Final report

การปรับปรุงวิธีการที่เหมาะสมต่อการจัดจำแนกวงศ์วานวิวัฒนาการของเชื้อราในกลุ่ม Colletotrichum ที่เป็นสาเหตุที่สำคัญของโรคพืช

By

Associate Professor Kevin David Hyde
Assistant Professor Ekachai Chukeatirote

This research was made possible by support of

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Associate Professor Dr. Kevin D. Hyde

บทสรุปผู้บริหาร (EXECUTIVE SUMMARY)

1. ความสำคัญและที่มาของปัญหาในการการวิจัย (Rationale and review)

Species concepts in the important pathogenic genus *Colletotrichum* are currently based largely on morphology in culture. Host-specificity is now considered to operate only at the microspecies level. Assumptions in the past that *Colletotrichum* species are host-specific has led to more than 650 species having been named (http://www.ukncc.co.uk/cabipages/Names/NAMES.ASP). This has severely constrained accurate exchange of information on *Colletotrichum* species.

Morphology and culture-based systems are generally appropriate for diagnosis of species aggregates, but are of little use to distinguish the evolutionary units of relevance to plant pathologists. Hence the outcome of the project is a significant refinement of current *Colletotrichum* taxonomy based on phylogenetic evidence potentially leading to molecular identification systems and diagnostic tools. This in turn will lead to a better understanding of the requirements for plant breeders who require knowledge of species they need to breed plant resistance against.

2. วัตถุประสงค์ของโครงการวิจัย (Objectives of the research)

i. To create a preliminary multigene-based phylogeny of the genus *Colletotrichum* using nuclear and mitochondrial genomes, ribosomal DNA, β-tubulin, TEF1α, and other appropriate genes.

ii. To determine the utility for phylogenetic inferences of single gene sequence data sets as compared to combined data sets at the generic level using *Colletotrichum* species.

iii. To indicated species concepts within the genus by linking molecular result and morphology.

3. ขอบเขตของโครงการวิจัย (Scope of the research)

At the start of this project the genus *Colletotrichum* contained about 50 species, which cause plant diseases often known as anthracnose. We collected more than 200 isolates in Thailand (mostly northern part) and some specimens from elsewhere in Thailand. In this study we developed a practical phylogeny-based approach for the identification of *Colletotrichum*, focused on Thai species and the *C. gloeosporioides* complex. Variations in the mitochondrial genome, ribosomal DNA, β-tubulin, TEF1α and other appropriate genes had investigated; all are in wide use in other fungal genera to resolve problems in identification. The successful outcome of this project had led to important practical implications to the plant pathology, plant breeding and quarantine communities and resulted in many important publications.

4. ระเบียบวิธีวิจัยและผลผลิตจากการวิจัย (Methodology and the research output)

Research Plan from October 2010 to September 2013

Year 3 (October 2012 to September 2013): (12 months).

Anthracnose diseased samples were collected in Thailand and specimens were procured from elsewhere as needed for this study. Diseased samples had observed and any the *Colletotrichum* species associated with the disease tissues were isolated by single spore isolation techniques. Cultures were grown in PDA at room temperature for one week and the morphology of selected species were observed and documented by stereomicroscope and compound microscope. More than 200 isolates were obtained. Molecular sequence data, initially ITS, were used to characterize species and decide which strains need further study. All the cultures were deposited at MFLU culture collection (MFLUCC, Mae Fah Luang University, Thailand). We are also worked towards epitypification of some species.

5. ประโยชน์ที่ได้รับ (Benefit)

Colletotrichum species are important pathogens causing disease of crops and ornamental plants and it is essential we can accurately identify species for plant disease control, plant breeding programs, quarantine regulations and important publications. We have surveyed *Colletotrichum* species infecting crops, fruits and other plants in Thailand and isolated endophytes from healthy grasses. By identifying the disease causal agents we were able to establish which species infect which plants in Thailand. Moreover, we published several new species and SCI publications and we have been working towards epitypification of other species. Phylogenetic analyses of ITS and morphology were used to characterize species that need further work and we have been looking towards establishing a single gene(s) that can readily identify species. Ultimately we have been developing a practical phylogeny for the identification of *Colletotrichum* species, by focusing on Thai species. Our research has involved international collaborations so that the techniques used to classify *Colletotrichum* species are globally accepted.

6. แผนการถ่ายทอดเทคโนโลยีหรือผลการวิจัยสู่กลุ่มเป้าหมาย

Systematics, plant pathologists, plant health practitioners, plant breeders and quarantine officers, since they cannot name organisms confidently

บทคัดย่อ

เชื้อราในสกุล *Colletotrichum* (หรือ "Glomerella") ซึ่งโดยส่วนใหญ่เป็นสาเหตุของโรคแอนแทรคโนสโดยเข้าทาลายพืชเศรษฐกิจ อาทิเช่น ธัญพืช พืชผัก พืชตระกูลถั่ว ไม้ดอกไม้ประดับ และ ไม้ผล และทางด้านการควบคุมและกักกัน อันรวมไปถึงการปรับปรุงพันธุ์พืช ซึ่งเชื้อราในสกุล *Colletotrichum* สามารถแพร่ระบาดได้อย่างกว้างขวาง โดยเชื้อราในสกุลนี้ยังเป็นสิ่งมีชีวิตที่ต้องการพืชอาศัยและจะปรากฏร่วมกับบริเวณที่เกิดอาการของโรคคล้ายเชื้อราเอนโดไฟต์ ในอดีตการจัดจาแนกนี้พิจารณาจากลักษณะทางด้านสัญฐานวิทยาและชนิดของพืชอาศัยซึ่งพบว่ามีความซับซ้อนเป็นอย่างมาก จึงได้มีการใช้ ITS ยีนส์ร่วมกับลักษณะทางสัณฐานวิทยา อย่างไรก็ตามก็ยังไม่สามารถจัดจาแนกในระดับสปีชี่ส์ได้อย่างแม่นยา เมื่อไม่นานมานี้ จึงมีการใช้ความหลากหลายของยีนส์ actin (act), calmodulin (cal), chitin synthase (chs1), glyceraldehyde-3-phosphate dehydrogenase (gapdh) และ ITS ร่วมกับการศึกษาทางด้านสัญฐานวิทยารวมไปถึงการทดลองความสามารถในการก่อโรค ทาให้ขณะนี้มีการรายงานจะนวนสปีชีส์กว่า 100 สปีชีส์ โดย 4 สปีชีส์ได้รับการยอมรับว่าเป็นสปีชีส์ที่มีความซับซ้อนโดยเฉพาะอย่างยิ่ง *C. gloeosporioides* ประกอบไปด้วยลักษณะทางสัณฐานวิทยา 22 ลักษณะที่มีความคล้ายคลึงกัน อย่างไรก็ตาม ITS, beta tubulin (tub2), DNA lyase และ the intergenic region of apn2 และ MAT1-2-1 genes (ApMat) ได้ใช้ในการจัด ดังนั้น 28 สปีชีส์ จึงได้รับการยอมรับ ในงานวิจัยฉบับนี้ผู้วิจัยได้ยืนยันผลการวิจัยทางด้านชีวิโมเลกุล มากกว่า 50 สปีชีส์ โดย 15 สปีชี่ส์ค้นพบภายในประเทศไทยและได้สารวจเชื้อราในกลุ่มดังกล่างที่เข้าทาลายพืชผลและเอนโดไฟต์ในพืชตระกูลหญ้า โดยได้มีการรวบรวมจากตัวอย่างสดในแต่ละสถานที่กว่า 200 ตัวอย่าง โดยผู้วิจัยได้มีการนาเสนอผลงานการวิจัย 5 ฉบับ ตั้งแต่เชื้อราในสกุลนี้ได้เป็นปัญหาที่พบได้ทั่วโลกดังนั้นทางผู้วิจัยจึงได้เลือกการติดต่อประสานงานกับคณะวิจัยในประเทศบลาซิล จีน และ อินเดีย เพื่อให้เกิดการศึกษาอย่างกว้างขวางและครอบคลุม ทางคณะผู้จัดมี

ความรู้สึกว่างานวิจัยในครั้งนี้ได้ประสบความสำเร็จอย่างดีเยี่ยง เนื่องจากโดยรวมได้มีการตีพิมพ์ผลงานวิจัยในวารสารนานาชาติรวมถึง 11 ชิ้นงานด้วยกันที่และยังคงเหลืออีก 3 ผลงานตีพิมพ์ที่อยู่ในกระบวนการตีพิมพ์ในขณะนี้ ในปัจจุบัน *Colletotrichum* มากกว่า 100 สายพันธ์ ได้รับการวิเคราะห์แต่ยังคงต้องการการศึกษาที่ลึกซึ่งยิ่งขึ้น ตัวอย่างเช่น *C. gloeosporioides* อย่างไรก็ตามทางคณะผู้จัดทำได้มีความมุ่งมั่นที่จะดำเนินงานวิจัยนี้ต่อไปโดยจะมีการเก็บรวบรวมตัวอย่าง ในประเทศไทย และรายงานหรือตีพิมพ์ผลงานเกี่ยวกับสายพันธ์ใหม่ ๆ เนื่องจากยังมีการค้นพบสายพันธ์อย่างต่อเนื่องเพื่อประโยชน์ในการปรับปรุงการจัดจำแนก ขณะนี้ IST สามารถที่จะใช้ในการแยกสายพันธ์ใน 7 สายพันธ์หลักที่มีความซับซ้อนได้ ในขณะเดียวกัน 6-7 ยีนส์ ยังคงจำเป็นอย่างยิ่งที่จะต้องศึกษาในความซับซ้อนนั้น ในลำดับต่อไปในการทำลองจีโนมทั้งหมดสามารถทำได้ใน *Colletotrichum* 3 สายพันธ์ และใช้ ข้อมูลนี้สร้าง 1 หรือ 2 ยีนส์ที่มีความสามารถในการระบุสปีชี่ส์อย่างแม่นยำได้ งานวิจัยที่กล่าวอ้างนี้ได้อยู่ภายใต้ความร่วมมือกับนักวิจัยใน Beijing และภายใน 1-2 ปี คณะผู้วิจัยคาดหวังว่าจะมีการตีพิมพ์ผลงานวิจัยที่เป็นประโยชน์ขึ้น โดยสรุปเกี่ยวกับสถานะของการจัดจำแนกของเชื้อราในกลุ่ม *Colletotrichum* ได้ระบุไว้ในงานตีพิมพ์ที่คณะผู้ทำวิจัยได้จัดเตรียมไว้เพื่อการตีพิมพ์ใน Hyde et al. (in prep).

ABSTRACT

The genus *Colletotrichum* (sexual state "Glomerella") are important pathogens causing serious disease of plants and infected crops are subjected of import control (quarantine) and plant breeding programs. *Colletotrichum* species have a worldwide distribution and are associated with leaf spots, fruit anthracnose and when serious infections occur they are responsible for reducing economic plant yields (e.g. of cereals, vegetables, legumes, ornamental plants and fruits). They are also obligate symbionts and occur in a symptomless parts of plants as endophytes and the relationships between life modes (i.e. can the fungi switch modes) is poorly establish. Previous identification and classification was based on host association and morphological characteristics. Molecular sequence data analysis has become commonplace in classifying plant pathogenic genera like *Colletotrichum*, which have been found to comprise several species complexes. Initially ITS and morphology was used to characterize species, however, they could not resolve species well. Recent multigene phylogenetic analysis have involved actin (*act*), calmodulin (*cal*), chitin synthase (*chs1*), glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) and ITS gene regions as well as morphology and pathogenicity testing so at present there are about 100 described species and this is increasing monthly. There are also four accepted species complexes and *C. gloeosporioides* is the most important. Recently multigene phylogenetic analysis confirmed that *C. gloeosporioides* is a species complex that comprises 22 morphologically similar, phylogenetically distinct species. However, ITS, beta tubulin (*tub2*), DNA lyase and the intergenic region of *apn2* and *MAT1-2-1* genes (*ApMat*)) have also been used to identify new lineages and new species within this species-complex and presently, there are 28 accepted species names within the species complex. There is however, yet no consensus among mycologists as to which gene markers should be used to define and delimit a species within the species complex.

