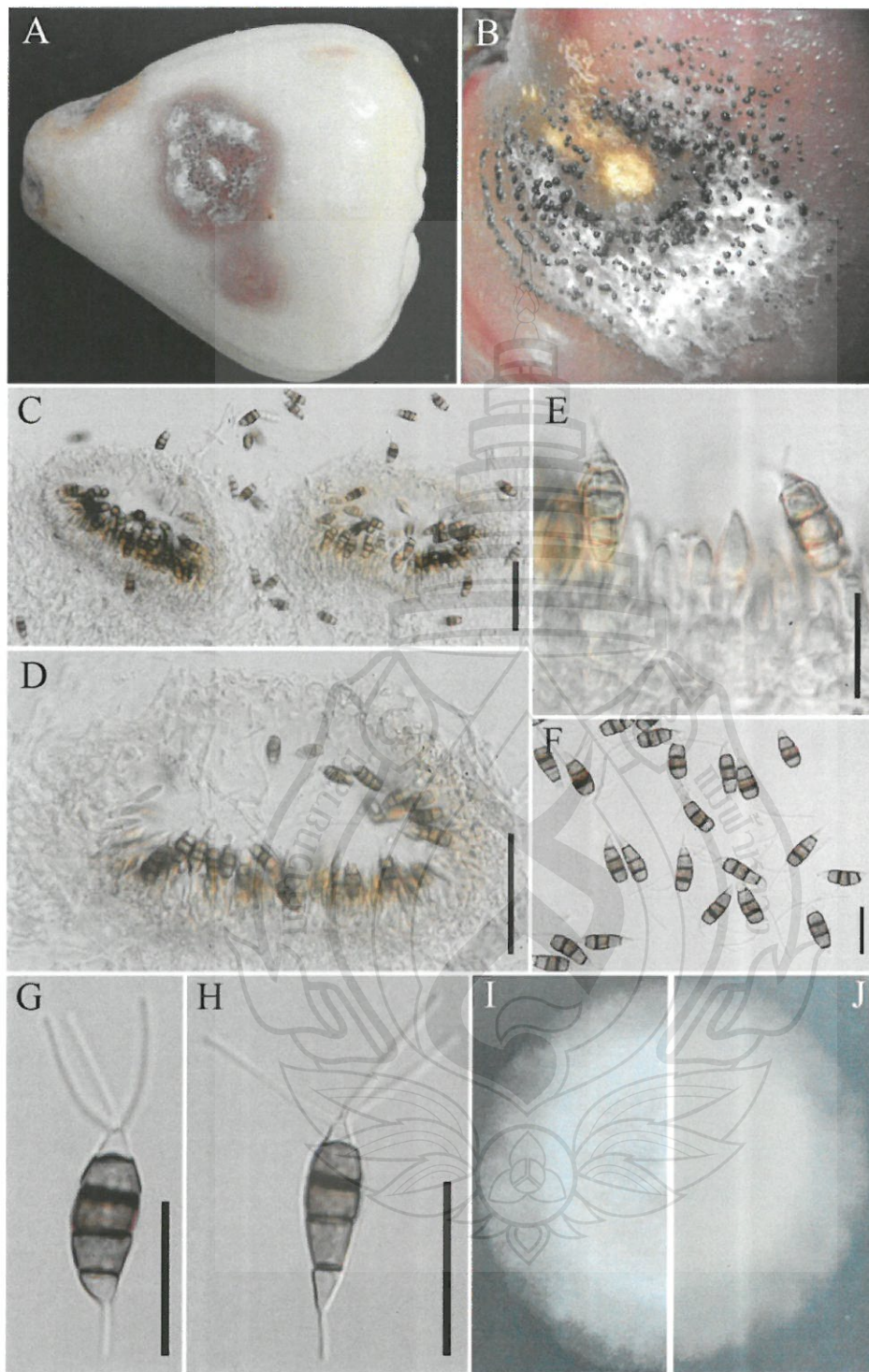


80–150 deep. *Conidiophores* reduced to conidiogenous cells arising within the acervuli. *Conidiogenous* cells discrete, simple, short, filiform. *Conidia* 18–21 × 6.5–7.5 µm ( $\bar{x}$  = 20 × 7 µm), fusiform to ellipsoid, broadly clavate, straight to slightly curved, 4-septate, versicoloured; basal cell conical, hyaline, thin and smooth-walled, 3.5–4.8 µm long ( $\bar{x}$  = 4 µm); apical cell 2.5–4.6 µm long ( $\bar{x}$  = 3.4 µm), conical, hyaline, thin and smooth-walled; three median cells 12.8–13.8 µm long ( $\bar{x}$  = 13.5 µm), with thick verruculose walls, dark brown, central cell darker than the cells on either side, the second cell from base pale brown, 4.3–5.3 µm long ( $\bar{x}$  = 4.8 µm); third cell darker brown, 3.7–5 µm long ( $\bar{x}$  = 4.1 µm); the fourth cell darkest, 4.5–5.3 µm ( $\bar{x}$  = 4.9 µm); three apical appendages 12–18 µm long ( $\bar{x}$  = 15 µm), tubular, arising from the upper portion of the apical cell; single basal appendage, 3.5–5.2 µm long, filiform.

Colonies on PDA reaching 7 cm diam after 6 days at 25°C, edge entire, whitish aerial mycelium, fruiting-bodies black, gregarious; reverse of culture white.

*Habitat/Distribution*: Known to cause fruits rots on *Syzygium samarangense*-in Thailand.

*Material examined*: THAILAND, Chiang Mai Prov., Chiang Mai, on fruits of *Syzygium samarangense*, 20 January 2010, S.S.N. Maharachchikumbura S200110b (MFLU 12-0133; holotype) - ex-type culture MFLUCC 12-0233; *ibid.*, 15 May 2011, S.S.N. Maharachchikumbura S200511 (MFLU 12-0134); Chiang Rai province, Chiang Rai, 15 September 2011, S.S.N. Maharachchikumbura S150911 (MFLU 12-0135).



**Figure 4.3** *Pestalotiopsis samarangensis* (holotype). A, B. Fruit rot of wax apple C, D. Acervular conidiomata, epidermal to superficial in origin E. Conidiogenous cells, F-H. Versicoloured conidia, I, J. Colony on PDA top (I) reverse (J). Scale bars: C, D = 50  $\mu\text{m}$ , E-H = 20  $\mu\text{m}$ .

## 4.2 Phylogenetic studies

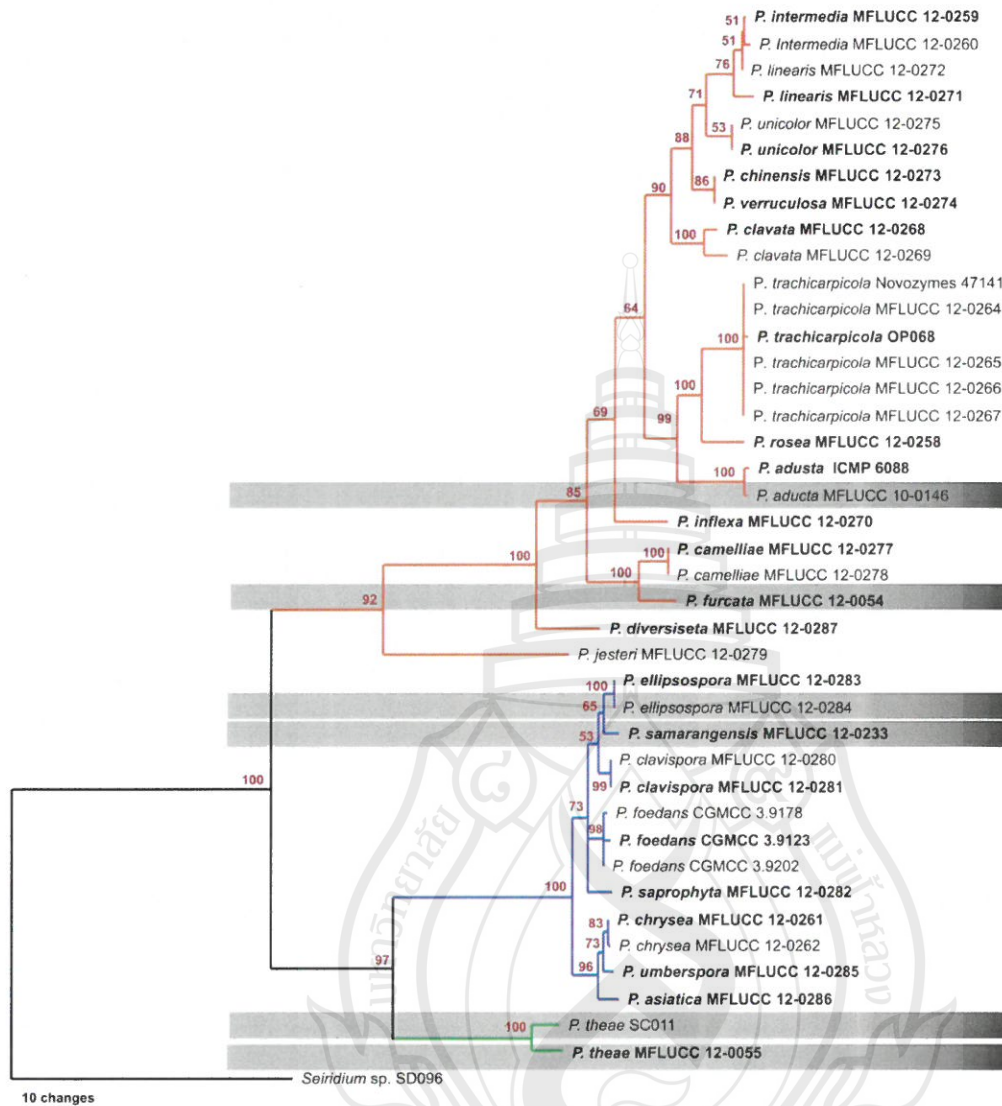
Of the 10 gene regions (ACT,  $\beta$ -tubulin, CAL, GPDH, GS, ITS, LSU, RPB 1, SSU and *tef1*) utilized to resolve cryptic *Pestalotiopsis* species, ITS,  $\beta$ -tubulin and *tef1* proved to be the better markers. The other gene regions were less useful due to poor success in PCR amplification and/or in their ability to resolve species boundaries. As a single gene *tef1* met the requirements for an ideal candidate and functions well for species delimitation due to its better species resolution and PCR success. Although  $\beta$ -tubulin showed fairly good differences among species, a combination of ITS,  $\beta$ -tubulin and *tef1* gene data gave the best resolution as compared to single gene analysis.

**Table 4.2** Comparison of gene regions used in our study

Region	ITS	$\beta$ -tub	<i>tef1</i>	Combined
PCR success/sequencing success	100%	90%	95%	-
Characters in aligned dataset	546	487	1005	2038
Parsimony-informative characters	78 (14.3 %)	154 (31.6 %)	195 (13 %)	427 (21 %)
Number of bootstrap support >50%	16	24	28	34

**Table 4.3** Comparison of gene regions tested but not used in the final phylogenetic studies

Region	Primer/s	PCR success (%)	Sequence success (%)	Species resolution
LSU	LROR/5	100	100	Very low
SSU	NS 1/4	100	100	Very low
Actin	ACT 512F/783R	95	100	Low
GS	GS F1/R1	0	-	-
GPDH	GDF1/GPD2LM	95	100	Low
RPB 1	RPB1 Af/Ac/Cr	60	50	High
CAL	CL 1/2; CAL 228F/737R	70	90	High



**Figure 4.4** Maximum parsimony phylogram generated from combine ITS,  $\beta$ -tubulin and *tef1* analysis. Data were analyzed with random addition sequence, unweighted parsimony and treating gaps as missing data. *Seiridium* spp. was used as the outgroup. Ex-type and ex-epitype sequences are in bold. Thai isolates are in darker.