At the beginning of this study (October 2010) there were more than 50 confirmed "molecular" species in the genus causing plant diseases often known as anthracnose with 15 species known from Thailand. We therefore initiated a survey of *Colletotrichum* species infecting fruits in Thailand and also those which are

endophytes of healthy grasses. In the first, second and third year we collected and isolated strains from more than 200 fresh specimens of various disease plants and fruits and asymptomatic grass endophytes from difference places in Thailand. We also sequenced these isolates and carried out morphological as well as pathogenicity studies. We identified several new species and also worked towards epitypification of other species and five publications (four SCI) appearing in year two (Oct 2011-Sept 2012) of the grant. In the final year of the project we published six SCI papers. We were involved in developing a practical phylogeny and morphology based approach for the identification of *Colletotrichum* species, focusing on Thai species. However, since this is a global problem we have also chosen to collaborate with Brazilian, Chinese and Indian colleagues in order to bring greater depth to the research and international agreement to the findings. The latter was achieved with the formation of the International Subcommission on *Colletotrichum* taxonomy (http://www.fungaltaxonomy.org/ subcommissions) of which our group is a founder member. We feel this has been a remarkably successful research project. In total we published 11 international papers. We also have three papers in preparation.

At the present time more than 100 species of *Colletotrichum* are recognized but there is still considerable work needed to establish how many species exist in species complexes such as *C. gloeosporioides.* We will continue this work and continue collecting in Thailand and publish new species as they are found and resolved. At present ITS can separate species into the seven main species complexes, while a 6-7 gene combined phylogeny is need to resolve species within these complexes. Identification is therefore difficult and costly. The next step is to examine the whole genome sequences that are available for three *Colletotrichum* species and use data mining to establish one or two genes that can more accurately resolve species. This work is now underway in collaboration with colleagues in Beijing and within 1-2 years we hope to publish these results. A summary of the status of *Colletotrichum* species identification with be given in a paper we are preparing for publication in Hyde et al. (in prep).

Keywords: *Colletotrichum*, Phylogeny, Plant pathogen, Taxonomy

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ABBREVIATION AND SYMBOLS

ACT	=	Actin
CTAB	=	Hexadecyltrimethylammonium bromide
DNA	=	Deoxyribonucleic acid
ITS	=	Internal transcribed spacer
LSU	=	Large subunit (28S rDNA)
PDA	=	Potato dextrose agar
RFLP	=	restriction fragment length polymorphism
Sp.	=	Species
TEF 1-α	=	Translation elongation factor 1-alpha
β-tubulin	=	Beta-tubulin
μm	=	micrometer

CHAPTER 1
INTRODUCTION

1.1 Background

Species concepts in the important pathogenic genus *Colletotrichum* are currently based largely on morphology in culture. Host specificity is now considered to operate only at the micro-species level. An assumption in the past that Colletotrichum species are host-specific has led to numerous problems in identification, with more than 650 species having been named (http://www.ukncc.co.uk/cabipages/Names/NAMES.ASP). This has severely constrained accurate exchange of information on *Colletotrichum* species.

Morphology and culture-based systems are generally appropriate for diagnosis of species aggregates, but are of little use to distinguish the evolutionary units of relevance to plant pathologists. Hence the outcome of the project will be a significant refinement of current *Colletotrichum* taxonomy based on phylogenetic evidence potentially leading to molecular identification systems and diagnostic tools. This in turn will lead to a better understanding of the requirements for plant breeders who require knowledge of species they need to breed plant resistance against.

1.2 Objectives

1.2.1 To create a preliminary multigene-based phylogeny of the genus *Colletotrichum* using nuclear and mitochondrial genomes, ribosomal DNA, β-tubulin, TEF1α, and other appropriate genes.

1.2.2 To determine the utility for phylogenetic inferences of single gene sequence data sets as compared to combined data sets at the generic level using *Colletotrichum* species.

1.2.3 To elucidate species concepts within the genus by linking molecular and morphological approaches.

1.3 Scope of research

The genus *Colletotrichum* contains about 50 species, which cause plant diseases often known as anthracnose. *Colletotrichum* species are worldwide in distribution and cause major damage to cereals, vegetables, legumes, ornamental plants and fruit trees. The current naming of *Colletotrichum* species is largely based on a combination of morphological and cultural characteristics. Due to limited numbers of morphological character-suites available in culture coupled with inherent phenotypic plasticity, precise identification of the species has always been difficult. Physiological specialization within species and overlapping host ranges mean that our current classification system is impracticable for users. This causes problems to systematics, plant pathologists, plant health practitioners, plant breeders and quarantine officers, since they cannot name organisms confidently. In our proposal we had developed a practical phylogeny-based approach for identification of *Colletotrichum*, focusing on Thai species and the *C. gloeosporioides* complex. Variations in the mitochondrial genome, ribosomal DNA, β-tubulin, TEF1α and other appropriate genes had investigated; all are in wide use in other fungal genera to resolve problems in identification and taxonomy. The successful outcomes of this project have important practical implications to the plant pathology, plant breeding and quarantine communities and important publications.

CHAPTER 2

LITERATURE REVIEW

2.1 The need for species recognition

The genus *Colletotrichum* causes various plant diseases often known as anthracnose and is worldwide in distribution (Sutton, 1992). *Colletotrichum* species cause major damage to crops in tropical, subtropical and temperate regions (Than *et al*., 2008 and Hyde *et al*., 2009a, 2010). Cereal, vegetables, legumes, ornamentals and fruit trees may be seriously affected by this pathogen (Freeman, 2000). Also, *Colletotrichum* sp. is cosmopolitan and has been shown that multiple species can infect single host or single species can infect multiple hosts (Cai *et al*., 2009 and Hyde *et al*. 2009). *Colletotrichum* species are also commonly isolated as endophytes, and latent and quiescent infections by these species on several hosts have been reported (Bills, 1996; Brown *et al*., 1998 and Photita *et al*., 2004, 2005). Their ability to cause latent infection i.e. infection without visible symptom makes them one of the most successful pathogens causing post-harvest disease in a wide range of crop species (Sutton, 1992).

At least nine different *Colletotrichum* species, *C. capsici*, *C. coccodes*, *C. crassipes*, *C. dematium*, *C. destructivum*, *C. gloeosporioides*, *C. lindemuthianum*, *C. trifolii* and *C. truncatum* for example, have been reported on legumes in tropical and temperate regions (Lenne, 1992). These legume hosts include many important human food sources including grain legumes, root crops and fruits, and pasture plants, medicinal plants, timber trees and ornamentals (Anonymous, 1979). All of these *Colletotrichum* species are reported to infect at least two hosts, and *C. capsici*, *C. gloeosporioides* and *C. lindemuthianum* are reported to have the widest host ranges among these nine. These reports however, are not backed up by voucher specimens and their occurrence cannot be verified. If we want to breed legumes that are resistant to anthracnose caused by *Colletotrichum* species we must know which species infect which hosts.

Colletotrichum gloeosporioides is a particularly large complex comprising taxa which cause diseases of a wide range of crops. The taxa have been isolated as pathogens, endophytes and saprobes and it is not clear whether these different lifestyles are associated with specific lineages or have evolved many times. It is therefore particularly important that we gain an understanding of the diversity of organisms within this complex.

Colletotrichum sp. is the anamorphic stage of several species of *Glomerella* sp. and has a taxonomic history of about 200 years (Corda, 1837). There are 17 acknowledged generic synonyms for *Colletotrichum* sp. and two further names are dubiously included, and there are about 900 species names assigned to this genus (Sutton, 1980, 1992). The identification and characterization of *Colletotrichum* species are mainly based on morphological and cultural criteria or a combination of both. It has become apparent that the classification system presently used has limited scope since some species names assigned to collection and isolates lack the precision required by users. The numbers of morphological characters derived from growth in culture are limited, and growth conditions have been rarely standardized. Moreover the inherent phenotypic plasticity of individual isolates creates confusion in identification. There are group species or species complexes such as *C. gloeosporioides*, *C. dematium*, *C. lindemuthianum* which are known to be represented by at least nine distinct subtaxa (Sutton, 1992).

Under these circumstances the species name has limited practical significance to the plant pathologist involved in disease management and quarantine, and the breeder involved in resistant breeding. The evolution of different systems for identification of species over time has largely been the result of subtle changes in the concept concerning the importance of different aspects of morphology combined with ideas about host range and host-pathogen relationships for particular taxa. Despite these amendments the current concept of *Colletotrichum* sp. systematics is still very broad, unreliable and unpredictable being based on the combination of classical criteria such as conidial shape and size, presence, absence and morphology of setae, presence of sclerotia and appressoria and symptom expression on host. Moreover, the current classification system for *Colletotrichum* in general is unsatisfactory because

the constituent species are inadequately defined (Cannon *et al.*, 2000; Than *et al.*, 2008a).

Molecular approaches are being used to resolve problems in fungal taxonomy and fungal identification by many workers (Ma *et al.*, 1997; Ranghoo *et al.*, 1999; Rollo *et al.*, 1995; Zhang *et* al., 1997; Than *et al*, 2008a.b). Because of the shortcomings of *Colletotrichum* sp. systematic based on cultural characteristics and morphology there is need for a combined approach including the use of molecular data. Various DNA-based systems have been used to study phylogeny, systematic, genetic diversity and population structure of *Colletotrichum* species. These markers include restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), amplified restriction fragment length polymorphisms (AFLP), rDNA internal transcriber spacer (ITS1 to ITS2) and small subunit ribosomal RNA (18S rDNA). RFLP was found extremely useful to determine genetic relationships within *C. gloeosporioides* from *Stylosanthes* spp (Braithwaite *et al.*, 1990). The same marker was used to study diversity in *C. gloeosporioides* from *Stylosanthes guianensis*, and avocado and almond (Freeman *et al.*, 1996; Kelemu *et al.*, 1999). RAPD was used to study genetic diversity and variability in *C. lindemuthianum*, *C. acuminatum* and *C. gloeosporioides* (Balardin *et al.*, 1997; Gonzalez *et al.*, 1998; Kelemu *et al.*, 1999; Lander *et al.*, 1999; Sicard *et al.*, 1997). Similarly AFLP was used to characterize *C. lindemuthianum* isolates (Gonzalez *et al.*, 1998), however these days these techniques are considered crude.

Cannon *et al.* (2000) stated that data derived from nucleic acid analyses should provide the most reliable framework to build a classification of *Colletotrichum* sp., as DNA characters were not directly influenced by environmental factors. Most fungal phylogenetic studies utilized sequences from the ribosomal gene cluster, since they were present in large numbers as tandem repeats and evolved as a single unit (Mitchell *et al.*, 1995). In particular, sequence analysis of the internal transcribed spacer (ITS) regions which lie between the 18S and 5.8S genes and the 5.8S and 28S genes, has proved useful in studying phylogenetic relationships of *Colletotrichum* species because of their comparative variability (Sreenivasaprasad *et al.*, 1994; 1996; Moriwaki *et al.*, 2002; Photita *et al.*, 2005; Shenoy *et al.*, 2007; Than *et al.*, 2008a,b).