## CHAPTER 5

### CONCLUSION

In the present study, we have collected 53 *Pestalotiopsis* isolates throughout Northern of Thailand from different habitats and hosts. Most of the *Pestalotiopsis* isolates were obtained from economically important cash crops such as *Artocarpus heterophyllus*, *Camellia sinensis*, *Musa paradisiaca*, *Phylanthus emblica*, *Saccharum* sp., and *Syzygium samarangense*. Of these important hosts, species isolated from *Camellia sinensis* (tea) are described as the new species *Pestalotiopsis furcata* and one species is epitypified as a *P. theae*. The species causing serious fruit rot disease to the *Syzygium samarangense* is described as a new species *Pestalotiopsis samarangensis*.

The morphological characterization of *Pestalotiopsis* isolates clearly show that isolates cluster in to different morphological groups. These morphological groups are highly supported by the sequence data and belong to three distinct clades. These clades corresponded to three conidial types: one clade with pale brown or olivaceous concolorous median conidial cells, the next with versicolorous median conidial cells and the last with dark-coloured concolorous median conidial cells and with knobbed apical appendages. Current research clearly shows that species of *Pestalotiopsis* comprise three species complexes. There are few morphological characters available to distinguish species belonging to these groups. Those morphological important characters include colour of the median cells, the size (length and width) of conidia and the characters within the apical appendages. However the morphological characters are not varied enough to identify species within these complexes. Therefore different gene regions were tested to separate the species within these complexes. Out of tested locus, *tefl* gave the highest species resolution as a single gene and combination of ITS,  $\beta$ -tubulin and *tefl* gave the best resolution. As a result of this study we have published four SCI papers describing 15 new species with three epitypes. Two papers are also in prep and one candidate is training as a PhD student.

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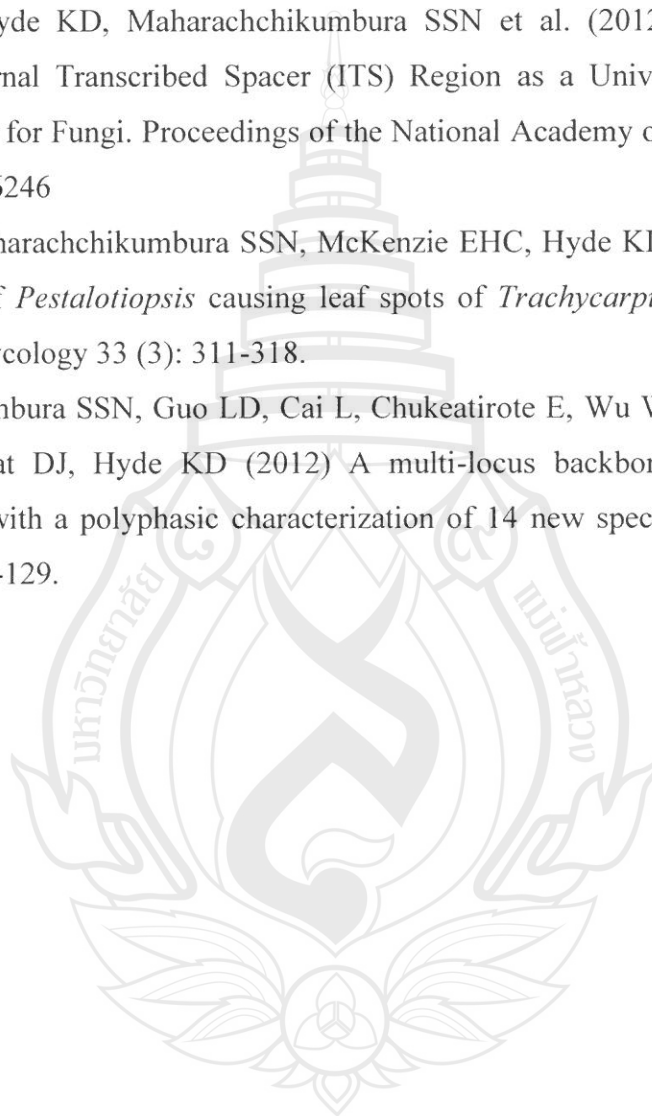
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## *Pestalotiopsis*—morphology, phylogeny, biochemistry and diversity

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**Abstract** The genus *Pestalotiopsis* has received considerable attention in recent years, not only because of its role as a plant pathogen but also as a commonly isolated endophyte which has been shown to produce a wide range of chemically novel diverse metabolites. Classification in the genus has been previously based on morphology, with conidial characters being considered as important in distinguishing species and closely related genera. In this review, *Pestalotia*, *Pestalotiopsis* and some related genera are evaluated; it is concluded that the large number of described species has resulted from introductions based on host association. We suspect that many of these are probably not good biological species. Recent molecular data have shown that conidial characters can be used to distinguish taxa; however, host association and geographical location is less informative. The taxonomy of the genera complex remains confused. There are only a few type cultures and, therefore, it is impossible to use gene sequences in GenBank to clarify species names reliably. It has not even been established whether *Pestalotia* and *Pestalotiopsis* are distinct genera, as no isolates of the type species of *Pestalotia* have been sequenced, and they

are morphologically somewhat similar. When selected GenBank ITS accessions of *Pestalotiopsis clavispora*, *P. disseminata*, *P. microspora*, *P. neglecta*, *P. photinia*, *P. theae*, *P. virgatula* and *P. vismia* are aligned, most species cluster throughout any phylogram generated. Since there appears to be no living type strain for any of these species, it is unwise to use GenBank sequences to represent any of these names. Type cultures and sequences are available for the recently described species *P. hainanensis*, *P. jesteri*, *P. kunningensis* and *P. pallidotheae*. It is clear that the important species in *Pestalotia* and *Pestalotiopsis* need to be epitypified so that we can begin to understand the genus/genera. There are numerous reports in the literature that various species produce taxol, while others produce newly discovered compounds with medicinal potential and still others cause disease. The names assigned to these novel compound-producing taxa lack an accurate taxonomic basis, since the taxonomy of the genus is markedly confused. Until the important species have been epitypified with living strains that have been sequenced and deposited in public databases, researchers should refrain from providing the exact name of species.

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**Keywords** Epitypify · Host occurrence · *Pestalotia* ·  
*Pestalotia* · Pigmentation · Secondary metabolites ·  
Taxol

### Introduction

*Pestalotiopsis* Steyaert is an appendage-bearing conidial anamorphic form (coelomycetes) in the family Amphispheariaceae (Barr 1975, 1990; Kang et al. 1998, 1999), and molecular studies have shown that *Pestalotiopsis* is monophyletic (Jeewon et al. 2002, 2003, 2004). Species of *Pestalotiopsis* are common in tropical and temperate

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## Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi*

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Six DNA regions were evaluated as potential DNA barcodes for *Fungi*, the second largest kingdom of eukaryotic life, by a multinational, multilaboratory consortium. The region of the mitochondrial cytochrome *c* oxidase subunit 1 used as the animal barcode was excluded as a potential marker, because it is difficult to amplify in fungi, often includes large introns, and can be insufficiently variable. Three subunits from the nuclear ribosomal RNA cistron were compared together with regions of three representative protein-coding genes (largest subunit of RNA polymerase II, second largest subunit of RNA polymerase II, and minichromosome maintenance protein). Although the protein-coding gene regions often had a higher percent of correct identification compared with ribosomal markers, low PCR amplification and sequencing success eliminated them as candidates for a universal fungal barcode. Among the regions of the ribosomal cistron, the internal transcribed spacer (ITS) region has the highest probability of successful identification for the broadest range of fungi, with the most clearly defined barcode gap between inter- and intraspecific variation. The nuclear ribosomal large subunit, a popular phylogenetic marker in certain groups, had superior species resolution in some taxonomic groups, such as the early diverging lineages and the ascomycete yeasts, but was otherwise slightly inferior to the ITS. The nuclear ribosomal small subunit has poor species-level resolution in fungi. ITS will be formally proposed for adoption as the primary fungal barcode marker to the Consortium for the Barcode of Life, with the possibility that supplementary barcodes may be developed for particular narrowly circumscribed taxonomic groups.

DNA barcoding | fungal biodiversity

The absence of a universally accepted DNA barcode for *Fungi*, the second most speciose eukaryotic kingdom (1, 2), is a serious limitation for multitaxon ecological and biodiversity studies. DNA barcoding uses standardized 500- to 800-bp sequences to identify species of all eukaryotic kingdoms using primers that are applicable for the broadest possible taxonomic group. Reference barcodes must be derived from expertly identified vouchers deposited in biological collections with online metadata and validated by available online sequence chromatograms. Interspecific variation should exceed intraspecific variation (the barcode gap), and barcoding is optimal when a sequence is constant and unique to one species (3, 4). Ideally, the barcode locus would be the same for all kingdoms. A region of the mitochondrial gene encoding the cytochrome *c* oxidase subunit 1 (*COI*) is the barcode for animals (3, 4) and the default marker adopted by the Consortium for the Barcode of Life for all groups of organisms, including fungi (5). In *Oomycota*, part of the kingdom *Stramenopila* historically studied by mycologists, the de facto barcode internal transcribed spacer (ITS) region is suitable for identification, but the default *COI* marker is more reliable in a few clades of closely related species (6). In plants, *COI* has limited value for differentiating species, and a two-marker system of chloroplast genes was adopted (7, 8) based on portions of the ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit gene and a maturase-encoding gene from

the intron of the *trnK* gene. This system sets a precedent for reconsidering *COI* as the default fungal barcode.

*COI* functions reasonably well as a barcode in some fungal genera, such as *Penicillium*, with reliable primers and adequate species resolution (67% in this young lineage) (9); however, results in the few other groups examined experimentally are inconsistent, and cloning is often required (10). The degenerate primers applicable to many *Ascomycota* (11) are difficult to assess, because amplification failures may not reflect priming mismatches. Extreme length variation occurs because of multiple introns (9, 12–14), which are not consistently present in a species. Multiple copies of different lengths and variable sequences occur, with identical sequences sometimes shared by several species (11). Some fungal clades, such as *Neocallimastigomycota* (an early diverging lineage of obligately anaerobic, zoospore gut fungi), lack mitochondria (15). Finally, because most fungi are microscopic and inconspicuous and many are unculturable, robust, universal primers must be available to detect a truly representative profile. This availability seems impossible with *COI*.

The nuclear rRNA cistron has been used for fungal diagnostics and phylogenetics for more than 20 y (16), and its components are most frequently discussed as alternatives to *COI* (13, 17). The eukaryotic rRNA cistron consists of the 18S, 5.8S, and 28S rRNA genes transcribed as a unit by RNA polymerase I. Posttranscriptional processes split the cistron, removing two internal transcribed spacers. These two spacers, including the 5.8S gene, are usually referred to as the ITS region. The 18S nuclear ribosomal small subunit rRNA gene (SSU) is commonly used in phylogenetics, and although its homolog (16S) is often used as a species diagnostic for bacteria (18), it has fewer hypervariable

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Data deposition: The sequences reported in this paper have been deposited in GenBank. Sequences are listed in Dataset S1.

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<sup>2</sup>A complete list of the Fungal Barcoding Consortium can be found in the SI Appendix. This article contains supporting information online at [www.pnas.org/lookup/suppl/doi:10.1073/pnas.1117018109/-DC5](http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1117018109/-DC5) supplemental.

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## A novel species of *Pestalotiopsis* causing leaf spots of *Trachycarpus fortunei*

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**Abstract** – Specimens of a new *Pestalotiopsis* species were obtained from leaves of *Trachycarpus fortunei* from Kunming Botany Garden, Kunming, Yunnan Province, China, where it caused serious leaf blotch and defoliation in the garden. Single ascospore isolates from the teleomorph produced identical colonies with black slimy conidial masses. Morphological characteristics of the conidia produced in culture accorded well with the genus *Pestalotiopsis*. Based on morphological characters and molecular analysis, *Pestalotiopsis trachicarpicola* sp. nov. is introduced and both its asexual and sexual forms are described.