The ITS region was used to analyze *Colletotrichum* species from various fruits, to study intraspecific diversity in *C. acuminatum* and *C. lindemuthianum* and to study phylogeny and systematic of several *Colletotrichum* spp. (Freeman *et al.*, 2000, 2001; Sicard *et al.*, 1997; Sreenivasaprasad *et al.*, 1996). Small subunit rRNA was used to infer phylogenetic placement of *Athelia bambacina*, *Aureobasidium pullunans* and *C. gloeosporioides* (Illingworth *et al.*, 1991). Besides these A+T rich DNA analysis, ß-tubulin genes have been used to study *Colletotrichum* species (Freeman *et al.*, 2000, 2001; Thon and Royse, 1999; Than *et al.*, 2008a,b). Sequence analysis of protein coding genes such as partial β-tubulin gene, has also been applied to resolve phylogenetic relationships among *C. acutatum* species complexes (Sreenivasaprasad and Talhinhas, 2005). Sequences of introns from two genes (glutamine synthase and glyceraldehyde-3-phosphate dehydrogenase) were also used to evaluate a diverse collection of isolates of *C. acutatum* (Guerber *et al.*, 2003; MacKenzie *et al.*, 2008). *C. acutatum* isolates clustered into groups (Guerber *et al.*, 2003; MacKenzie *et al.*, 2008; Peres *et al.*, 2008). These groups might represent phylogenetically distinct species of *C. acutatum sensu lato* (Guerber *et al.*, 2003). Yun *et al.* (1999) stated that, because of the high intra-species variability and the low inter-species variability, MAT1-2 mating type sequences gave strong support for branches, allowing differentiation of closely related *Cochliobolus* spp. whose relationships were not resolved by ITS sequences alone. Consequently, Du *et al.*(2005) confirmed that MAT1-2 mating type was useful in differentiating the groups of isolates from the species complexes (*C. graminicola*, *C. gloeosporioides* and *C. acutatum*).

A combined application of molecular diagnostic tools along with traditional morphological characterization is an appropriate and reliable approach for studying *Colletotrichum* species complexes (Cannon *et al.*, 2000). Than *et al.* (2008a) differentiated isolates of chilli anthracnose from Thailand into three species: *C. acutatum*, *C. capsici* and *C. gloeosporioides*, based on morphological characterization, sequencing based on rDNA-ITS region and partial ß tubulin gene and pathogenicity testing. Hong and Kim (2007) reported that Korea isolates of *C. acutatum* were phylogenetically separated from the global groups of *C. acutatum* A1 to A8 based on the sequences in partial beta-tubulin 2 (exons 3-6). Restriction

fragment length polymorphisms (RFLP) of ITS region resulting from *Alu*I, *Rsa*I and *Bam*HI digestions have also been employed to differentiate *Colletotrichum* species from chilli anthracnose in Taiwan region (Sheu *et al.*, 2007).

The current classification of *Colletotrichum* species is broad and has a limited practical significance. It is well accepted that the systematic of the genus *Colletotrichum* awaits a detailed investigation and refinement. We had refined *Colletotrichum* taxonomy focusing on Thailand taxa and in particular the *C. gloeosporioides* complex. Various molecular techniques such as PCR amplification of rDNA; β-tubulin, TEF1α and other appropriate gene sequences were used in this study. We had also determined the utility for phylogenetic inferences of single gene sequence data sets as compared to combined data sets at the generic level using *Colletotrichum* species.

CHAPTER 3

MATERIAL AND METHODS

3.1 Fungal isolates and cultures

Strains of *Colletotrichum* spp. were isolated by single spore isolation method on the water agar (WA) and Potato dextrose agar (PDA, Difgo). After that cultures were grown on PDA at room temperature (25-30°C) for one week. And then they were deposited at MFLU culture collection (MFLUCC, Mae Fah Luang University, Thailand).

3.2 Molecular analysis

3.2.1 DNA extraction

DNA extracted from the fungal mycelium that were grown on PDA petridis (one week). Re-suspended fungal mycelium in 1-2 ml of sterile water. Suspensions vortexed briefly and left in the dark at 25°C for 24 hrs to encourage spore germination and/or mycelium were softened. Total genomic DNA extracted by the adapted CTAB method used to extract fungal genomic DNA from fungi (Ford *et al.*, 2000).

3.2.2 Polymorphisms in nuclear DNAs

A nuclear DNA probe, GcpR1, from the bean anthracnose pathogen *C. lindemuthianum* had been found very useful to differentiate between species of *Colletotrichum* (Rodriguez and Yoder, 1991). This probe was also used to differentiate between *C. gloeosporioides* from Almond and Avocado (Freeman *et al.*, 1996). The same probe used to study inter-specific differences between these *Colletotrichum* species.

3.2.3 Analysis of A + T-rich DNA associated with mt DNA

Polymorphisms in A+T rich DNA revealed by restriction enzyme H*ae*III used to characterize and differentiate various *Colletotrichum* isolates (Freeman and Shabi, 1996; Freeman *et al.*, 1996; Hodson *et al.*, 1993; Sreenevasaprasad *et al.*, 1992). The restriction enzyme H*ae*III recognizes and cleaves the DNA sequence GGCC. Hence, most of the nuclear DNA digested to the

fragment of less than 2 kb whereas A+T rich DNA is cleaved infrequently by this enzyme (Freeman *et al.*, 1996).

3.2.4 DNA fragment amplification (for ITS, β-tubulin and TEF 1-α genes)

Universal PCR primers available for amplification of ITS 1 and ITS 2 regions between the small and large nuclear rDNA including the 5.8S rDNA used as previously described by (White *et al.*, 1990). Similarly conserved regions of β-tubulin and TEF 1-α genes amplified using specific primers. Amplification reactions carried out in an automated thermal cycler using the basic amplification protocol described by Guo *et al.* (2001).

3.2.5 DNA sequencing

PCR products purified by Promega Wizard DNA purification kits. The purified DNA sequenced on an automated sequencer using fluorescent dye-labeled sequencing primers (ALFExpress, Pharmacia). The amplification product with multiple bands were appearing by electrophoresis in 1% low-melting temperature agarose gel, visualized with ethidium bromide, excised separately, purified and sequenced. When the two products were too closed for gel elution they cloned into pGEM-T Easy vector with an overnight ligation. Recombinants vector could identified and plasmid DNA did extracted, purified and sequenced. All of the data made available to the scientific community via submission to GenBank.

3.2.6 Data analysis

RFLP auto-radiograms of nDNA RFLP and gel photographs of mtDNA were scored for the presence (1) or absence (0) of band separately. A multi-locus genotype based on these two set of data were constructed for each study isolate. Genetic similarity between and within species were computed to construct UPGMA tree using the NTSYS-pc version 2.1 (Rolf, 2000). nDNA RFLP data for *C. lindemuthianum* populations from Hong Kong and Southern China were subjected to similar analysis, and the genetic diversity within and between the study populations were estimated by Gleason and Shannon indices.

PCR sequenced data of each gene was aligned for all isolates and with published sequences from GenBank and EMBL databases with computer program (eg. SeqApp, Clustal X) and then aligned manually. Non-informative sites was omitted or re-coded in the analysis. All characters were treated as unordered.

Alignment data was subjected to three methods of phylogenetic analysis; maximum Parsimony (MP), Weighted parsimony (WP) and Maximum-likelihood (ML) using PAUP* 4.0 (Swofford, 1998). Out group rooting was used to determine polarity. Bootstrapping and decay indices were used to measure branch robustness.

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 Crous and Rampai had hand-on experience in the specific molecular and physiological evaluation techniques. Dr Hyde had many years experience in identification of *Colletotrichum* species and in relating molecular and morphological species concepts.

CHAPTER 4

RESULTS

4.1 Morphological study

Basic morphological characters of *Colletotrichum* species on various plant are the fruiting bodies called acervuli which may have dark setae. Orange masses of conidia may from on the acervuli. Examples of some species we have documented are illustrated in Figs 1-5. Conidia are unicellular, hyaline and differently shaped.

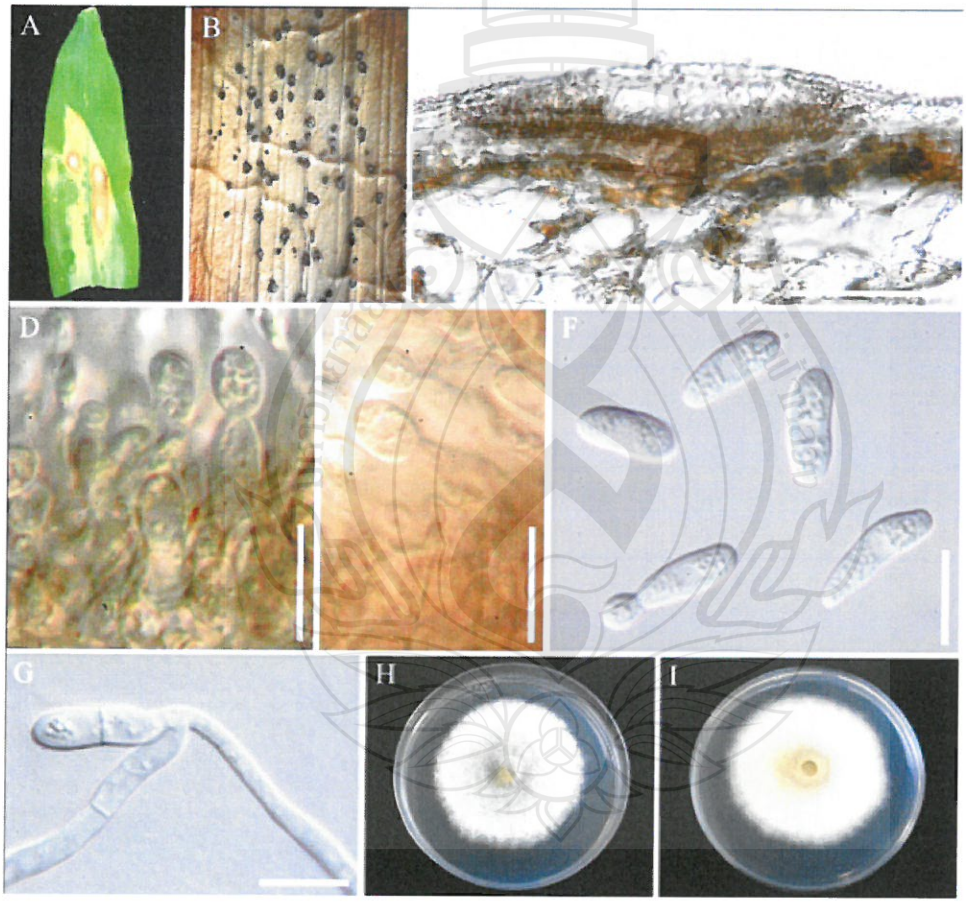

Fig 1. ***Colletotrichum gloeosporioides*** (MFLUCC12-0205, NTCL045): **A.** Specimen on living leaf. **B.** Conidiomata on the host surface. **C.** Longitudinal section of a conidioma. **D-E.** Conidiogenous cells with developing conidia. **F.** Conidia. **G.** Germinating conidium. **H-I.** Colonies on PDA; **H.** From top; **I.** From reverse. **Scale bar: C** = 100 μm; **D-G** = 10 μm.

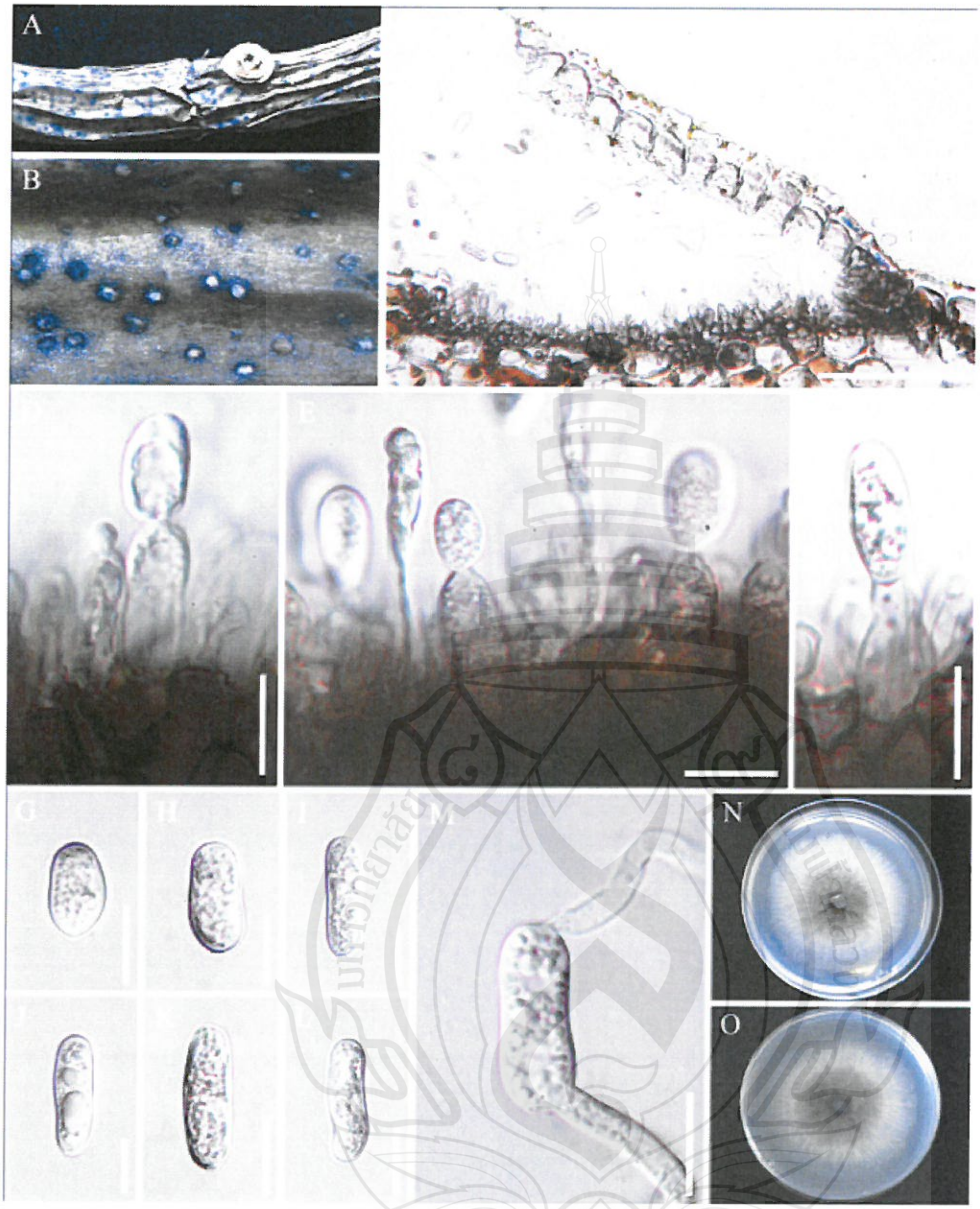

Fig 2. ***Colletotrichum ignotum*** (MFLUCC12-0138, NTCB001): **A.** Specimen on dead bark. **B.** Conidiomata on the host surface. **C.** Longitudinal section of a conidioma. **D-F.** Conidiogenous cells with developing conidia. **G-L.** Conidia. **M.** Germinating conidium. **N-O.** Colonies on PDA; **N.** From top; **O.** From reverse. **Scale bar: C** = 100 μm; **D-N** = 10 μm.

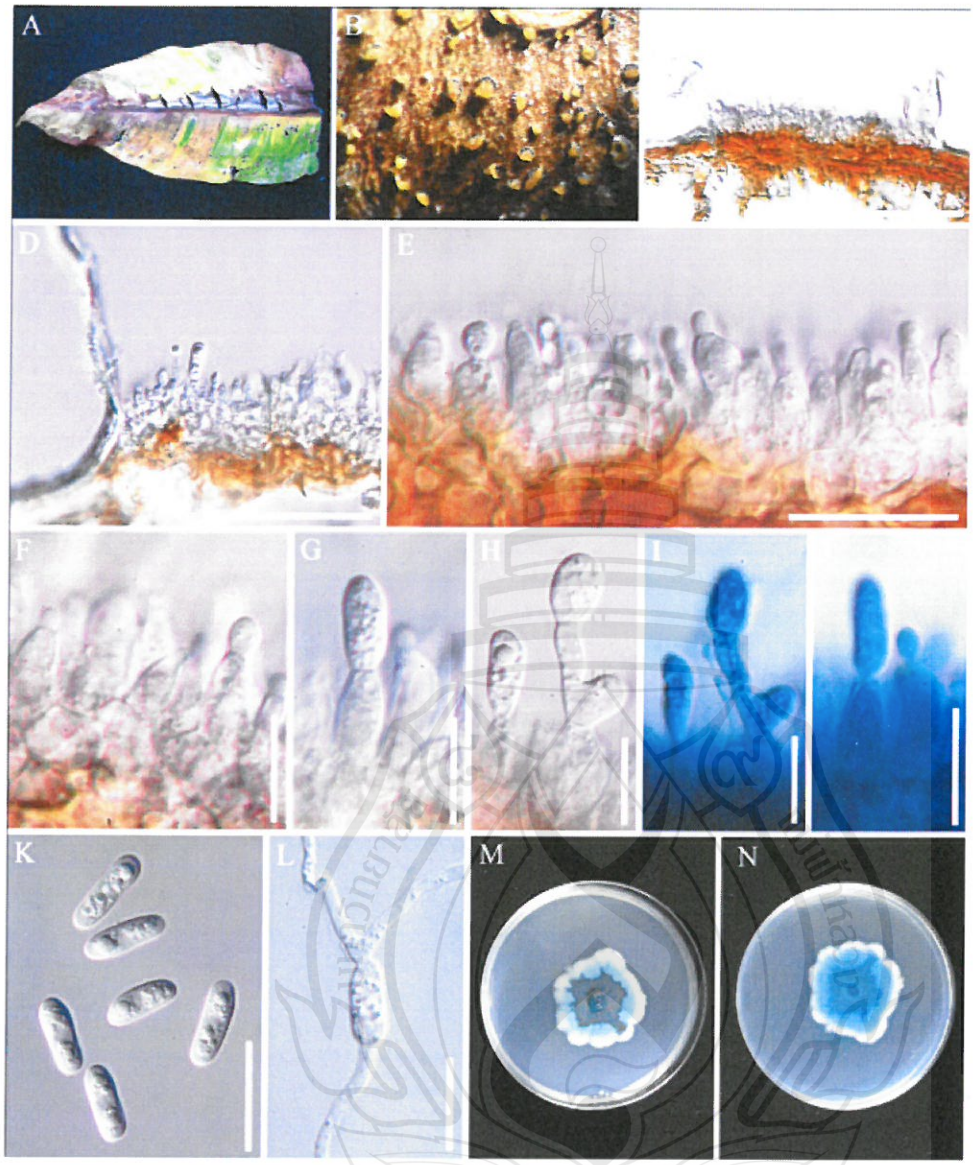

Fig 3. ***Colletotrichum boninense*** (MFLUCC12-0148, NTCL031): **A.** Specimen on living leaf. **B.** Conidiomata on the host surface. **C.** Longitudinal section of a conidioma. **D.** Longitudinal section of a conidioma wall. **E-H.** Conidiogenous cells with developing conidia. **I-J.** Conidiogenous cells stained with lactophenol cotton blue. **K.** Conidia. **L.** Germinating conidium. **M-N.** Colonies on PDA; **M.** From top; **N.** From reverse. **Scale bar: C** = 100 μm; **D** =50 μm; **E, K-L** = 20 μm; **F-J** = 10 μm.

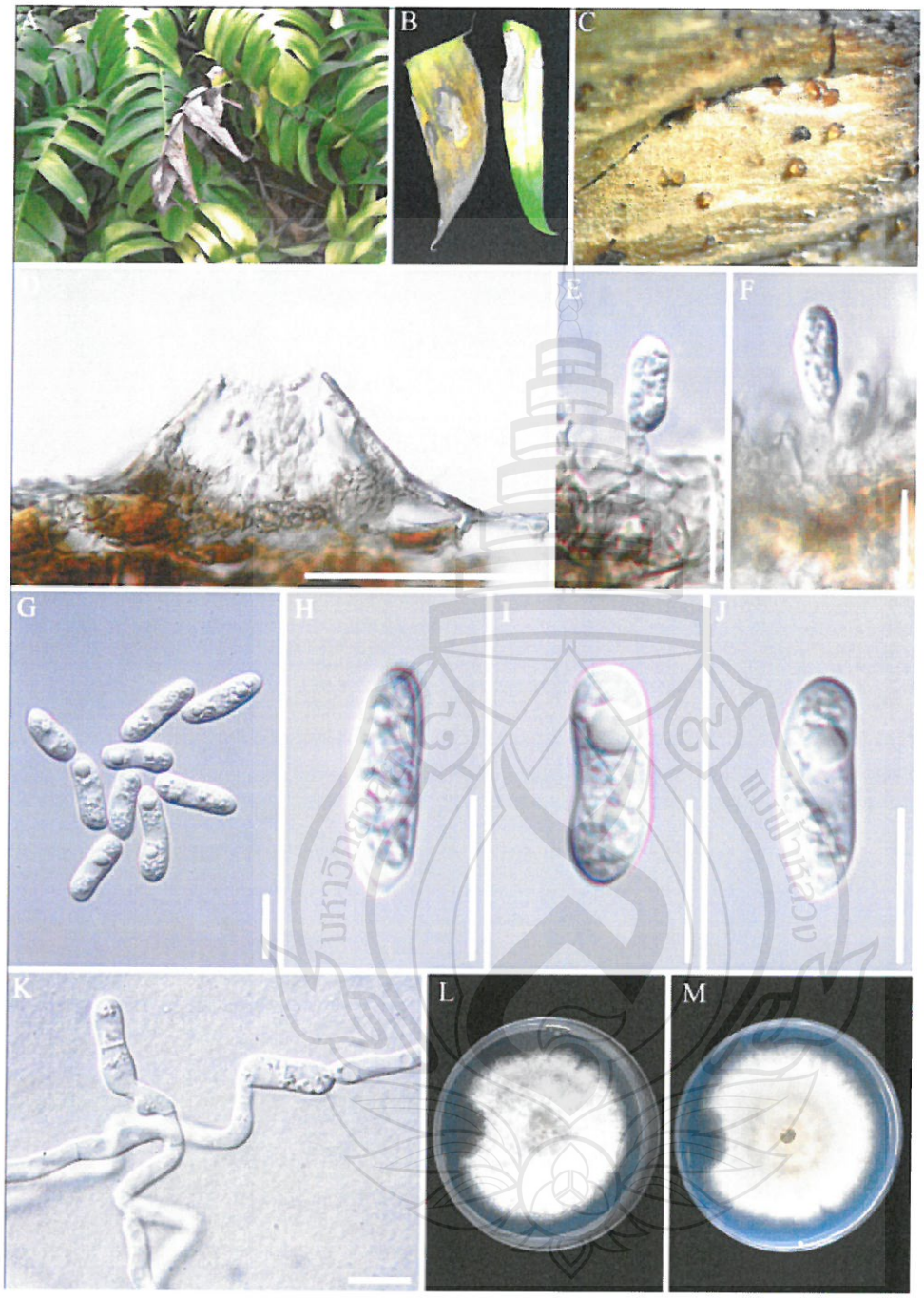

Fig 4. ***Colletotrichum gloeosporioides*** (MFLUCC12-0203, NTCL042): **A-B.** Specimen on living leaf. **C.** Conidiomata on the host surface. **D.** Longitudinal section of a conidioma. **E-F.** Conidiogenous cells with developing conidia. **G-J.** Conidia. **K.** Germinating conidium. **L-M.** Colonies on PDA; **L.** From top; **M.** From reverse. **Scale bar: D** = 100 μm; **E-K** = 10 μm.