**Coelomycetes / new species / holomorph / *Pestalosphaeria***

### INTRODUCTION

We are in the process of studying the pathogens of ornamental plants in Yunnan Province. The study involves collecting fresh specimens, isolation, and molecular analysis, and reporting the known and the novel pathogens, so as to strengthen plant quarantine, integrated pest management and diagnosis of fungal diseases of these plants. In this paper we address a species of *Pestalotiopsis* causing serious leaf spotting disease of *Trachycarpus fortunei* (Chinese windmill palm, Arecaceae).

*Pestalotiopsis* is a confused genus with 234 names (<http://www.indexfungorum.org/names/names.asp>; accession date, 2012.02.25), which is in need of molecular characterization. Maharachchikumbura *et al.* (2011) reviewed the genus and noted there were only four sequenced type or epitype strains available and

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## A multi-locus backbone tree for *Pestalotiopsis*, with a polyphasic characterization of 14 new species

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**Abstract** *Pestalotiopsis* is a taxonomically confused, pathogenic and chemically creative genus requiring a critical re-examination using a multi-gene phylogeny based on ex-type and ex-epitype cultures. In this study 40 isolates of *Pestalotiopsis*, comprised of 28 strains collected from living and dead plant material of various host plants from China were studied by means of morphology and analysis of ITS,  $\beta$ -tubulin and *tefl* gene sequence data. Based on molecular and morphological data we describe 14 new species (*Pestalotiopsis asiatica*, *P. chinensis*, *P. chrysea*, *P. clavata*, *P. diversiseta*, *P. ellipsozona*, *P. inflexa*, *P. intermedia*, *P. linearis*, *P. rosea*, *P. saprophyta*, *P. umberspora*, *P. unicolor* and *P. verruculosa*) and three species are epitypified (*P. adusta*, *P. clavispora* and *P. foedans*). Of the 10 gene regions (ACT,  $\beta$ -tubulin, CAL, GPDH, GS, ITS, LSU, RPB 1, SSU and *tefl*) utilized to resolve cryptic *Pestalotiopsis* species, ITS,

$\beta$ -tubulin and *tefl* proved to be the better markers. The other gene regions were less useful due to poor success in PCR amplification and/or in their ability to resolve species boundaries. As a single gene *tefl* met the requirements for an ideal candidate and functions well for species delimitation due to its better species resolution and PCR success. Although  $\beta$ -tubulin showed fairly good differences among species, a combination of ITS,  $\beta$ -tubulin and *tefl* gene data gave the best resolution as compared to single gene analysis. This work provides a backbone tree for 22 ex-type/epitypified species of *Pestalotiopsis* and can be used in future studies of the genus.

**Keywords**  $\beta$ -tubulin · Epitype · ITS · Phylogeny · Saprobe · *tefl*

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(Tanaka et al. 2011) and *Seiridium* (Barnes et al. 2001). *Tefl* is a widely used taxonomic marker and this has been successfully utilized to investigate the kingdom-level phylogeny of eukaryotes (Roger et al. 1999; Baldauf et al. 2000) and species in fungal genera such as *Diaporthe* (Santos et al. 2010; Udayanga et al. 2012), *Fusarium* (Geiser et al. 2004; O'Donnell et al. 2010) and *Trichoderma* and *Hypocrea* (Druzhinina et al. 2005). In the present study,  $\beta$ -tubulin and *tefl* gene regions proved to be favorable taxonomic markers for *Pestalotiopsis* since they resolved the taxonomic relationships of most species studied. Further, *tefl* had better PCR amplification success rates (95 %) and was found to be superior to  $\beta$ -tubulin (90 %). *Tefl* is therefore a powerful tool to resolve lineages within *Pestalotiopsis*. Because of the better PCR and sequencing success rate and fewer difficulties with alignment, editing and better resolution, the *tefl* gene appears to be a very good molecular marker for phylogenetic investigation of *Pestalotiopsis*.

Combined sequence analysis of ITS,  $\beta$ -tubulin and *tefl* genes successfully resolved most of the *Pestalotiopsis* species used in this study with high bootstrap support. Hu et al. (2007) and Liu et al. (2010) have previously shown that a combination of  $\beta$ -tubulin and ITS genes gave better species resolution than a single gene and they suggested that at least two genes should be used to resolve species in *Pestalotiopsis*. Similar results have been shown in *Fusarium* (Summerell et al. 2010); *Calonectria* (Lombard et al. 2010), *Phyllosticta* (Wikee et al. 2011), and *Colletotrichum* (Phoulivong et al. 2010), however the genes best suited for each genus differed. In addition to the above three genes, we tested LSU, SSU, ACT and GPDH (low resolution), GS and RPB1 (cannot be synthesized using available primers or multiple copies) and Calmodulin (species resolution is high, PCR success rate low) and these were less successful in PCR amplification and/or resolving species.

Three epitypes based on live cultures derived from fresh collections from China and Fiji were chosen. When choosing epitypes it is desirable (but not mandatory) to use fresh collections with living isolates from the original host, with the same symptoms and as near to the original location of the holotype as possible (Zhang and Hyde 2008). However, this is not straightforward for *Pestalotiopsis* species which may be endophytes, weak pathogens or saprobes on a wide range of hosts. There are also numerous cryptic species, very few distinct species, species with wide host ranges, those with cosmopolitan distribution and some species being opportunistic pathogens. We have, therefore, been pragmatic in choosing epitypes so that we can advance *Pestalotiopsis* understanding. In this way future workers can pin names to isolated endophytic *Pestalotiopsis* species (Ko Ko et al. 2011) which may be important for chemical bioprospecting and other research in the genus (Xu et al. 2010; Maharachchikumbura et al. 2011).

In the present study multi-locus phylogeny for *Pestalotiopsis* species is presented and strives towards providing biological species concepts based on taxonomically important morphological characters. This backbone tree needs expanding by re-examining type materials of some of the important species described in *Pestalotiopsis* and using multi-locus analysis to establish epitypes. We have not epitypified some of the more commonly known *Pestalotiopsis* names (e.g. *P. disseminata*, *P. fiji*, *P. longiseta*, *P. microspora*, *P. neglecta*, *P. pauciseta*, *P. photinae* and *P. uvicola*) due to lack of fresh collections and living material. Our new species, however, differ from putatively named examples of the common species in GenBank (e.g. *P. microspora*, *P. neglecta*) and thus we are confident that the species newly introduced in this paper are distinct.

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# BIOGRAPHY

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**School of Science, Mae Fah Luang University, Chiang Rai, Thailand;**

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## ***QUALIFICATIONS***

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

## **EXPERIENCE**

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- **Associate Professor**, School of Science, Mae Fah Luang University, Chiang Rai, Thailand – January 2008-present (9 months per year)
- **Associate Professor (Senior Lecturer)**, Department of Ecology & Biodiversity, The University of Hong Kong – 1992-2007.
- **Director**, Centre for Research in Fungal Diversity, Department of Ecology & Biodiversity, The University of Hong Kong – 1998-2007
- **Senior Plant Pathologist**, Queensland Department of Primary Industries – 1989-1991.



## INTERNATIONAL STANDING

### Prestigious committees and awards

1. President of the Asian Mycological Committee

### Journals - Editor in Chief (EC) and Editorial board (EB)

Fungal Diversity (EC)  
 Fungal Diversity Research Series (EC)  
 Agricultural Technology (EC)  
 Mycology (EC)  
 Mycological Research (EB)  
 Biotropica (EB)  
 Cryptogamie Mycologie (EB)  
 Journal of Forest Research (EB)  
 Persoonia (EB)  
 Fungal Ecology (EB)  
 Australian Mycologist (EB)  
 Mycosphere (EB)

## PUBLICATIONS

- I have published more than 600 books and refereed papers and more than 120 abstracts. Of these 440 were in SCI journals.
- I have written, co-authored or co-edited 18 books
- I have edited 3 conference abstract books
- I am Editor-in-Chief and produced 42 volumes of Fungal Diversity
- I am Editor-in-Chief and produced 20 volumes of the Fungal Diversity Research Series
- I was Editor-in-Chief and produced 8 issues of the International Journal of Agricultural Biotechnology

## TEACHING

Taught and designed courses in:

- Plant Pathology (MFLU), Food Biotechnology (MFLU), Mycology (MFLU), Special Project in Mushroom Growing for MSc in Biotechnology (MFLU), Seminar course for MSc in Biotechnology (MFLU), Introductory Microbiology (HKU), Environmental Microbiology (HKU), Applied Microbiology (HKU)

### Main CV (Thailand emphasis)

Graduate students supervision of Thai students or students registered in Thai Universities

### Postdoctoral Fellows Supervised

1. Dr Po Po Than. January 2008 – July 2009. *Colletotrichum*.
2. Dr Sutheera Thongkantha. December 2006 – May 2008. Basidiomycete expert and Scientific Manager at Mushroom Research Centre.
3. Dr Edward Grand. May 2004 – December 2005. Basidiomycete expert and scientific manager at Mushroom Research Centre.
4. Dr Yu Jiang. June 2000 – August 2002. Fungal Biotechnologist (Now Assistant Professor at Baptist University).
5. Dr Ho Wai Hong. August 1998 – June 2006. Culture Collection Manager /Teaching Instructor. Samuel is developing Biotechnological Projects and supervising Undergraduate Students.
6. Dr Sally Fryar. February 1998 - November 2000. Ecology of Freshwater Fungi. Sally was co-supervised by Dr Hodgkiss.
7. Dr E.C.Y. Liew. January 1997 – December 2001. Molecular Phylogeny (Now Principal Scientist in Sydney University).
8. Dr W.S. Wong. September 1996 – December 1997. Ultrastructure of the Xylariaceae and Culture Collection Manager. (Now Project Manager, CK Life Science Limited, HK).

9. Dr T.K. Goh. July 1996 – June 1999. Molecular taxonomy of *Bipolaris*-like fungi (Now Assistant Director, Planning and Coordination Office, HKUST)
10. Dr B. Tread. August 1995 – April 1997. Culture collection and herbarium development. (Now Pharmacist with a French drug company).

### MSC and PhD students completed (Thai Universities)

#### Thai students

1. **Aom Pinnoi.** October 2004-May 2008. **Palm fungi.** (PhD student, jointly supervised at Princess Songkla University, Thailand).
2. **Rampai Kodsueb.** June 2002 – September 2007. **Biodiversity of Saprobic Fungi on Woody Litter.** (PhD student, jointly supervised at Chiang Mai University, Thailand).
3. **Itthayakorn Promputtha** June 2001 – May 2006. **Fungal succession and diversity.** (PhD student, jointly supervised at Chiang Mai University, Thailand).
4. **Sutheera Thongkantha.** June 2002 – July 2006. **Biodiversity of fungi on *Pandanus*.** (PhD student, jointly supervised at Chiang Mai University, Thailand).
5. **B. Bussaban,** July 1999 – October 2005. **Fungi on *Zingiberaceae* in Thailand.** (PhD student, jointly supervised at Chiang Mai University, Thailand).
6. **P. Wipornpan,** July 1998 – May 2004. **Fungi on *Musa acuminata* (banana) in northern Thailand** (PhD student, jointly supervised at Chiang Mai University).
7. **Umpava Pinruan.** October 2004. **Biodiversity and antifungal production by fungi on the palm *Eleiodoxa conferta* in Sirindhorn peat swamp forest, Narathiat, Thailand.** (MSc student, jointly supervised at Chiang Mai University, Thailand).
8. **Aom Pinnoi.** April 2004. **Biodiversity and antifungal production by fungi on the palm *Eleiodoxa conferta* in Sirindhorn peat swamp forest, Narathiat, Thailand.** (MSc student, jointly supervised at Chiang Mai University, Thailand).
9. **Warin Techa.** May 2001. **Diversity of saprobic fungi from *Calamus kerrianus* and *Walichia caryotoides* at Suthep-Pui National Park,**

- Thailand.** (MSc student, jointly supervised at Chiang Mai University, Thailand).
10. **Weraphol Bhilabutra** June 2002 – present. **Grass Fungi.** (Royal Golden Jubilee PhD student, jointly supervised at Chiang Mai University, Thailand) – should submit within 2-3 months.
  11. **Kanchalika Ratanacherdchai**, June 2005-present. **Induced plant immunity to control anthracnose in organic crop production** (Royal Golden Jubilee PhD student, jointly supervised at KMITL, Thailand).
  12. **Mongkol Wongsawas**, June 2007 – present. **Freshwater fungi in Zhejiang Province, China** (PhD student, jointly supervised at Zhejiang University, China).
  13. **Saithong Kaewchay**, June 2006-present. **Biological Control of White Root Disease of Rubber Tree** (Royal Golden Jubilee PhD student, jointly supervised at Chiang Mai University, Thailand).
  14. **Hongli Hu**, September 2005-present. **Brown spored bitunicate ascomycetes** (PhD student, HKU).