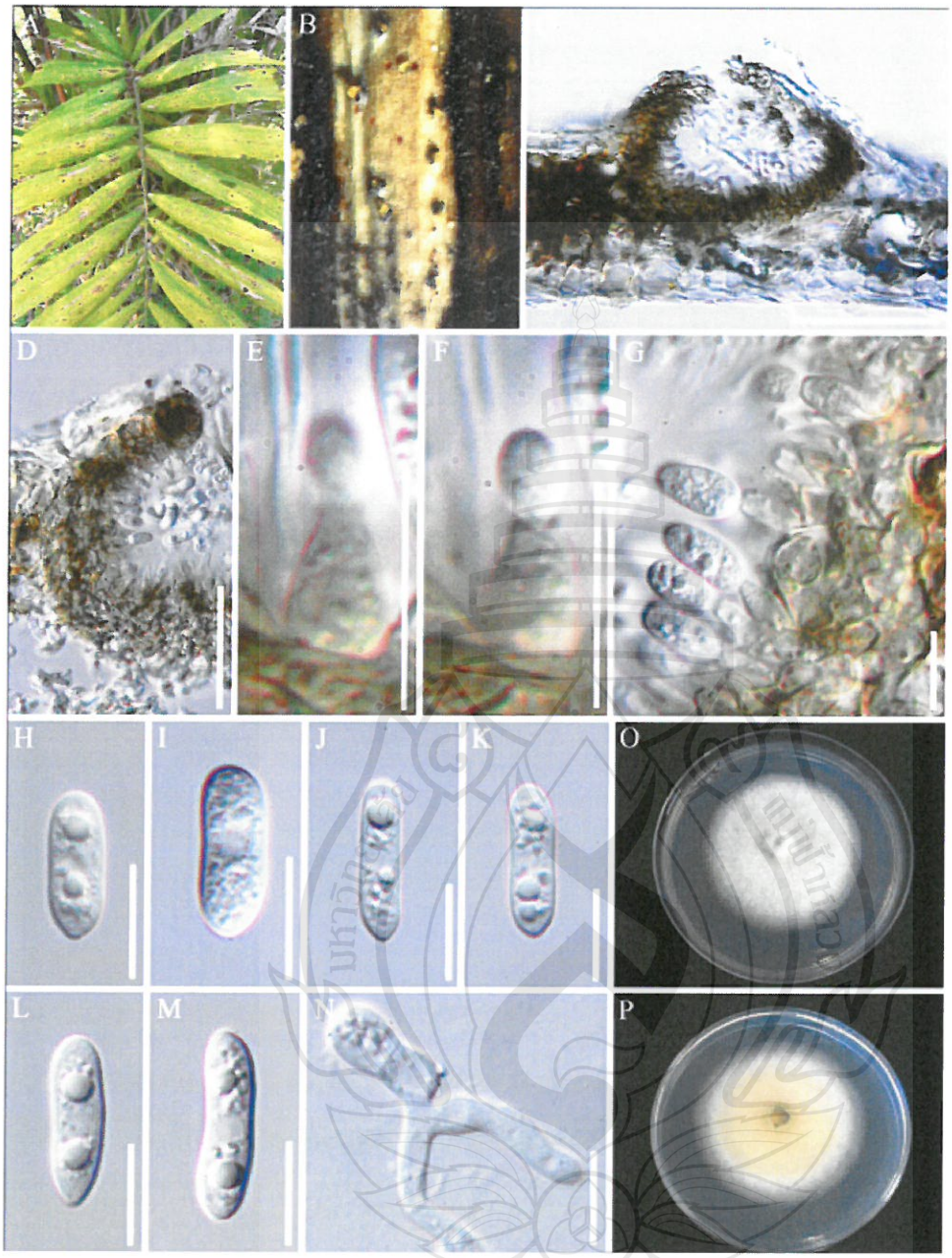

Fig 5. ***Colletotrichum gloeosporioides*** (MFLUCC12-0204, NTCL043): **A.** Specimen on living leaf. **B.** Conidiomata on the host surface. **C.** Longitudinal section of a conidioma. **D.** Longitudinal section of a conidioma wall. **E-G.** Conidiogenous cells with developing conidia. **H-M.** Conidia. **N.** Germinating conidium. **O-P.** Colonies on PDA; **O.** From top; **P.** From reverse. **Scale bar: C** = 100 μm; **D** = 50 μm; **E-N** = 10 μm.

4.3 Molecular analysis

In this project, we did PCR and sequenced the ITS, Actin, CAL, GPDH, GS and TUB gene regions of most strains (Table 4.3). Much of this data has analyzed and publish while a small amount is still being worked up. All sequence data has been or will be deposited in NCBI and is listed reported in subsequent publications (see publications in attachment) and in Table 4.3.

Table 4.3 Some of the PCR results for *Colletotrichum* strains collected.

MFLUCC	Region							
	ITS	SSU	LSU	Actin	CAL	GAPDH	GS	TUB
10-0610	x	x	x	x	x	x	x	x
10-0611	x	x	x	x	x	x	x	x
10-0612	x	x	x	x	x	x	x	x
10-0613	x	x	x	x	x	x	x	x
10-0614	x	x	x	x	x	x	x	x
10-0615	x	x	x	x	x	x	x	x
10-0616	KF24211	x	x	KF15782	KF25487	KF24218	KF24214	KF25489
10-0617	x	x	x	x	x	x	x	x
10-0618	x	x	x	x	x	x	x	x
10-0619	x	x	x	x	x	x	x	x
10-0620	x	x	x	x	x	x	x	x
10-0621	KF24209	x	x	KF15780	KF25485	KF24215	KF24212	KF25487
10-0622	x	x	x	x	x	x	x	x
10-0623	x	x	x	x	x	x	x	x
10-0624	KF24209	x	x	KF15780	KF25485	KF24215	KF24212	KF25488
10-0625	KF24209	x	x	KF15780	KF25485	KF24215	KF24212	KF25485
10-0626	x	x	x	x	x	x	x	x
10-0627	x	x	x	x	x	x	x	x
10-0628	x	x	x	x	x	x	x	x
10-0629	KF24209	x	x	KF15780	KF25485	KF24215	KF24212	KF25485
10-0630	KF24209	x	x	KF15780	KF25486	KF24215	KF24212	KF25488
10-0631	x	x	x	x	x	x	x	x
10-0632	x	x	x	x	x	x	x	x

Table 4.3 Some of the PCR results for *Colletotrichum* strains collected (Continued)

MFLUCC	Region							
	ITS	SSU	LSU	Actin	CAL	GAPDH	GS	TUB
10-0633	x	x	x	x	x	x	x	x
10-0634	x	x	x	x	x	x	x	x
10-0635	KF242107	x	x	KF157814	KF254868	KF242169	KF242138	KF254889
10-0636	KF242105	x	x	KF157812	KF254866	KF242167	KF242136	KF254887
10-0637	x	x	x	x	x	x	x	x
10-0638	x	x	x	x	x	x	x	x
10-0639	x	x	x	x	x	x	x	x
10-0640	KF242121	x	x	KF157829	KF254844	KF242179	KF242152	KF254903
10-0641	x	x	x	x	x	x	x	x
10-0642	x	x	x	x	x	x	x	x
10-0643	x	x	x	x	x	x	x	x
10-0644	KF242109	x	x	KF157816	KF254870	KF242171	KF242140	KF254891
10-0645	x	x	x	x	x	x	x	x
10-0646	KF242120	x	x	KF157828	KF254843	KF242178	KF242151	KF254902
10-0647	x	x	x	x	x	x	x	x
10-0648	x	x	x	x	x	x	x	x
10-0649	x	x	x	x	x	x	x	x
10-0650	x	x	x	x	x	x	x	x
10-0651	KF242119	x	x	KF157826	KF254878	KF242177	KF242150	KF254901
10-0652	KF242096	x	x	KF157803	KF254882	KF242158	KF242127	KF254882
10-0653	KF242099	x	x	KF157806	KF254851	KF242161	KF242130	KF254855
10-0654	KF242100	x	x	KF242100	KF254852	KF242162	KF242131	KF254856
10-0655	KF242110	x	x	KF157817	KF254871	KF242172	KF242141	KF254892
10-0656	KF242116	x	x	KF157823	KF254875	KF242185	KF242147	KF254898
10-0657	KF242111	x	x	KF157818	KF254872	KF242173	KF242142	KF254893
10-0658	x	x	x	x	x	x	x	x
10-0659	x	x	x	x	x	x	x	x
10-0660	KF242103	x	x	KF157810	KF254864	KF242165	KF242134	KF254885

Table 4.3 Some of the PCR results for *Colletotrichum* strains collected (Continued)

MFLUCC	Region							
	ITS	SSU	LSU	Actin	CAL	GAPDH	GS	TUB
10-0661	x	x	x	x	x	x	x	x
10-0662	x	x	x	x	x	x	x	x
10-0663	x	x	x	x	x	x	x	x
10-0664	x	x	x	x	x	x	x	x
10-0665	x	x	x	x	x	x	x	x
10-0666	x	x	x	x	x	x	x	x
10-0667	x	x	x	x	x	x	x	x
10-0668	KF24212	x	x	KF15783	KF25484	KF242180	KF24215	KF25490
10-0669	KF24211	x	x	KF15781	KF25487	KF242174	KF24214	KF25489
10-0670	x	x	x	x	x	x	x	x
10-0671	x	x	x	x	x	x	x	x
10-0672	x	x	x	x	x	x	x	x
10-0673	KF24210	x	x	KF15781	KF25486	KF242170	KF24213	KF25489
10-0674	KF24211	x	x	KF15782	KF25489	KF242175	KF24214	KF25489
10-0675	x	x	x	x	x	x	x	x
10-0676	KF24212	x	x	KF15782	KF25484	KF242181	KF24215	KF25485
10-0677	KF24210	x	x	KF15781	KF25486	KF242166	KF24213	KF25488
10-0678	x	x	x	x	x	x	x	x
10-0679	KF24211	x	x	KF15782	KF25484	KF242182	KF24214	KF25489
10-0680	KF24211	x	x	KF15782	KF25484	KF242183	KF24214	KF25489
10-0681	KF24210	x	x	KF15780	KF25486	KF242163	KF24213	KF25488
10-0682	x	x	x	x	x	x	x	x
10-0684	x	x	x	x	x	x	x	x
11-0456	x	x	x	x	x	x	x	x
11-0460	x	x	x	x	x	x	x	x
11-0534	x	x	x	x	x	x	x	x
11-0535	x	x	x	x	x	x	x	x
11-0536	x	x	x	x	x	x	x	x

Table 4.3 Some of the PCR results for *Colletotrichum* strains collected (Continued)

MFLUCC/ LC code	Region							
	ITS	SSU	LSU	Actin	CAL	GAPDH	GS	TUB
11-0538	x	x	x	x	x	x	x	x
11-0539	x	x	x	x	x	x	x	x
11-0541	x	x	x	x	x	x	x	x
13-0418	KC633854	x	x	x	KC810018	KC832854	x	x
13-0417	KC633853	x	x	KC692467	KC810017	KC832853	x	x
13-0419	KC633855	x	x	KC692468	KC810016	KC832846	x	x
10-0676	KC835393	x	x	KC835394	KC835395	KC835396	x	x
LC0542	KC633852	x	x	KC692466	KC810019	KC832841	x	x
LC0540	KC633859	x	x	KC669628	KC835391	KC832842	x	x
LC0548	KC633856	x	x	KC669625		KC832849	x	x
LC1212	KC633857	x	x	KC669626	KC692471	KC832848	x	x
LC0532	KC633858	x	x	KC669627	KC692472	KC832852	x	x
LC1232	KC633860	x	x	KC676709	KC692473	KC832845	x	x
LC0326	KC633861	x	x	KC676710	KC692474	KC832856	x	x
LC0347	KC633862	x	x	KC676711	x	KC832851	x	x
LC0329	KC633863	x	x	KC676712	KC692475	KC832843	x	x
LC1235	KC633865	x	x	KC676714	KC692477	KC832850	x	x
LC0323	KC633866	x	x	KC676715	KC692478	KC832855	x	x
LC1234	KC633867	x	x	x	KC692479	KC832844	x	x
LC1225	KC633869	x	x	KC676716	x	KC832847	x	x
LC0328	KC633868	x	x	x	KC692480	KC832858	x	x
LC0537	KC633864	x	x	KC676713	KC692476	KC832857	x	x
LC0551	KC633850	x	x	KC633871	KC692470	KC835389	x	x
LC1238	KC633851	x	x	KC633870	KC692469	KC835390	x	x

x= will be submitted to GenBank as publication arise.