#### Asian students at Thai Universities

15. **Ohmar Myo Aung**, May 2004 - June 2008. **Entomophagous fungi in northern Thailand** (PhD student, jointly supervised at KMITL, Thailand).
16. **Ruilin Zhao** July 2004 - June 2008. **Agaricus in northern Thailand** (PhD student, jointly supervised at KMITL, Thailand).
17. **Po Po Than**. October 2004 – February 2008. **Interaction of *Colletotrichum capsici* and *C. gloeosporioides* in anthracnose in chilli.** (PhD student, jointly supervised at Mae Jo University, Thailand).
18. **Duong Minh Lam**. May 2003 – October 2006. **Diversity of litter fungi.** (PhD student, jointly supervised at Chiang Mai University, Thailand).
19. **Tran Thi My Hanh**. October 2004 – June 2006. **Slime molds.** (MSc student, jointly supervised at Kasetsart University, Thailand).
20. **Thida Win Ko Ko**, June 2006-present. **Ecology of myxomycetes in northern Thailand** (PhD student, jointly supervised at Chiang Mai University, Thailand).
21. **Elvie Kurniawati**, October 2008-present. **Diversity of freshwater fungi in**

- streams in northern Thailand** (MS student, Mae Fah Luang University).
22. **Melati P. Hapsari**, October 2008-present. **Freshwater Trichomycetes in northern Thailand** (MS student, Mae Fah Luang University).
23. **Haryudian Prihastuti**, November 2007 – present. ***Colletotrichum* species on coffee in Thailand**. (MS student, Mae Fah Luang University).

### MSC and PhD students in progress (Thai Universities)

#### Thai students

1. **Umpava Pinruan**. October 2004 - present. **Physiology of Palm fungi** (PhD student, jointly supervised at Chiang Mai University, Thailand).
2. **Ratchadawan Cheewangkoon**, June 2006-present **Eucalyptus fungi in Thailand** (PhD student, jointly supervised at Pretoria University, South Africa).
3. **Maythasith Konkarn**, June 2008-present. **Fungi associated with pine-infesting ambrosia beetles in Thailand** (PhD students, jointly supervised at Preoria University, South Afrcia).
4. **Putarak Chomnunti**, October 2008-present. **Biodiversity and phylogeny of selected Dothideomycetes in Thailand 1** (PhD student, MFLU, Thailand).
5. **Saranyaphat boonmee**, October 2008-present. **Biodiversity and phylogeny of selected Dothideomycetes in Thailand 2** (PhD student, MFLU, Thailand).
6. **Pornthip Ruanpanun**, June 2007-present. **Biodiversity and application of actinomycetes and fungal parasites of invertebrates in organic agricultural system** (Royal Golden Jubille PhD student, jointly supervised at Chiang Mai University, Thailand).
7. **Nathacha Seehanan**, June 2008-present. **Biodiversity and utilization of forest litter saprobes** (PhD student, jointly supervised at KMITL, Thailand).
8. **Tanapak Inyod**, June 2008-present. **Biological Control of White Root Disease of Oil Palm** (Royal Golden Jubille PhD student, jointly supervised at KMITL, Thailand).

9. **Parinn Noireung**, June 2009 – present. **Phylogenetic biodiversity of pathogenic *colletotrichum* on plant leaves in northern thailand.** (thailand graduate institute of science and technology (tgist))
10. **Jutamart Monkai**, June 2009 - present. **Utilizing Thailand's biodiversity: ascomycetes taxonomy, phylogeny and screening for insecticides.** (basic research (trf))
11. **Rungtiwa Phookamsak**, June 2009 – present. **Biodiversity,taxonomy and phylogenetic studies on dothideomycetes monocotyledons in thailand.**( royal golden jubille)
12. **Supalak Yacharone**, October 2009 – present. **Utilizing Thailand's biodiversity: ascomycetes taxonomy, phylogeny and screening for insecticides.** (basic research (trf))
13. **Naritsada Thongklang**, October 2009 – present. **Cultivation of agaricus species endemic to thailand and their medicinal properties.**(brn)
14. **Saowanee Wikee**, June 2010 – present. **taxonomy and phylogeny of phyllosticta, diplodia and stemphylium species in northern thailand.**

#### Asian students at Thai Universities

15. **Sitthisack Phoulivong**, November 2007 – present. **A study of the diversity of *Colletotrichum acutatum* in Thailand.** (PhD student, Mae Fah Luang University).
16. **Samantha Karunarathna**, October 2008-present. **Diversity and taxonomy of *Lentinus* and *Panus* in Northern Thailand and Sri Lanka**(PhD student, Mae Fah Luang University).
17. **Nilam Wulandari**, June 2006-present. **A world monograph of *Guignardia*.** (PhD student, jointly supervised at Chiang Mai University, Thailand).
18. **Phongun Sysouphanthong**, October 2006-present. **Diversity of *Lepiota* and *Leucoagaricus* (BASIDIOMYCOTA) in Northern Thailand** (MS student, Mae Fah Luang University).
19. **Paul Mungai**, October 2008-present. **Diversity of dung fungi on wild anamils in Kenya** (MS student, Mae Fah Luang University).

20. **Manamgoda Gamage Dimuthu Sandarenu Manamgoda** June 2010 – present **Taxonomy and phylogenetics of cochliobolus curvularia bipolaris and helminthosporium** (PhD student Mae Fah Luang University).
21. **Dhanushka Udayanga** June. 2010 – present Taxonomy and phylogeny of the genera phomopsis/diaporthe and colletotrichum (PhD student Mae Fah Luang University)
22. **Sajeewa Maharachchikumbura** June.2010 – present **Phylogenetic evaluation of genus pestalotiopsis using both morphological and molecular character and studies in the natural product chemistry of selected species of pestalotiopsis** (PhD student Mae Fah Luang University)
23. **Jiankui Liu** October 2009 – present. **The phylogeny of higher fungi (ascomycota and basidiomycota) from palms.** (PhD student Mae Fah Luang University).
24. **Pheng Phengsintham** October 2009 – present. Cercospora and allied genera from northern thailand and laos (PhD student Mae Fah Luang University)

#### **Other student supervision**

#### **Research Assistant Professor Supervised**

- 1) **Dr Stephen Pointing**. October 1998 - July 2001. **Fungal Enzymology** (now **Assistant Professor** in the Department of Ecology & Biodiversity).

#### **Postdoctoral Fellows Supervised**

1. **Dr Sutheera Thongkantha**. December 2006 – 2008. Basidiomycete expert and Scientific Manager at Mushroom Research Centre
2. **Dr Rajesh Jeewon**. April 2003-present. **Fungal Molecular Biologist**.
3. **Dr Edward Grand**. May 2004 – December 2005. **Basidiomycete expert and scientific manager at Mushroom Research Centre**.
4. **Dr Yu Jiang**. June 2000 – August 2002. **Fungal Biotechnologist** (Now **Assistant Professor** at Baptist University).

5. **Dr Ho Wai Hong.** August 1998 – June 2006. **Culture Collection Manager /Teaching Instructor.** Samuel is developing Biotechnological Projects and supervising Undergraduate Students.
6. **Dr Sally Fryar.** February 1998 - November 2000. **Ecology of Freshwater Fungi.** Sally was co-supervised by Dr Hodgkiss.
7. **Dr E.C.Y. Liew.** January 1997 – December 2001. **Molecular Phylogeny** (Now **Principal Scientist** in Sydney University).
8. **Dr W.S. Wong.** September 1996 – December 1997. **Ultrastructure of the Xylariaceae and Culture Collection Manager.** (Now Project Manager, CK Life Science Limited, HK).
9. **Dr T.K. Goh.** July 1996 – June 1999. **Molecular taxonomy of Bipolaris-like fungi** (Now Assistant Director, Planning and Coordination Office, HKUST).
10. **Dr B. Tread.** August 1995 – April 1997. **Culture collection and herbarium development.** (Now **Pharmacist** with a French drug company).

#### PhD students completed

11. **Shenoy Belle Damodar.** September 2003-September 2007. **Grass endophytes.** (PhD student, HKU).
12. **Alvin Tang Ming Chak.** September 2003-October 2006. **Multigene analysis of Sordariomycetes** (PhD student, HKU).
13. **Justin Bahl.** January 2002-June 2006. **Molecular phylogenetics of Hyponectriaceae.** (PhD student, HKU).
14. **Cai Lei.** October 2002- May 2006. **Aquatic fungi.** (PhD student, HKU).
15. **Dhanasekaran Vijaykrishna,** January 2002 - February 2005. **Freshwater Fungi: Biodiversity, Origins and molecular taxonomy.** (PhD student, HKU).
16. **B. Paulus,** June 2000 – October 2004. **Measuring Biodiversity of Fungi in North Queensland, Australia** (PhD student, jointly supervised at James Cooke University, Cairns, NQ, Australia).
17. **S.S.K. Durairajan,** July 2000 – Jan 2004. **Biological screening and isolation of immunomodulatory compounds from endophytic fungi of *Tripterygium***



*wilfordii* (PhD student, HKU).

18. **V. Bucher**, September 1999 – May 2003. **Enzymes from aquatic fungi** (PhD student, HKU).
19. **G. Smith**, August 1998 – April 2003. **Phylogenetic studies on the Xylariaceae** (PhD student, HKU).
20. **Dr S.R. Ghimire**, Nepal, January 1999 – December 2001. ***Phytophthora infestans* population in Nepal** (Post Doctoral Fellow, USA).
21. **Dr R. Jeewon**, Mauritius, January 1999 – December 2001. ***Pestalotiopsis* Taxonomy: Molecular Phylogenetics, Species nomenclature and Telemorph Relationships**. (Post Doctoral Fellow, HKU).
22. **Dr O.H.K. Lee**. February 1998 – January 2001. **The Feeding Ecology of *Littoraria* species in Hong Kong Mangroves** (PhD HKU, now Assistant Environmental Protection Officer, Environmental Protection Department, HKSAR).
23. **Dr Yanna**. February 1998 – January 2001. **Biodiversity, Ecology and Taxonomy of Saprobic Fungi on Palm Fronds** (PhD HKU, now Environmental Education Officer, Environmental Protection Department, HKSAR).
24. **Assistant Professor Dr Y.Z. Wang**. December 1997 – November 2000. **Revision of the Ascomycete Genus *Amphisphaeria*** (PhD HKU, now Chief Manager, China General Microbiological Culture Collection, Chinese Academy of Sciences, Beijing).
25. **Dr D. Zhou**. December 1997- November 2000. **Biodiversity of Saprobic Microfungi Associated with Bamboo in Hong Kong and Kunming, China** (PhD student, HKU, now Dean and Professor, Faculty of Conservation Biology, Southwest Forestry University, Kunming, China).
26. **Professor B.H. Lu**. May 1997 - April 2000. **A World Monograph of *Anthostomella*** (PhD HKU, now Professor, Department of Plant Protection, Faculty of Agriculture, Shanxi Agricultural University, Taigu, China).
27. **Dr M.K.M. Wong**. March 1997 – May 2000. **Diversity, Host Preference , and Vertical Distribution of Saprobic Fungi on Grasses and Sedges in Hong Kong** (PhD HKU, now Post Doctoral Fellow in Department of Zoology, HKU).
28. **Dr L.D. Guo**. February 1997 – January 1999. **Identification of Endophytic Fungi**

in *Livistona chinensis* (Palmae) (PhD HKU, now a scientist at the Lichen and Mycology Laboratory, Academia Sinica, Beijing).