4.4. Resolving species complexes

We can now resolve *Colletotrichum* into species complexes with ITS (see Fig 6) but to resolve species complexes is much more difficult (see Fig 6).

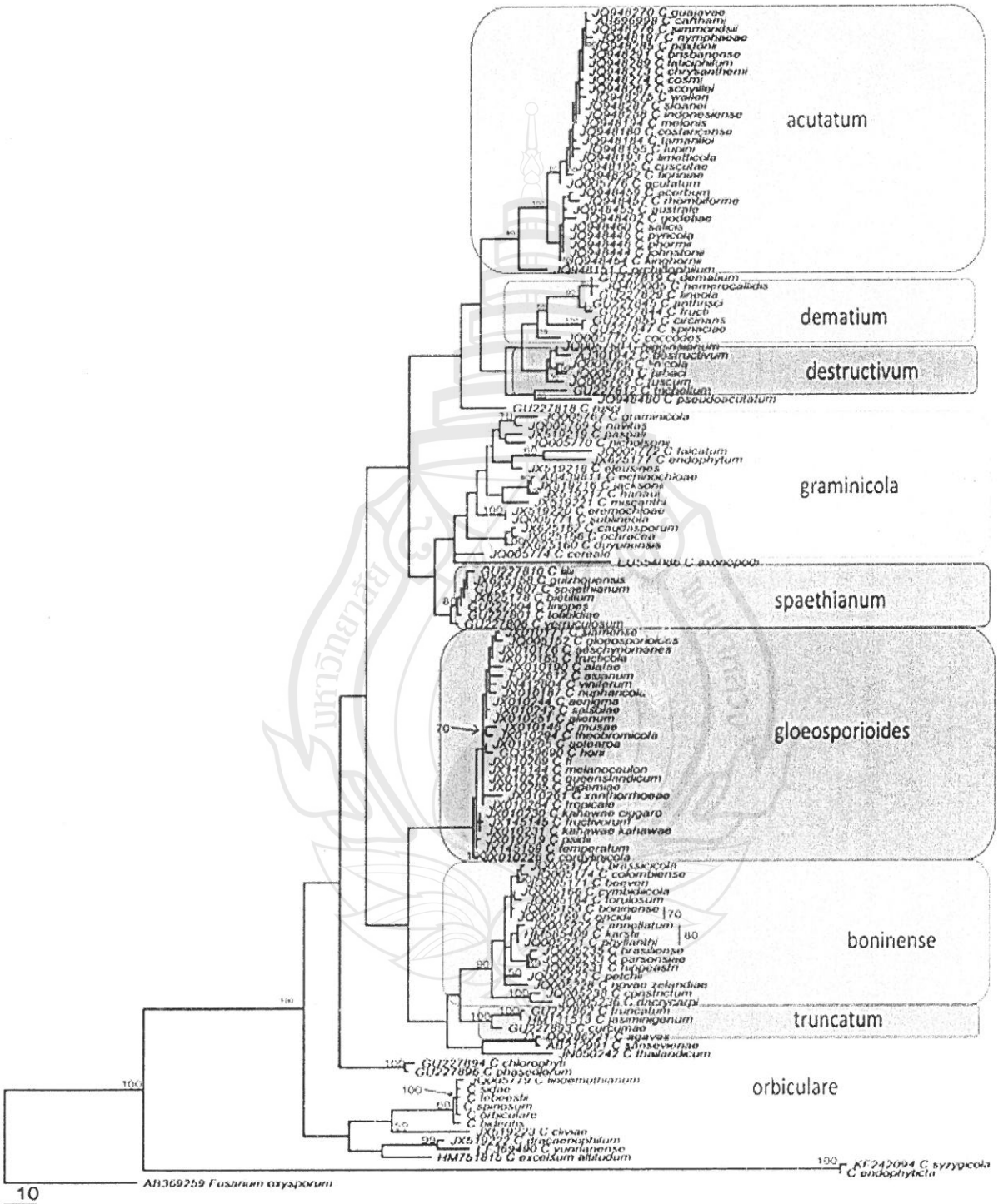

Fig 6. ITS tree resolving species complexes.

For example, species of *Colletotrichum* on grapes can be resolved into three species including *C. aenigma, C. hebeiense* and *C. viniferum*. However, *C. viniferum* is a species complex comprising several species. Better genes are needed to resolve the species in this complex.

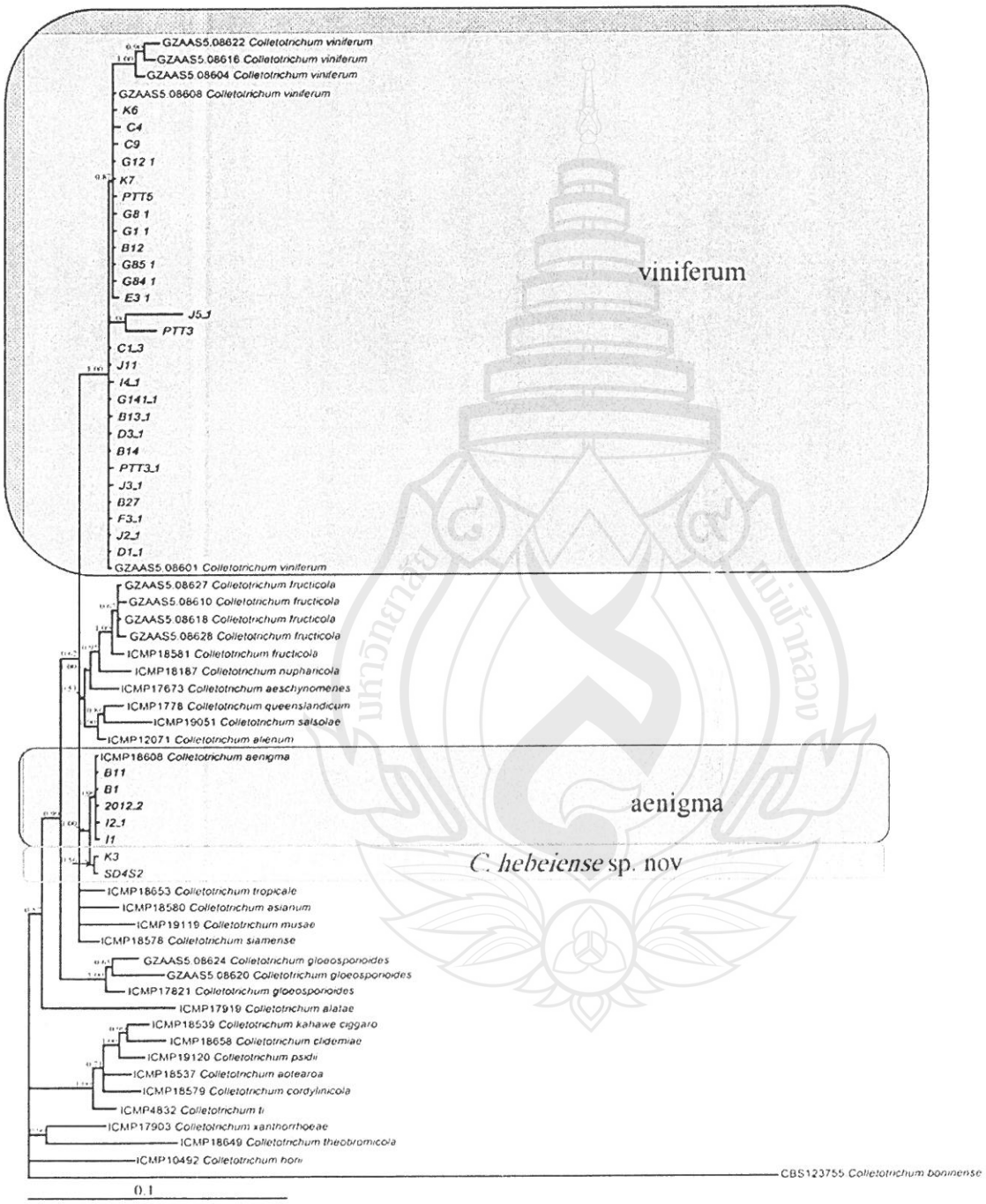

Fig 7. Species of *Colletotrichum* from grape.

CHAPTER 5
CONCLUSION

Colletotrichum species have a worldwide distribution and are important pathogens subjected to import control and plant breeding programs. Previous identification and classification was based on host-association and morphological characteristics. Molecular sequence data, initially ITS and morphology was used to characterize species, however, they could not resolve species well. Recent multigene phylogenetic analysis as well as morphology and pathogenicity testing have resolved about 100 *Colletotrichum* species. There are four important species complexes. *C. gloeosporioides* species complex is the most important and multigene phylogenetic analysis have confirmed that *C. gloeosporioides* comprises 22 morphologically similar, phylogenetically distinct species. However, ITS, beta tubulin (*tub2*), DNA lyase and the intergenic region of *apn2* and *MAT1-2-1* genes (*ApMat*)) have also used shown new lineages within this species-complex and presently, there are 28 accepted species names within the complex. No consensus among mycologists as to which gene markers should be used to define and delimit a species within the species complex yet exists. We have collected more than 200 strains of *Colletotrichum* from various diseased plants and also grasses as asymptomatic endophytes. We have sequence and characterized many new species and have published these. Between October 2010 and September 2013 we published eleven manuscripts of which all nut one were SCI (Table 5.1). Since *Colletotrichum* is a global problem we have chosen to collaborate with Brazilian, Chinese and Indian colleagues in order to bring greater depth to the research and international agreement to the findings. The latter was achieved with the formation of the International Subcommission on *Colletotrichum* taxonomy (http://www.fungaltaxonomy.org/ subcommissions) of which our group is a founder member.

Table 5.1 Publication list

Year	Publications
2011	Ko Ko TW, Moslem MA, Abdelsalam K, Chamyuang S, Cheewangkoon R, Chukeatirote E, Jonglaekha N, Kobsueb R, McKenzie EHC, Promputtha I, Soytong K, To-anun C, Wikee S, Wulandari NF, Hyde KD (2011) The need for re-inventory of Thai phytopathogens. Chiang Mai Journal of Science 38(4): 625-637.
2012	Jadrane I, Kornievsky M, Desjardin DE, He ZH, Cai L, Hyde KD (2012) First Report of Flower Anthracnose Caused by *Colletotrichum karstii* in White *Phalaenopsis* Orchids in the United States. Plant Disease 96 (8):1227
	Noireung P, Phoulivong S, Liu F, Cai L, Mckenzie EHC, Chukeatirote E, Jones EBG, Bahkali AH, Hyde KD (2012) Novel species of *Colletotrichum* revealed by morphology and molecular analysis. Cryptogamie, Mycologie 33(3): 347-362.
	Peng LJ, Yang YL, Hyde KD, Bahkali AH, Liu ZY (2012) *Colletotrichum* species on Citrus leaves in Guizhou and Yunnan provinces, China. Cryptogamie Mycologie 33(3): 267-283.
	Phoulivong S, McKenzie EHC, Hyde KD (2012) Cross infection of *Colletotrichum* species; a case study with tropical fruits. Current Research in Environmental & Applied Mycology 2(2): 99–111 DOI 10.5943/cream/2/2/2.
	Yang Y, Liu Z, Cai L, Hyde KD (2012) New species and notes of *Colletotrichum* on daylilies (*Hemerocallis* spp.). Tropical Plant Pathology 37(3): 165-174.
2013	Huang F, Chen GQ, Hou X, Fu YS, Cai L, Hyde KD, Li HY (2013) *Colletotrichum* species associated with cultivated citrus in China. Fungal Diversity 61:61–74
	Lima N, Batista MV de A, Morais, MA De Morais Jr, Barbosa MAG, Michereff SJ, Hyde KD, Câmara MPS (2013) Five *Colletotrichum*

species are responsible for mango anthracnose in northeastern Brazil. Fungal Diversity 61:75–88

Manamgoda DS, Udayanga D, Cai L, Chukeatirote E, Hyde KD (2013) Endophytic *Colletotrichum* from tropical grasses with a new species *C. endophytica*. Fungal Diversity 61(1): 107-115.