29. **Dr C. Pearce.** February 1996 – Decemebr 1999. **The *Phyallachoraceae* of Australia: a Taxonomic Treatise** (PhD HKU, now **Director** of the Australian Tropical Rainforest Research Centre, Cairns).
30. **Dr C.K.M. Tsui.** Septemebr 1996 – August 1999. **Biodiversity and Longitudinal Distribution of Fungi on Submerged Wood, with Reference to Human Disturbance** (PhD HKU, now **Post Doctoral Fellow** in Botany, HKU).
31. **Dr M. Ranghoo.** December 1995 – November 1998. **Phylogeny of Freshwater Ascomycetes** (PhD HKU, now Molecular Biologist with the Mauritius Sugar Research Institute).
32. **Dr S.R. Whitton.** October 1995 – August 1999. **Microfungi on the *Pandanaceae*** (PhD HKU. **Post Doctoral Fellow** at Landcare Research, New Zealand).
33. **Dr W.H. Ho.** July 1995 – June 1998. **Biodiversity, Ecology and Ultrastrcture Observations of fungi on wood submerged in tropical streams** (PhD HKU, **Post Doctoral Fellow** and **Culture Collection Manager** in Ecology & Biodiversity, HKU).
34. **Dr T.K. Yeun.** July 1995 – June 1998. **Wood Decomposition and Competition in Tropical Freshwater Fungi** (PhD HKU, now Consultant, N. Law and Associates Management Consultancy Ltd, Hong Kong).
35. **Dr A. Poonyth.** May 1995 – August 1998. **Biodiversity of Mangrove Fungi in Mauritius.** (PhD University of Mauritius, cosupervisod, now **Research Officer** for Mauritius Wildlife and Parks).
36. **Dr T. Dalisay.** January 1995 – February 1998. **Biodiversity of Microfungi Associated with Species of *Bambusa* and *Dendrocalamus*** (PhD HKU, now **Associate Professor** in Plant Pathology at The University of the Philippines, Los Banos).
37. **Dr J. Wright.** October 1994 – March 1998. **The Role of Endophytes in Citrus Stem End Rots** (PhD HKU, now **Plant Pathologist** in Fiji).
38. **Dr J. Taylor.** January 1994 – September 1997. **Biodiversity of distribution of Microfungi on Palms** (PhD HKU, now **Lecturer** at Botswana University).
39. **Dr Kang Ji Chuan.** December 1993 – March 1997. **Molecular and**

**Morphological Studies on the *Amphisphaeriaceae*** (PhD HKU, now a **Post Doctoral Fellow** at the University of Stellenbosch in South Africa).

40. **Dr W.S. Wong.** August 1993 – July 1996. **Ultrastructural and Taxonomic Studies of Freshwater Ascomycetes** (PhD HKU).
41. **Dr J. Fröhlich.** June 1993 – June 1997. **Biodiversity of Microfungi Associated with Palms in the Tropics** (PhD HKU, now Principal Scientist with Landcare Research in Auckland, New Zealand working on biocontrol of weeds).
42. **Aung Swe.** September 2004 – present. **Nematode trapping fungi.** (PhD student, HKU) – will submit at the end of August.

#### **MPhil students completed**

1. **Hu Dianming.** June 2003-May 2006. **Dung fungi.** (MSc student, jointly supervised at Yunnan University, China).
2. **Li Yan.** June 2003-May 2006. **Nematodes trapping fungi in China.** (MSc student, jointly supervised at Yunnan University, China).
3. **Hong Zhu.** June 2003-May 2006. **Aquatic fungi in Yunnan China.** (MSc student, jointly supervised at Yunnan University, China).
4. **Hongli Hu.** June 2003 – June 2005. **Chinese pine fungi.** (MSc student, jointly supervised at Yunnan University, China).
5. **QuinYeung Sze Yuen.** September 2002-January 2005. **The fungal diversity of Pinaceae in Hong Kong** (MPhil, HKU).
6. **P. Alva.** January 2000 – February 2005. **Internal fungi from seagrasses and their ability to produce enzymes** (MSc student, jointly supervised at Ateneo de Manila University of the Philippines).
7. **Sin Kai Wai.** July 2004. **Molecular biology, physiology and metal resistance of the ligninolytic enzyme system in a newly isolated basidiomycete from a Hong Kong forest.** (Mphil, HKU)
8. **Luo Jing.** May 2004. **Taxonomic and ecological studies on freshwater fungi associated with identified substrates.** (MSc student, jointly supervised at Yunnan University, Kunming, China).
9. **Nguyễn văn Diễm.** December 2003. **Saprobe ascomycetes on *Nypa fruticans***

- in Can Gio Mangrove Biosphere Reserve, Vietnam** (Student, jointly supervised at Hanoi University of Education).
10. **A. Besitulo**. April 2002. **Occurrence and distribution of fungi in a mangrove forest at Siargao Island, Philippines** (MSc student, jointly supervised at St Carlos University, Cebu).
  11. **D. Lacap**. March 2001. **Biodiversity of fungal endophytes on *Taxus baccata* (Taxaceae) and *Polygonum multiflorum***. (MSc student, jointly supervised at Ateneo de Manila University of the Philippines).
  12. **A.M.C. Tang**, January 2001 – present. **Secondary metabolites of wild fruits in Hong Kong: Implications for antimicrobial defense and seed dispersal** (MPhil student, HKU).
  13. **C. Lei**, July 2000 – present. **Freshwater Fungi in Yunnan, China** (MSc student, jointly supervised at Yunnan University, Kunming, China).
  14. **Y.W. Choi**, January 1999 – present. **Endophytes on *Brucia javanica*** (M.Phil student, HKU).
  15. **S.W. Lee**, January 2000 – present. **Post Harvest Diseases of Citrus** (M.Phil student, HKU).

**Graduate students** (MS and PhD students in Progress)

25. **Zhang Ying**, September 2006 – present. **Revision of the Pleosporales using morphology and gene sequencing**. (PhD student, HKU).
26. **Hu Dianming**. June 2008-present. **Freshwater fungi in Yunnan Province, China**. (PhD student, IFRD, China).
27. **Yang Youlian**. June 2007-present. **Colletotrichum species in Guizhou Province in China**. (PhD student, jointly supervised at Guizhou University).
28. **Zhang Huang**, September 2009 – present. **Freshwater huculoasconycales**. (PhD student, Kunming University of science & technology)

### Community Service

- I was **Coordinator of EASIANET** from 2004 until 2007. This was an elected position for the body designated with the role to remove the taxonomic impediment to CBD from the East Asia region.
- I am **President** of the Asian Mycological Committee. This committee aims to promote the study of mycology throughout the Asian region.
- In 1997 **the Mycological Association of Hong Kong** was inaugurated. I was **Chairman** of this organization between 2002-2007.
- I have also been **external examiner** for students at many Universities.
- I have given more than 20 **keynote and guest lectures** and numerous invited lectures.

I have **organised** (as Chair or on committee) more than 10 international conferences and numerous workshops

### Publication list

Year	International Publications	SCi Papers	Thailand collaboration
1985	2	2	
1986	8	5	
1987	2	2	
1988	8	4	
1989	14	9	1
1990	8	4	1
1991	10	6	
1992	21	9	
1993	22	9	

1994	18	7	1
1995	27	19	
1996	36	26	
1997	37	16	
1998	50	33	
1999	60	30	
2000	51	27	1
2001	56	48	5
2002	39	31	9
2003	43	33	9
2004	48	26	16
2005	24	21	4
2006	31	27	11
2007	23	19	7
2008	24	24	11
2009	23	23	8
2010			

#### Books (17)

1. **Hyde, K.D.** (ed.) (1997). *Biodiversity of Tropical Microfungi*. Hong Kong University Press, Hong Kong, 421p.
2. **Hyde, K.D.** and Cannon, P.F. (1999). *Fungi Causing Tar Spots on Palms*. IMI, UK, 114p.
3. **Hyde, K.D.** and Pointing, S.B. (eds.) (2000). *Marine Mycology - A Practical Approach*. [Fungal Diversity Research Series 1], Fungal Diversity Press, Hong Kong, 377p.

4. **Hyde, K.D.**, Taylor, J.E. and Fröhlich, J. (2000). *Genera of Ascomycetes from Palms*. [Fungal Diversity Research Series 2], Fungal Diversity Press, Hong Kong, 247p.
5. Fröhlich, J. and **Hyde, K.D.** (2000). *Palm Microfungi*. [Fungal Diversity Research Series 3], Fungal Diversity Press, Hong Kong, 393p.
6. Lu, B.H. and **Hyde, K.D.** (2000). *A World Monograph of Anthostomella*. [Fungal Diversity Research Series 4], Fungal Diversity Press, Hong Kong, 376p.
7. Lu, B.H., **Hyde, K.D.**, Ho, W.H., Tsui, K.M., Taylor, J.E., Wong, K.M., Yanna and Zhou, D.Q. (2000). *Checklist of Hong Kong Fungi*. [Fungal Diversity Research Series 5], Fungal Diversity Press, Hong Kong, 207p.
8. **Hyde, K.D.**, Ho, W.H. and Pointing, S.B. (2000). *Aquatic Mycology across the Millennium*. [Fungal Diversity 5], Fungal Diversity Press, Hong Kong, 207p.
9. Pointing, S.B. and **Hyde, K.D.** (eds.) (2001). *Bio-Exploitation of Filamentous Fungi*. [Fungal Diversity Research Series 6], Fungal Diversity Press, Hong Kong, 467p.
10. **Hyde, K.D.** (ed.) (2002). *Fungi in Marine Environments*. [Fungal Diversity Research Series 7], Fungal Diversity Press, Hong Kong, 397p.
11. Tsui, C.K.M. and **Hyde, K.D.** (2003). *Freshwater Mycology*. *Fungal Diversity Research Series* 10: 1-350.
12. Taylor, J.E. and **Hyde, K.D.** (2003). *Microfungi on Tropical and Temperate Palms*. *Fungal Diversity Research Series* 1-459.
13. Wang, Y.Z., Aptroot, A. and **Hyde, K.D.** (2004). *Review of the Genus Amphisphaeria*. *Fungal Diversity* 13: 1-180.
14. E.B.G. Jones, M. Tantichareon and **K.D. Hyde** (eds.) (2004). *Thai Fungal Diversity*. BIOTEC, Thailand: 281p.
15. Pearce, C. and **Hyde, K.D.** (2006). *Phyllachoraceae of Australia*. *Fungal Diversity Research Series* 17: 1-308.
16. Cai, L. **Hyde, K.D.** and Tsui, C.K.M.T. (2006). *Genera of Freshwater Fungi*. *Fungal Diversity Research Series* 18: 1-261.
17. Sridhar, K.R., Barlocher, F. and **Hyde, K.D.** (eds.) (2008). *Novel Techniques and Ideas in Mycology*. *Fungal Diversity Research Series* 20: 1-373.

### Journal Articles, Book Chapters and Other Published Papers

- Between 1985-2003 I published 316 *SCI* publications, 86 publications in *non-SCI* International journals and 18 Book chapters.
- I have published more than 140 abstracts

#### 2004

- 48 Publications (30 SCI)
1. **Hyde, K.D.** (2004). Fungal Conservation: Issues and Solutions. *The Quarterly Review of Biology* **79**: 80-81.
  2. Bucher, V.V.C., **Hyde, K.D.**, Pointing, S.B. and Reddy, C.A. (2004). Production of wood decay enzymes, mass loss and lignin solubilization in wood by diverse freshwater fungi. *Microbial Ecology* **48**: 331-337.
  3. Fryar, S.C., Davies, J., Booth, W., Hodgkiss, I.J. and **Hyde, K.D.** (2004). Succession of fungi on dead and live wood in brackish water. *Mycologia* **96**: 219-225.
  4. Ho, W.H. **Hyde, K.D.**, Hodgkiss, I.J. and Yanna (2004). *Cataractispora receptaculorum*, a new freshwater ascomycete from Hong Kong. *Mycologia* **96**: 411-417.
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## 2005

- 23 Publications (20 SCI)

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## 2006

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15. Li, Y., Jeewon, R., **Hyde, K.D.**, Mo, M., and Zhang, K. (2006). Two new species of nematode trapping fungi relationships inferred from morphology, rDNA and protein gene sequence analyses. *Mycological Research* **110**: 790-800.
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17. Cai, L., Jeewon, R. and **Hyde, K.D.** (2006). Phylogenetic investigations of Sordariaceae based on multiple gene sequences and morphology. *Mycological Research* **110**: 137-150.
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20. Paulus, B., Gadek, P. and **Hyde, K.D.** (2005). *Discostroma ficicola* sp. nov. (Amphisphaeriaceae) and a key to species of *Discostroma*. *Sydowia* **58**: 76-90.
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22. Aung, O.M., Kang, J.C., Liang, Z.Q., Soyong, K. and **Hyde, K.D.** (2006). *Cordyceps mrciensis* sp. nov. from a spider in Thailand. *Mycotaxon* **97**: 253-240.
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25. Zhu, H., Cai, L., Zhang, K.Q. and **Hyde, K.D.** (2006). A new species of *Acrogenospora* from submerged bamboo in Yunnan, China *Mycotaxon* **95**: 348-358.
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  27. 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.
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  29. Yeung, Q.S.Y., Jeewon, R. and **Hyde, K.D.** (2006). *Cancellidium pinicola* sp. nov. from *Pinus massoniana* and its phylogeny. *Cryptogamie Mycologie* **27**: 295-304.
  30. Bhilabutra, W., Lumyong, S., Jeewon, R., McKenzie, E.H.C. and **Hyde, K.D.** (2005). *Neolinocarpon penniseti* sp. nov. on the grass *Pennisetum purpureum* (Poaceae). *Cryptogamie Mycologie* **27**: 305-310.
  31. **Hyde, K.D.** and Sarma, V.V. (2006). Biodiversity and ecological observations on filamentous fungi of mangrove palm *Nypa fruticans* Wurumb (Liliopsida-Arecales) along the Tutong River, Brunei. *Indian Journal of Marine Science* **35**: 297-307.
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## 2007

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1. Tang, A.M.C., Jeewon, R. and **Hyde, K.D.** (2007). Phylogenetic utility of protein (RPB2, B-tubulin) and ribosomal (18S, 28S) gene sequences in the systematics of Sordariomycetes (Ascomycota, Fungi). *Antonie van Leeuwenhoek International Journal of General and Molecular Microbiology* **91**: 327-394.
2. Promputtha, I., Jeewon, R., Lumyong, S., McKenzie, E.H.C. and **Hyde, K.D.** (2007). A phylogenetic evaluation of whether endophytes become saprotrophs at host senescence. *Microbial Ecology* **53**: 579-590.
3. Kodsueb, R., Jeewon, R., **Hyde, K.D.**, McKenzie, E.H.C. and Lumyong, S. (2007). Morphological and molecular characterization of Aquaticheirospora and phylogenetics of Massarinaceae (Pleosporales). *Botanical Journal of the Linnean Society* **155**: 283-296.
4. Zhao, R.L., Jeewon, R., Grand, E., Desjardin, D., Soyong, K. and **Hyde, K.D.** (2007). Ribosomal DNA phylogenetics of *Cyathus*; is the current infrageneric classification appropriate? *Mycologia* **99**: 385-395.
5. Pinnoi A, Jeewon R, Sakayaroj J, **Hyde, K.D.** and Jones, E.B.G. (2007). *Berkleasium crunisia* sp. nov. and its phylogenetic affinities to the Pleosporales based on 18S and 28S rDNA sequence analyses. *Mycologia* **99**: 378-384.
6. Hu HL, Jeewon R, Zhou DQ, Zhou, T.X. and **Hyde, K.D.** (2007). Phylogenetic diversity of endophytic *Pestalotiopsis* species in *Pinus armandii* and *Ribes* spp.: evidence from rDNA and beta-tubulin gene phylogenies. *Fungal Diversity* **24**: 157-173.
7. Pinruan, U., Lumyong, S., **Hyde, K.D.** and Jones, E.B.G. (2007). Occurrence of fungi on tissues of the peat swamp palm *Licuala longecalycata*. *Fungal Diversity* **25**: 157-173.
8. Shenoy, B.B., Jeewon, R., and **Hyde, K.D.** (2007). Morpho-molecular characterisation and epitypification of *Colletotrichum capsici* (Glomerellaceae, Sordariomycetes), the causative agent of anthracnose in chilli. *Fungal Diversity* **27**: 197-211.
9. Tang, A.M.C., Jeewon, R. and **Hyde, K.D.** (2007). Phylogenetic relationships of *Nemania plumbia* sp. nov. and related taxa based on ribosomal ITS and RPB2

- sequences. *Mycological Research* **111**: 392-402.
10. **Hyde, K.D.** Bussaban, B., Paulus, B., Crous, P.W., Lee, S., Mckenzie, E H.C., Photita, W. and Lumyong, S. (2007). Biodiversity of saprobic fungi. *Biodiversity and Conservation* **16**: 17-35.
  11. Hibbitt, D.S., **Hyde, K.D.** et al (2007). A higher-level phylogenetic classification of the fungi. *Mycological Research* **111**: 509-547.
  12. Wang HK, Aptroot A, Crous PW, **Hyde KD**, Jeewon R (2007) The polyphyletic nature of Pleosporales: an example from *Massariosphaeria* based on rDNA and RBP2 gene phylogenies. *Mycological Research* **111**: 1268-1276.
  13. Cai, L. and **Hyde, K.D.** (2007). Anamorphic fungi from China freshwater habitats: *Dictyosporium tetrasporum* sp. nov., *Exserticlava yunnanensis* sp. nov., and new records *Pseudofuscophialis lignicola* and *Pseudobotrytis terrestris*. *Cryptogamie Mycologie* (In press).
  14. 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.
  15. Cabanela MV, Jeewon R, **Hyde KD** (2007) Morphotaxonomy and phylogeny of *Paoayensis lignicola* gen. et sp nov (ascomycetes) from submerged wood in Paoay Lake, Ilocos Norte, the Philippines. *Cryptogamie Mycologie* **28**, 301-310.
  16. Cai L, **Hyde KD** (2007) *Ascorhombispora aquatica* gen. et sp nov from a freshwater habitat in China, and its phylogenetic placement based on molecular data. *Cryptogamie Mycologie* **28**, 291-300.
  17. Hu DM, Zhu H, Cai L, **Hyde KD**, Zhang KQ (2007) *Sirothecium triseriale*, a new chirosporous anamorphic species from China. *Cryptogamie Mycologie* **28**, 311-314.
  18. **Hyde, K.D.** and Soyong, K. (2007) Understanding microfungus diversity - a critique. *Cryptogamie Mycologie* **28**, 281-289.
  19. Kodsueb R, McKenzie EHC, Ho WH, **Hyde KD**, Lumyong P, Lumyong S (2007) New anamorphic fungi from decaying woody litter of *Michelia baillonii* (Magnoliaceae) in northern Thailand. *Cryptogamie Mycologie* **28**, 237-245.
  20. Huang, W.Y., Cai, Y.Z., **Hyde, K.D.**, Corke, H. and Sun, M. (2007). Endophytic

fungi from *Nerium oleander* L (Apocynaceae): main constituents and antioxidant activity. *World Journal of Microbiology and Biotechnology* **23**: 1253-1263.

21. Cai, L. and **Hyde, K.D.** (2007). New species of *Clohesia* and *Paraniesslia* collected from China freshwater habitats. *Mycoscience* (In press).
22. Cai, L. and **Hyde, K.D.** (2007). Anamorphic fungi from China freshwater habitats: *Dictysporium tetrasporum* sp. nov., *Exserticlava yunnanensis* sp. nov., and new records *Pseudofuscophialis lignicola* and *Pseudobotrytis terrestris*. *Mycoscience* (In press).
23. Jeewon, R. and **Hyde, K.D.** (2007). Detection and diversity of fungi from environmental samples: traditional versus molecular approaches. In: *Soil Biology*, Volume 11 (eds. A. Varma and R. Oelmüller). Springer-Verlag, Heidelberg: 1-15.

## 2008

- 18 Publications (18 SCI)

1. Aung OM, Soyong K, **Hyde KD**, 2008. Diversity of entomopathogenic fungi in rainforests of Chiang Mai Province, Thailand. *Fungal Diversity* 30, 15-22.
2. Cai L, Guo XY, **Hyde KD**, 2008. Morphological and molecular characterisation of a new anamorphic genus *Cheirosporium*, from freshwater in China. *Persoonia* 20, 53-58.
3. Duong LM, McKenzie EHC, Lumyong S, **Hyde KD**, 2008. Fungal succession on senescent leaves of *Castanopsis diversifolia* in Doi Suthep-Pui National Park, Thailand. *Fungal Diversity* 30, 23-36.
4. **Hyde KD**, Zhang Y, 2008. Epitypification: should we epitypify? *Journal of Zhejiang University-Science B* 9, 842-846.
5. Kodsueb R, McKenzie EHC, Lumyong S, **Hyde KD**, 2008a. Diversity of saprobic fungi on Magnoliaceae. *Fungal Diversity* 30, 37-53.
6. Kodsueb R, McKenzie EHC, Lumyong S, **Hyde KD**, 2008b. Fungal succession on woody litter of *Magnolia liliifera* (Magnoliaceae). *Fungal Diversity* 30, 55-72.

7. Pinruan U, Sakayaroj J, **Hyde KD**, Jones EBG, 2008. *Thailandiomyces bisetulosus* gen. et sp nov (Diaporthales, Sordariomycetidae, Sordariomycetes) and its anamorph *Craspedodidymum*, is described based on nuclear SSU and LSU rDNA sequences. *Fungal Diversity* 29, 89-98.
8. Swe A, Jeewon R, Pointing SB, **Hyde KD**, 2008. Taxonomy and molecular phylogeny of *Arthrotrrys mangrovispora*, a new marine nematode-trapping fungal species. *Botanica Marina* 51, 331-338.
9. Than PP, Jeewon R, **Hyde KD**, Pongsupasamit S, Mongkolporn O, Taylor PWJ, 2008a. Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose on chilli (*Capsicum* spp.) in Thailand. *Plant Pathology* 57, 562-572.
10. Than PP, Prihastuti H, Phoulivong S, Taylor PWJ, **Hyde KD**, 2008b. Chilli anthracnose disease caused by *Colletotrichum* species. *Journal of Zhejiang University-Science B* 9, 764-778.
11. Than PP, Shivas RG, Jeewon R, Pongsupasamit S, Marney TS, Taylor PWJ, **Hyde KD**, 2008c. Epitypification and phylogeny of *Colletotrichum acutatum* J.H. Simmonds. *Fungal Diversity* 28, 97-108.
12. Thongkantha S, Lumyong S, McKenzie EHC, **Hyde KD**, 2008. Fungal saprobes and pathogens occurring on tissues of *Dracaena lourieri* and *Pandanus* spp. in Thailand. *Fungal Diversity* 30, 149-169.
13. Tran HTM, Stephenson SL, **Hyde KD**, Mongkolporn O, 2008. Distribution and occurrence of myxomycetes on agricultural ground litter and forest floor litter in Thailand. *Mycologia* 100, 181-190.
14. Wang HK, **Hyde KD**, Soyong K, Lin FC, 2008. Fungal diversity on fallen leaves of *Ficus* in northern Thailand. *Journal of Zhejiang University-Science B* 9, 835-841.
15. Wongsawas M, Wang HK, **Hyde KD**, Lin FC, 2008. New and rare lignicolous hyphomycetes from Zhejiang Province, China. *Journal of Zhejiang University-Science B* 9, 797-801.
16. Zhang Y, Fournier J, Jeewon R, **Hyde KD**, 2008a. *Quintaria microsporum* sp nov., from a stream in France. *Cryptogamie Mycologie* 29, 179-182.