Nilsson RH, Hyde KD, Alias SA et al. (2013) A distributed third-party annotation effort of fungal ITS sequences from plant pathogenic fungi. Plos One (in prep)

Peng LJ, Sun T, Yang L, Cai L, Hyde KD, Bahkali AH, Yi Liu ZY (2013) *Colletotrichum* species on grape in Guizhou and Yunnan provinces, China. Mycoscience 54:29-41.

Sharma G, Kumar N, Weir BS, Hyde KD, Shenoy BD (2013) The ApMat marker can resolve *Colletotrichum* species: a case study with *Mangifera indica*. Fungal Diversity 61:117–138

Udayanga D, Manamgoda DS, Liu X, Chukeatirote E, Hyde KD (2013) What are the common anthracnose pathogens of tropical fruits? Fungal Diversity 61:165-179

Yan JY, Jayawardena MMRS, Wang Y, Zhang W, Liu M, Yan H, Huang JB, Wang ZY, Shang JJ, Peng YL, Bahkali A, Hyde KD, Li XH (2013) Diverse species of *Colletotrichum* associated with grapevine anthracnose in China. Fungal Diversity (in prep)

Vieira WAS, Nascimento RJ, Michereff SJ, Hyde KD, Câmara MPS (2013) First Report of Papaya Fruit Anthracnose Caused by *Colletotrichum brevisporum* in Brazil. Plant Disease (in prep)

APPENDIX

Chiang Mai J. Sci. 2011; 38(4) : 625-637
http://it.science.cmu.ac.th/ejournal/
Contributed Paper

The need for re-inventory of Thai phytopathogens

Thida Win Ko Ko [a], Eric Hugh Charles McKenzie [b], Ali Hassan Bahkali [c], Chaiwat To-anun [d], Ekachai Chukeatirote [a], Ithayakorn Promputtha [a], Kamal Ahmed Abd-Elsalam [c], Kasem Soytong [f], Nilam Fadmaulidha Wicaksari [d,g], Niwat Sanoamuang [h], Nuchsart Jongkaewha [i], Rampai Kodsueb [j], Ratchadawan Cheewangkoon [d], Saowanee Wikee [a], Somita Chamyoung [a] and Kevin David Hyde*[a,c]

[a] School of Science, Mae Fah Luang University, Thasud, Chiang Rai 57100, Thailand.

[b] Landcare Research, Private Bag 92170, Auckland, New Zealand.

[c] King Saud University, College of Science, Botany and Microbiology Department, P.O. Box: 2455, Riyadh 1145, Saudi Arabia.

[d] Department of Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand

[e] School of Cosmetic Science, Mae Fah Luang University, Thasud, Chiang Rai 57100, Thailand.

[f] Division of Plant Pest Management Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand.

[g] Microbiology Division, Research Centre for Biology (PKSB), Indonesian Institute of Sciences (LIPI), Jl. Raya Bogor, KM 46, Cibinong Science Centre, Cibinong 16911, Indonesia.

[h] Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand.

[i] Plant Protection Center, Royal Project Foundation, Chiang Mai, Thailand.

[j] Biology Programme, Faculty of Science and Technology, Pibulsongkram Rajabhat University, Phitsanulok, Thailand.

*Author for correspondence; e-mail: kdhyde3@gmail.com

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ABSTRACT

Plant disease associated fungi are of concern to plant pathologists, plant breeders, post harvest disease experts, quarantine officials and farmers in Thailand. Checklists with sound morphological identifications are paramount to work by these specialists. In recent years molecular techniques have been applied to species identification and many species have been shown to comprise numerous cryptic species. In this paper, the need for modern systematic treatments of several important phytopathogenic genera are highlighted and a recommendation for future research of plant pathogens in Thailand is discussed.

Keywords: plant pathogenic fungi, quarantine, systematics.

1. INTRODUCTION

The Index of Plant Diseases in Thailand [1] lists fungi and other pathogens of plants in Thailand recorded up to 1994 and is based on many years of sound morphological identifications [2], and was the best methodology that was applicable at that time.

in species definition [34, 51, 71]. On the other hand, use of phylogeny only would become questionable as DNA extraction from the correct identified specimens or culture is essential. Nilsson *et al.* [103] found many sequences deposited in GenBank with incorrect taxon names and assumed that these were generated from wrongly identified specimens or cultures. While this may be the case, many of the earlier identifications were based on morphology, which could not recognize cryptic species or separate species complexes. Therefore, an integrated approach using morphological characters as well as molecular tools is recommended as an appropriate technique to define plant pathogenic taxa [100, 102].

Present knowledge of the systematics of Thai phytopathogens is mainly based on morphology or on a host-association nomenclature system, and no longer applicable names, are often used [1]. In order to develop a better understanding of Thai phytopathogens, a modern polyphasic approach is necessary. As DNA extraction from dry herbarium specimens is often difficult or impossible, re-collection of fresh specimens is required. Identification of fresh specimens by comparison with type specimens or newly chosen epitypes will reduce the potential for confusion. The retention of living cultures is not a common practice in plant pathology. Thus, there is a need to collect fresh material, where possible culture the suspected pathogen, isolate, extract, sequence and analyse DNA from the fungi. Results must be compared with those obtained from authenticated isolates, and combined with a precise morphological study of the pathogen. However, there are likely to be difficulties in obtaining research funding to re-determine all known plant pathogenic fungi from Thailand since species identification using molecular techniques is costly. Therefore, serious plant pathogens on commercial crops, such as *Fusarium* on rice, *Colletotrichum* on chili and *Phyllosticta* on Citrus should be given priority.

Acknowledgements

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Plant Disease

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Disease Notes

First Report of Flower Anthracnose Caused by *Colletotrichum karstii* in White *Phalaenopsis* Orchids in the United States

I. Jadrane, M. Kornievsky, D. E. Desjardin, and **Z.-H. He,** Department of Biology, San Francisco State University, CA 94132; **L.Cai,** State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Science, Beijing, China; and **K. Hyde,** School of Science, Mae Fah Luang University, Muang, Chiang Rai, 57100, Thailand

In October 2010, a *Colletotrichum* species was isolated from white *Phalaenopsis* flowers growing in a greenhouse in San Francisco, CA. This *Phalaenopsis* is a common commercial orchid hybrid generated mostly likely from *Phalaenopsis amabilis* and *P. aphrodite*. The white petals showed anthracnose-like lesions where necrotic tissue is surrounded by a ring of green tissue. The green halo tissues around the necrotic tissue contain functional chloroplasts. One-centimeter disks were cut around the necrotic sites and surface-sterilized with 95% ethanol and 0.6% sodium hypochlorite. The disks were placed on potato dextrose agar (PDA) medium to establish cultures. Pure cultures were obtained by subculturing hyphal tips onto fresh PDA plates. The generated colonies had white aerial mycelia and orange conidial mass. The color of the reverse colony varies between colorless and pale orange. Microscopic observations identified the conidia as cylindrical, straight, and rounded at both ends. In addition, the conidia were approximately 15.0 to 18.0 µm long and 5.0 to 6.5 µm in diameter. These observed morphological features suggested that these isolates possessed the same characteristics as previously described for *Colletotrichum karstii*, a species considered as part of the *C. boninense* species complex (1). Four putative independent *Colletotrichum* isolates were recovered (DED9596, DED9597, DED9598, and DED9599). To confirm the *Colletotrichum* isolates as the causative pathogen, healthy white *Phalaenopsis* flowers (five total) in a whole plant were sprayed with a conidial suspension (approximately 1.2×10^5 conidia/ml) of the isolates and incubated at 20°C and 100% relative humidity with cycles of 16 h light and 8 h of darkness. Approximately 1 ml of conidial suspension solution was used for each flower. The plants were watered regularly and flowers were sprayed with sterile double-distilled water daily. As negative controls, five flowers in a whole plant were sprayed with water. Fifteen to twenty days after inoculation, lesions started to form on the petals sprayed with the putative *Colletotrichum* isolates. All controls remained healthy. The *Colletotrichum*-inoculated flowers remained alive and did not die as a result of the infection. This same experiment was repeated and the same results were obtained. DNA was extracted from the necrotic regions of the petals infected by the pure cultures of the four isolates and used to sequence the 18S rRNA ITS (internal transcribed spacer) region. All four isolates gave identical ITS sequences. Analysis of the obtained representative sequences (GenBank Accession No. JQ277352) suggested that the isolated pathogen as *C. karstii*. Using the published ITS data for the *C. boninense* species complex (1), a phylogenetic tree was generated via the maximum likelihood method. This created tree places the isolates in the same group as *C. karstii*. This type of *C. karstii* infection in *Phalaenopsis* orchid petals was not documented in the U.S. before, although it has been reported in China and Thailand (2). To our knowledge, this is the first report of infection and green island formation caused by *C. karstii* on orchid flower in the United States.

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Novel species of *Colletotrichum* revealed by morphology and molecular analysis

Parinn NOIREUNG [a, b], *Saisamorn PHOULIVONG [a, b]*, *Fang LIU [c]*, *Lei CAI [c]*, *Eric H.C. MCKENZIE [d]*, *Ekachai CHUKEATIROTE [a, b]*, *E. B. G. JONES [e]*, *Ali H. BAHKALI [f]* & *Kevin D. HYDE [a, b, f, g]*

[a] *Institute of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand*

[b] *School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand*

[c] *State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, P.R. China, mrcailei@gmail.com*

[d] *Landcare Research, Private Bag, 92170, Auckland, New Zealand*

[e] *Institute of Ocean and Earth Sciences (IOES), C308, University of Malaya, 50603, Kuala Lumpur, Malaysia*

[f] *King Saud University, College of Science, Botany and Microbiology Department, Riyadh 1145, Saudi Arabia*

[g] *International Fungal Research & Development Centre, The Research Institute of Resource Insects, Chinese Academy of Forestry, Bailongsi, Kunming 650224, PR China*

Abstract – *Colletotrichum* species are widely known as key anthracnose pathogens of several economic plants. In this study, *Colletotrichum* species associated with leaf anthracnose isolated from various plants in Thailand were subjected to morphological and molecular analyses. The ITS rDNA regions of these strains were sequenced and aligned with those of type strains in the genus in order to establish if they can be assigned to any known species. Strains that could not be identified were further sequenced for partial actin (ACT), β-tubulin (TUB2) and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) genes and employed in a phylogenetic analysis to reveal their relationships with other closely related taxa. The multilocus sequence analysis, together with a critical examination of the phenotypic characters, revealed three new species. These are introduced as *C. brevisporum*, *C. tropicicola* and *C. thailandicum* and formally described, illustrated and compared with similar taxa.