17. Zhang Y, Jeewon R, Fournier J, **Hyde KD**, 2008b. Multi-gene phylogeny and morphotaxonomy of *Amniculicola lignicola*: a novel freshwater fungus from France and its relationships to the Pleosporales. *Mycological Research* 112, 1186-1194.
18. Zhao RL, Desjardin DE, Soyong K, **Hyde KD**, 2008. Advances in the phylogenesis of Agaricales and its higher ranks and strategies for establishing phylogenetic hypotheses. *Journal of Zhejiang University-Science B* 9, 779-786.
19. Cheewangkoon R, Crous PW, **Hyde KD**, Groenewald JZ, To-Anan C, 2008. Species of *Mycosphaerella* and related anamorphs on Eucalyptus leaves from Thailand. *Persoonia* 21, 77-91.
20. Hu DM, Dai XH, Guo DY, **Hyde KD**, Zhang KQ, 2008. The diversity of coprophilous fungi from Dahuadian and Zhongdian grasslands, Yunnan, China. *Cryptogamie Mycologie* 29, 355-364.
21. Huang WY, Cai YZ, **Hyde KD**, Corke H, Sun M, 2008. Biodiversity of endophytic fungi associated with 29 traditional Chinese medicinal plants. *Fungal Diversity* 33, 61-75.
22. **Hyde KD**, Soyong K, 2008. The fungal endophyte dilemma. *Fungal Diversity* 33, 163-173
23. Swe A, Jeewon R, **Hyde KD**, 2008a. Nematode-Trapping fungi from mangrove habitats. *Cryptogamie Mycologie* 29, 333-354.
24. Zhang Y, Fournier J, Pointing SB, **Hyde KD**, 2008b. Are *Melanomma pulvis-pyrius* and *Trematosphaeria pertusa* congeneric? *Fungal Diversity* 33, 47-60.
25. Zhao RL, Desjardin DE, Soyong K, **Hyde KD**, 2008a. A new species of bird's nest fungi: characterisation of *Cyathus subgloblisporus* sp nov based on morphological and molecular data. *Persoonia* 21, 71-76.
26. Tao G, Liu ZY, **Hyde KD**, Liu XZ, Yu ZN, 2008. Whole rDNA analysis reveals novel and endophytic fungi in *Bletilla ochracea* (Orchidaceae). *Fungal Diversity* 33, 101-122.

2009

- 23 Publications (23 SCI)

1. Cai L, Wu WP, and **Hyde KD**, (2009). Phylogenetic relationships of *Chalara* and allied species inferred from ribosomal DNA sequences. *Mycological Progress* 8, 133-143. (IF = 1.79)
2. Huang WY, Cai YZ, Surveswaran S, **Hyde KD**, Corke H, and Sun M, (2009). Molecular phylogenetic identification of endophytic fungi isolated from three *Artemisia* species. *Fungal Diversity* 36, 69-88. (IF = 2.28)
3. Lumyong S, Techa W, Lumyong P, McKenzie EHC, and **Hyde KD**, (2009). Endophytic Fungi from *Calamus kerrianus* and *Wallichia caryotoides* (Arecaceae) at Doi Suthep-Pui National Park, Thailand, *1st CMU-UNSW Science Challenges Symposium*, Chiang Mai, THAILAND: *Chiang Mai Journal of Science* 36: 158-167. (SCI but no IF)
4. Swe A, Jeewon R, Pointing SB, and **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. (IF = 1.47)
5. Tang AMC, Jeewon R, and **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. (IF = 2.28)
6. Thongkantha S, Jeewon R, Vijaykrishna D, Lumyong S, McKenzie EHC, and **Hyde KD**, (2009). Molecular phylogeny of Magnaporthaceae (Sordariomycetes) with a new species *Ophioceras Chiangdaoense* from *Dracaena loureiroi* in Thailand. *Fungal Diversity* 34, 157-173. (IF = 2.28)
7. 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. (IF = 2.28)
8. Zhang Y, and **Hyde KD**, (2009). Transfer of *Pseudoparodia pseudopeziza* to Patellariaceae (Patellariales). *Nova Hedwigia* 88, 211-215. (IF = 0.62)
9. Zhang, Y., Wang, H.K., Fournier, J., Crous, P.W., Jeewon, R., Pointing, S.B. and Hyde, K.D. (2009). Towards a phylogenetic clarification of *Lophiostoma* / *Massarina* and morphologically similar genera in the Pleosporales. *Fungal Diversity* 38: 225-251. (IF = 2.28)



10. Huang, W.Y., Cai, Y.Z., Surveswaran, S., **Hyde, K.D.**, Corke, H. and Sun, M. (2009). Molecular phylogenetic identification of endophytic fungi isolated from three *Artemisia* species. *Fungal Diversity* 36: 69-88 (IF = 2.28)
11. Kaewchai, S., Soyong, K. and **Hyde, K.D.** (2009). Mycofungicides and fungal biofertilizers. *Fungal Diversity* 38: 25-50. (IF = 2.28)
12. Phengsintham, P., **Hyde, K.D.** and Braun, U. (2009). *Cercospora* and allied genera from Laos. 1. Notes on *Zasmidium* (*Stenella* sensu lato). *Cryptogamie Mycologie* 30: 243-262. (IF = 0.55)
13. Wannathes, N., Desjardin, D.E., **Hyde K.D.**, Perry, B.A. and Lumyong, S. (2009). A monograph of *Marasmius* (Basidiomycota) from Northern Thailand based on morphological and molecular (ITS sequences) *Fungal Diversity* 37: 209-306. (IF = 2.28)
14. Ko, T.W.K., Stevenson, S.L., Jeewon, R., Lumyong, S. and **Hyde, K.D.** (2009). Molecular diversity of myxomycetes associated with decaying wood and forest floor leaf litter. *Mycologia* 101: 592-598. (IF = 2.36)
15. Pinnoi, A., Phongpaichit, P., **Hyde, K.D.** and Jones, E.B.G. (2009). Biodiversity of fungi on *Calamus* (Palmae) in Thailand. *Cryptogamie Mycologie* 30: 181-190. (IF = 0.55)
16. Hapsari, A., White, M.M., Chukeatirote, E. and **Hyde, K.D.** (2009). Seasonality of *Harpella melusinae* Leger and Duboscq (Harpellales) in black fly larvae in Northern Thailand. *Cryptogamie Mycologie* 30: 191-198. (IF = 0.55).
17. Theantana T., **Hyde K.D.**, Lumyong S. (2009). Asparaginase production by endophytic fungi from Thai medicinal plants: Cytotoxicity properties. *International Journal of Integrative Biology* 7: 1-8.
18. Jeewon R., Yeung S.Y.Q., **Hyde K.D.** (2009). A novel phylogenetic group within *Thozetella* (Chaetosphaeriaceae): A new taxon based on morphology and DNA sequence analyses. *Canadian Journal of Microbiology*, 55: 680-687.
19. **Hyde, K.D.**, Cai, L., McKenzie, E.H.C., Yang, Y.L., Zhang, J.Z. and Prihastuti, H. (2009). *Colletotrichum*: a catalogue of confusion. *Fungal Diversity* 39: 1-17.
20. Prihastuti, H., Cai, L., Chen, H., McKenzie, E.H.C. and **Hyde, K.D.** (2009). Characterization of *Colletotrichum* species associated with coffee berries in northern Thailand. *Fungal Diversity* 39: 89-109.

21. Yang, Y.L., Liu, Z.Y., Cai, L., **Hyde, K.D.**, Yu, Z.N. and McKenzie, E.H.C. (2009). *Colletotrichum* anthracnose of *Amaryllidaceae*. Fungal Diversity 39: 123-146.
22. **Hyde, K.D.**, Cai, L., Cannon, P.F., Crouch, J.A., Crous, P.W., Damm, U., Goodwin, P.H., Chen, H., Johnston, P.R., Jones, E.B.G., Liu, Z.Y., McKenzie, E.H.C., Moriwaki, J., Noireung, P., Pennycook, S.R., Pfenning, L.H., Prihastuti, H., Sato, T., Shivas, R.G., Taylor, P.W.J., Tan, Y.P., Weir, B.S., Yang, Y.L. and Zhang, J.Z. (2009). *Colletotrichum* – names in current use. Fungal Diversity 39: 147-182.
23. Cai, L., **Hyde, K.D.**, Taylor, P.W.J., Weir, B., Waller, J., Abang, M.M., Zhang, J.Z., Yang, Y.L., Phoulivong, S., Liu, Z.Y., Prihastuti, H., Shivas, R.G., McKenzie, E.H.C. and Johnston, P.R. (2009). A polyphasic approach for studying *Colletotrichum*. Fungal Diversity 39: 183-204.

## 2010

- 1 Publications (\*\* SCI)

1. Cai L., Kurniawati E., **Hyde K.D.** (2010). Morphological and molecular characterization of *Mariannaea aquaticola* sp. nov. collected from freshwater habitats. Mycological Progress, pp. 1-7. Article in Press.

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**Date of birth:** 17 September 1972

**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<sup>CAG</sup> 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 University, 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 abroad
- 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 University, 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, Apichartsrangkoon A and **Chukeatirote E**. 2011. Free-amino acid profiles of thua nao, a Thai fermented soybean. Food Chemistry 125: 342–347.
- Dajanta K, Apichartsrangkoon A and **Chukeatirote E**. 2011. Volatile profiles of thua nao, a Thai fermented soy product. Food Chemistry 125: 464-470.
- Wikee S, Cai L, Noireung P, McKenzie EHC, Su YY, **Chukeatirote E**, Thi HN, Bahkali AH, Moslem MA, Abdelsalam K and Hyde KD. 2011. *Colletotrichum* species from Jasmine (*Jasminum sambac*). Fungal Diversity 46: 171–182.
- Dajanta K, **Chukeatirote E** and Apichartsrangkoon A. 2011. Analysis and characterisation of amino acid contents of thua nao, a traditionally fermented soybean food of Northern Thailand. International Food Research Journal 18: 588-592.

- Chang-ngern P, Sardsud U, Pathom-aree W, Chantrasri P and **Chukeatirote E**. 2010. Diversity of moulds in fresh longan. *Agricultural Science Journal* 41 (1S): 322-324.
- **Chukeatirote E**, Dajanta K and Apichartsrangkoon A. 2010. *thua nao*, indigenous Thai fermented soybean: a review. *Journal of Biological Sciences* 10: 581-583.
- Pripdeevech P and **Chukeatirote E**. 2010. Chemical compositions, antifungal and antioxidant activities of essential oil and various extracts of *Melodorum fruticosum* L. flowers. *Food and Chemical Toxicology* 48: 2754–2758.
- Kurniawati E, Zhang H, **Chukeatirote E**, Sulistyowati L, Moslem MA and **Hyde KD**. 2010. Diversity of freshwater ascomycetes in freshwater bodies at Amphoe Mae Chan, Chiang Rai. *Cryptogamie Mycologie* 31: 323-331.
- Phengsintham P, **Chukeatirote E**, McKenzie EHC, Moslem MA, **Hyde KD** and Braun U. 2010. Two new species and a new record of cercosporoids from Thailand. *Mycosphere* 1: 205-212.
- Jannok P, Apichartsrangkoon A and **Chukeatirote E**. 2010. Effect of ultra-high pressure on physical, chemical and microbiological qualities of pennywort juice. *Food* 40: 71-79. (in Thai)
- Panyada Y, Apichartsrangkoon A, Rattanapitikorn P and **Chukeatirote E**. 2009. Effects of high pressure and heat treatment on physical, chemical and microbiological qualities of carrot juice. *Food* 39: 331-337 (in Thai).
- Niraphai K and **Chukeatirote E**. 2009. Biology of *Lasiodiplodia* spp. *SWU Sci J* 25(2): 119-134 (in Thai).
- Dajanta K, **Chukeatirote E**, Apichartsrangkoon A and Frazier RA. 2009. Enhanced aglycone production of fermented soybean products by *Bacillus* species. *Acta Biologica Szegediensis* 53: 93-98.
- **Chukeatirote E** and Saisavoey T. 2009. Antimicrobial property and antioxidant composition of crude extracts of *Pueraria mirifica* , *Butea superba* and *Mucuna macrocarpa* . *Maejo Int J Sci Technol* 3: 212-221.
- Dajanta K, Wongkham S, Thirach P, Baophoeng P, Apichartsrangkoon A, Santithum P and **Chukeatirote E** . 2009. Comparative study of proteolytic

activity of protease-producing bacteria isolated from thua nao . Maejo Int J Sci Technol 3: 269-276.

- Wisitrasamewong K, Jongmahasavat J, Lumyong S and **Chukeatirote E**. 2009. Towards understanding in molecular taxonomy using an in silico approach: a case study in lactic acid bacteria. Suranaree J Sci Technol 16: 53-62.
- Hapsari M P, White M M, **Chukeatirote E** and **Hyde K D**. 2009. Seasonality of Harpella melusinae Leger and Duboscq (Harpellales) in black fly larvae in Northern Thailand. Cryptogamie Mycologie , 30: 191-198.
- Siriwong N and **Chukeatirote E**. 2009. Antibiotic resistance in Staphylococcus aureus and controlling. Songkla Med J , 27: 347-358. (in Thai)
- **Chukeatirote E** , Wisitrasamewong K and Jongmahasavat J. 2008. In silico PCR-RFLP of Bacillus species: Problem-based case of teaching Bioinformatics. Kasetsart J (Nat Sci) 42: 693-7000
- Hanmoungjai W, **Chukeatirote E** , Yamada Y, Sahachaisaree V, Lumyong P, Takata G, Izumori K and Lumyong S. 2008. L-sorbose production by acidotolerant acetic acid bacteria isolated from Thailand sources. Chiang Mai J Sci 35: 382-390.
- 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.
- **Chukeatirote E** , Hanpattanakit P, Kaprom A and Tovaranton J. 2007. Antimicrobial Activity of Senna spectabilis and S. tora. J Plant Sci 2: 123-126.
- Hanmoungjai W, **Chukeatirote E** , Pathom-aree W, Yamada Y and Lumyong S. 2007. Identification of Acidotolerant Acetic Acid Bacteria Isolated from Thailand Sources. Res J Microbiol 2: 194-197.

- **Chukeatirote E** and Thakang P. 2006. Chemical composition of thua nao—a fermented soybean food of Northern Thailand. *Chiang Mai J Sci* 33: 243-245.
- **Chukeatirote E** , Chainun C, Siengsubchart A, Moukamnerd C, Chantawannakul P, Lumyong S, Boontim N and Thakang P. 2006. Microbiological and biochemical changes in thua nao fermentation. *Res J Microbiol* 1: 38-44

### **ITTHAYAKORN PROMPUTTHA, Ph.D.**

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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)

Current Employment Lecturer of School of Cosmetic Science, Mae Fah Luang University, Muang, Chiang Rai, 57100, Thailand. October 2009-current

Past Employment Post-doctoral mycologist for Department of Botany, The Field Museum of Natural History, Chicago, IL, USA, April 2008-August 2009

Post-doctoral mycologist for the Illinois Natural History Survey, University of Illinois at Urbana-Champaign, Champaign, IL, USA, April 2007-April 2008

Research assistant in the Department of Medical Technology, Faculty of Associated Medical Science, Chiang Mai University, Chiang Mai, Thailand, October 2006-March 2007

### **Scientific Interests/Expertise**

1. Field research including fungal succession, plot and collecting endophytic and, saprobic fungi
2. Fungal taxonomy, fungal isolation, identification, fungal cultures
3. Molecular biology including technique of DNA extraction from mycelium and fruitbody, PCR techniques, DNA sequencing, DNA bar coding
4. Use of computer software to analyze genetic data
5. Enzymatic study from endophytic and saprobic fungi
6. Digital imaging of fungi
7. Produce online interactive key for ascomycete fungi for [www.discoverlife.org](http://www.discoverlife.org)
8. Immunology techniques, ImmunoSorbent Assay (ELISA), Western blot
9. Cosmetic technology

### **Field Works**

1. Doi Suthep-Pui National Park, Chiang Mai, Thailand
2. Great Smoky Mountains National Park, Tennessee and North Carolina, USA

### **Synergistic Activity**

DiscoverLife interactive identification keys:

- Bertiaceae (<http://www.discoverlife.org/mp/20q?guide=Bertiaceae>)
- Chaetosphaeriaceae  
(<http://www.discoverlife.org/mp/20q?guide=Chaetosphaeriaceae>)
- Hysteriaceae (<http://www.discoverlife.org/mp/20q?guide=Hysteriaceae>)
- Tubeufiaceae (<http://www.discoverlife.org/mp/20q?guide=Tubeufiaceae>)
- Xylariaceae (<http://www.discoverlife.org/mp/20q?guide=Xylariaceae>)

Photograph and partial identify of some Pyrenomycete fungi of the project Pyrenomycete of the World:



- <https://www-s.life.illinois.edu/pyrenos/>

### **Publications**

1. Promptuttha 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 (Submitted).
2. Promptuttha I. and Miller A.M. 2009. Three new species of *Acanthostigma* (Tubeufiaceae, Pleosporales) from the Great Smoky Mountains National Park. Mycologia (In press)
3. Promptuttha 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.
4. Promptuttha 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.
5. Promptuttha I., Lumyong S., Lumyong P., McKenzie E.H.C. and Hyde K.D. 2005. A new species of *Anthostomella* on *Magnolia liliifera* from northern Thailand. Mycotaxon 91: 413–418.
6. Promptuttha 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.
7. Promptuttha 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.
8. Promptuttha I., Lumyong S., Lumyong P., McKenzie E.H.C. and Hyde K.D. 2004. A new species of *Pseudohalonectria* from Thailand. Cryptogamie Mycologie 25: 43–47.
9. Promptuttha I., Hyde K.D., Lumyong P., McKenzie E.H.C. and Lumyong S. 2002. *Dokmaia monthadangii* gen. et sp. nov., a synnematous anamorphic fungus on *Manglietia garrettii*. Sydowia 55: 99–103.

10. Promptuttha I., Lumyong S., Lumyong P., McKenzie E.H.C. and Hyde K.D. 2002. Fungal succession on senescent leaves of *Manglietia garrettii* in Doi Suthep-Pui National Park, northern Thailand. *Fungal Diversity* 10: 89–100.

### **Presentations at international conferences**

1. Promptuttha I., Hyde K.D., Peberdy J.F. and Lumyong S. 2007. How can endophytes survive as saprobes after host senescence? MSA Annual Meeting and Foray, 6-9 August 2007, Louisiana State University, Baton Rouge, LA, USA (Poster presentation).
2. Promptuttha I., Hyde K.D., Peberdy J.F. and Lumyong S. 2006. Enzymatic activity of endophytic fungi on leaf decomposition. 8th International Mycological Congress, 21-25 August 2006, Cairns Convention Centre, Queensland, Australia (Poster presentation).
3. Promptuttha I., Jeewon R., Hyde K.D., Vijaykrishna D., McKenzie E.H.C. and Lumyong S. 2006. Do fungal endophytes of *Magnolia liliifera* become saprobes at host senescence? RGJ-Ph.D. Congress VII, 20–22 April 2006, Jontein Palmbeach Resort, Pattaya, Chonburi, Thailand (Oral presentation).
4. Promptuttha I., Lumyong P., Hyde K.D., Peberdy J.F. and Lumyong S. 2005. The production pattern of carbohydrases during the decomposition of *Magnolia liliifera* leaves. British Mycological Society Annual Scientific Meeting, 5–8 September 2005, Hulme Hall, University of Manchester, England (Oral presentation).
5. Promptuttha I., Lumyong S., Lumyong P., McKenzie E.H.C. and Hyde K.D. 2004. Diversity and succession of fungi on senescent leaves of *Meliosma simplicifolia* (Sabiaceae). The IV Asia-Pacific Mycological Congress and The IX International Marine and Freshwater Mycology Symposium, 14–19 November 2004, Chiang Mai, Thailand (Poster presentation).
6. Promptuttha I., Lumyong S., Lumyong P., McKenzie E.H.C. and Hyde K.D. 2002. Fungal succession on senescent leaves of *Manglietia garrettii* in Doi Suthep-Pui National Park, northern Thailand. 3rd Asia-Pacific Mycological Conference on Biodiversity and Biotechnology (AMC2002), 4–8 November 2002, Yunnan University, Kunming, China (Oral presentation).

2. Promputtha I., Lumyong S., Lumyong P., McKenzie E.H.C. and Hyde K.D. 2002. Fungal saprobes on dead leaves of *Manglietia garrettii* in Thailand. The 14th Annual Meeting of the Thai Society for Biotechnology “BIOTECHNOLOGY FOR BETTER LIVING IN THE NEW ECONOMY”, 12–15 November 2002, Khonkaen University, Khonkaen, Thailand (Poster presentation).
8. Promputtha I., Lumyong S., Lumyong P., McKenzie E.H.C. and Hyde K.D. 2001. Saprobic Fungi on *Magnolia garrettii*. BioThailand 2001: From Research to Market, 7–10 November 2001, Queen Sirikit National Convention Center, Bangkok, Thailand (Poster presentation).

#### **Workshops in field specialization**

1. Workshop on “Unculturable Microbes: Molecular Techniques and Biotechnology Application”. At National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), and Ministry of Science and Technology (MOST). At BIOTEC auditorium room, BIOTEC Building, Thailand Science Park, Pathumthani, Thailand. 9–10 January 2006.
2. Workshop on “GBIF and EASIANET Proposed Collection/Names/Images Digitization”. At Centre for Research in Fungal Diversity, Department of Ecology and Biodiversity, The University of Hong Kong, Hong Kong SAR, China. 14–19 March 2005.
3. Workshop on “Molecular Phylogenetics”. At Department of Ecology and Biodiversity, The University of Hong Kong, Hong Kong SAR, China. 17–22 March 2004.
4. Workshop on “Denaturing Gradient Gel Electrophoresis”. At Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand. 4–6 May 2004.
5. Workshop on “Microbial Production of Surfactants and Their Applications”. At Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand. 10–14 May 2004.

6. Workshop on “Mycology Taxonomy, Molecular Systematics and Using Key Isolation and Preservation of Fungi”. At the Mushroom Research Centre, Chiang Mai, Thailand. 7–27 July 2003.
7. Workshop on “Fungal Diversity of Thailand: Towards a Checklist of Thai Fungi, Fungi and Their Biotechnological Application”. At National Science and Technology Development Agency (NSTDA), Bangkok, Thailand. 15–16 November 2001.