Anthracnose / multilocus phylogeny / plant disease / systematics / pathogenicity

INTRODUCTION

Colletotrichum is an important, cosmopolitan, phytopathogenic genus causing anthracnose disease of a wide range of economically important crops, ornamentals, perennials, herbaceous plants and grasses (Sutton, 1992; Freeman

* Corresponding author:

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Colletotrichum species on *Citrus* leaves in Guizhou and Yunnan provinces, China

*Lijuan PENG [a,b], Youlian YANG [c], Kevin D. HYDE [d,e], Ali H. BAHKALI [f] & Zuoyi LIU [g,h]**

[a] *The Provincial Key Laboratory for Agricultural Pest Management of Mountainous Region, Guizhou University, Guiyang, Guizhou 550025, P. R. China. E-mail: plj777@yahoo.com.cn*

[b] *Agricultural College of Guizhou University, Guiyang, Guizhou 550025, P. R. China*

[c] *Department of Biology and Geography, Liupanshui Teachers College, Shuicheng, Guizhou 553006, P. R. China. E-mail: yangyoulian@hotmail.com*

[d] *Institute of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand. E-mail: kdhyde3@gmail.com*

[e] *School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand*

[f] *King Saud University, College of Science, Botany and Microbiology Department, Riyadh, Saudi Arabia*

[g] *Guizhou Academy of Agricultural Sciences, Guiyang, Guizhou 550006 P. R. China. E-mail: liuzuoyi@yahoo.com.cn*

[h] *Guizhou Key Laboratory of Agricultural Biotechnology, Guiyang, Guizhou 550006 P. R. China*

Abstract – Thirty-eight strains of *Colletotrichum* were isolated from diseased *Citrus* leaves in Guizhou and Yunnan provinces in China. Based on morphological characters and multilocus sequence data, the strains were identified as *C. boninense* (1 strain), *C. brevispora* (1), *C. fructicola* (2), *C. gloeosporioides* (29), *C. karstii* (2), *C. siamense* (1) and *Colletotrichum murrayae*, the latter represents a new species which is described in this paper. *Colletotrichum gloeosporioides* was originally isolated from *Citrus sinensis* in southern Italy and was the most common species associated with diseased leaves of *Citrus* in China.

Anthracnose / morphology / multilocus phylogeny / plant disease

* Corresponding author: Zuo-Yi Liu: e-mail: liuzuoyi@yahoo.com.cn

Colletotrichum simmondsii has been reported to cause fruit rot or fruit anthracnose on chili pepper (*Capsicum frutescens*), strawberry (*Fragaria* × *ananassa*), papaya (*Carica papaya*), chinese pulsatilla (*Anemone chinensis*), litchi (*Litchi chinensis*), tree tomato (*Cyphomandra betacea*), avocado (*Persea americana*), mango (*Mangifera indica*), high bush blueberry (*Vaccinium corymbosum*) and tomato (*Lycopersicon esculentum*) (Shivas & Tan 2009). It also infected celery (*Apium graveolens* var. *dulce*) causing stunt anthracnose (Fujinaga *et al.*, 2011). We isolated one strain of *C. simmondsii* from a *Murraya* sp. leaf lesion.

Colletotrichum fructicola was isolated from coffee berries (*Coffea arabica*) as a pathogen and endophyte (Prihastuti *et al.*, 2009), leaves of spider lily (*Crinum asiaticum*) (Yang *et al.*, 2009) and fruits of table grape (*Vitis vinifera*).

Colletotrichum gloeosporioides is the most common species on *Citrus* plants, comprising 29 (76%) of 38 strains isolated in this study. Some species of *Colletotrichum* produce secondary conidia in culture directly from germinated primary conidia (Cannon *et al.*, 2000; Slade *et al.*, 1987). Leng *et al.* (1984) observed secondary conidia forming in eight isolates of *C. gloeosporioides* from *Citrus* spp. in China. Secondary conidia also can be formed in *C. acutatum* (Barbosa *et al.*, 2006; Leandro *et al.*, 2001). When inducing conidial appressoria in *C. gloeosporioides* on a glass slide, there were three ways in which conidia germinated, forming conidial appressoria, secondary conidia or both. We selected eight strains of *C. gloeosporioides* to observe the formation of secondary conidia (Fig. 4 e, f). All strains produced this phenomenon. Thus secondary conidia formation may be an important character of *C. gloeosporioides*.

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Current Research in Environmental & Applied Mycology Doi 10.5943/cream/2/2/2

Cross infection of *Colletotrichum* species; a case study with tropical fruits

Phoulivong S, McKenzie EHC and Hyde KD

Phoulivong S, McKenzie EHC, Hyde KD 2012 – Cross infection of *Colletotrichum* species: a case study with tropical fruits. Current Research in Environmental & Applied Mycology 2(2), 99–111, Doi 10.5943/cream/2/2/2

Strains of *Colletotrichum* were isolated from the fruits of chili, coffee, longan, mango, papaya and rose apple, collected from orchards and markets in Laos and Thailand. Isolates were identified using morphological characters, colony growth rate, and confirmed with DNA sequence data analysis of combined multi-gene loci. Pathogenicity testing of ten strains representing five species of *Colletotrichum* was carried out on *Capsicum* sp. (chili), *Carica papaya* (papaya), *Citrus reticulata* (orange), *Eugenia javanica* (rose apple), *Mangifera indica* (mango) and *Psidium guajava* (guava) using a wound drop technique. Pathogenicity and potential for cross infectivity of *Colletotrichum asianum*, *C. cordylinicola*, *C. fructicola*, *C. siamense* and *C. zimmondii* were tested on the hosts. The *Colletotrichum* strains belonging to different species tested were generally shown to infect a wide host range.

Key words – anthracnose – fruit infection – pathogenicity

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*Corresponding author: K.D. Hyde – e-mail – kdhyde3@gmail.com

Introduction

Colletotrichum is one of the most economically important pathogenic genera causing anthracnose of fruits, affecting a wide range of hosts in the tropics and subtropics (Cai et al. 2009, Cannon et al., 2012; Damm et al., 2012; Fujinaga et al., 2012; Hyde et al. 2009a, Phoulivong et al. 2010a, Noireung et al. 2012, Weir et al., 2012; Yang et al. 2012a, b). The above-ground plant parts of crops as well as fruit trees can be affected by *Colletotrichum* anthracnose and in the case of fruit infection, there is a reduction in yield quantity or quality (Phoulivong et al. 2010a). Hosts of *Colletotrichum* species in Thailand include fruits such as chili (*Capsicum* sp.), guava (*Psidium guajava*), jujube (*Zizyphus mauritiana*), mango (*Mangifera indica*), papaya (*Carica papaya*) and rose apple (*Eugenia javanica*) (Freeman & Shabi 2000, Peres et al. 2002, Ratanacherdchai et al. 2010, Sreenivasaprasad and Talhinhas, 2005). *Colletotrichum* species are cosmopolitan with either multiple species occurring on a single host or a single species occurring on multiple hosts (Sander & Korsten, 2003). Fungus-host relationships are broad, imprecise and often overlapping (Freemand & Shabi 2000). *Colletotrichum* species can infect many hosts and may adapt to new environments (Sanders & Korsten 2003, Photita et al. 2004), leading to serious cross infection problems in plant production. The study of pathogenic variability of *Colletotrichum* species is therefore important and the understanding of the host range of a particular pathogen may help in efficient disease control and management (Whitelaw-Weckert et al. 2007).

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RESEARCH ARTICLE / *ARTIGO*

New species and notes of *Colletotrichum* on daylilies (*Hemerocallis* spp.)

Youlian Yang[1,4], Zuoyi Liu[1], Lei Cai[2] & Kevin D. Hyde[4]

[1]Guizhou Key Laboratory of Agricultural Biotechnology, Guizhou Academy of Agricultural Sciences, Guiyang, Guizhou 550006, P. R. China; [2]Department of Life Science, Liupanshui Normal University, Shuicheng, Guizhou 553006, P. R. China; [3]State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, No.10, North 4th Ring Road West, Beijing 100190, P. R. China; [4]Institute of Excellence in Fungal Research, School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand; Visiting Professor, Botany and Microbiology Department, College of Science, King Saud University, Riyadh, Saudi Arabia

Author for correspondence: Kevin D. Hyde, e-mail: kdhyde3@gmail.com

ABSTRACT

Nine *Colletotrichum* strains were isolated from diseased and dead stalks of *Hemerocallis* species (daylilies) from Guizhou, Guangxi, and Liaoning provinces in China. Morphological characteristics and multilocus phylogenetic analyses of ACT, CHS-1, GPDH, ITS, and TUB-2 indicate that these strains represent four taxa. *Colletotrichum hemerocallidis* is a new species that is described, illustrated, and compared with similar species. *Colletotrichum gloeosporioides*, *C. fioriniae*, and *C. spaethianum* are also recorded on *Hemerocallis* species.

Key words: Anthracnose, multilocus phylogeny, systematics.

INTRODUCTION

Hemerocallis species (including *H. fulva* (L.) Linn., *H. citrina* Baroni, and other species or cultivars) are economically important as food plants in China, Japan, Korea, Thailand, and Vietnam, being known as "yellow flower vegetables" or "golden needles" in China (Zhou et al., 1994; Staples & Kristiansen, 1999; Zhang & Chen, 2008). Species are also cultivated and bred worldwide for their showy flowers and ability to adapt to a wide range of soils and climates (Munson, 1989; Tomkins et al., 2010), and are used as Traditional Chinese Medicine (Zhu et al., 2008; Ma et al., 2010).

Hemerocallis production has often been limited by anthracnose disease (Jiang et al., 1993). Disease outbreaks can be severe with 100% of some ornamental *Hemerocallis* species being infected (Jiang et al., 1993). Six *Colletotrichum* species have previously been reported as causal agent of anthracnose of *Hemerocallis* including *C. dematium* (Pers.) Grove on *Hemerocallis* sp. in the United States (Farr & Rossman, 2011), *C. gloeosporioides* (Penz.) Penz. & Sacc. on *H. citrina* in China (Gu et al., 2007), *C. lilioctonum* Ferraris on *H. fulva* var. *kwanso* Regel in China (Jiang et al., 1993; Farr & Rossman, 2011), *C. lilii* Plakidas ex Boerema & Hamers on *Hemerocallis* sp. in the United States (Farr & Rossman, 2011), *C. spaethianum* (Allesch.) Damm, P.F. Cannon, & Crous, on *Hemerocallis* sp. in New Zealand, and *Colletotrichum* sp. (CBS 125338) on *H. fulva* in Canada (Damm et al., 2009). There is, however, little knowledge concerning the *Colletotrichum* species associated with *Hemerocallis* in China. The objective of this paper was to characterize *Colletotrichum* species associated with these plants in China based on morphology and multilocus DNA sequence data.

MATERIALS AND METHODS

Isolation of *Colletotrichum*

Dead leaves and stalks of *Hemerocallis citrina*, *H. fulva*, and *H. fulva* var. *kwanso* with anthracnose lesions were collected in Guizhou, Guangxi, and Liaoning provinces in China from 2008 to 2011 (Table 1). Single-spore isolates were obtained using the procedure described by Choi et al. (1999) and Chomnunti et al. (2011). Pure cultures were stored at 4°C on PDA slants. Isolates are deposited in Guizhou Academy of Agricultural Sciences, China, and the China General Microbiological Culture Collection Center (CGMCC).

Morphological and cultural characterization

Starter cultures were prepared by growing each isolate on PDA at 25°C in darkness for five days. Five replicate cultures of each isolate were prepared by aseptically cutting disks from the actively growing edge of the starter culture using a sterile cork borer. Each plug was placed onto PDA plates (90 mm × 15 mm) and grown in alternating light and dark at 25°C (Sutton, 1980). To induce sporulation, plugs of actively growing mycelium were placed on to the surface

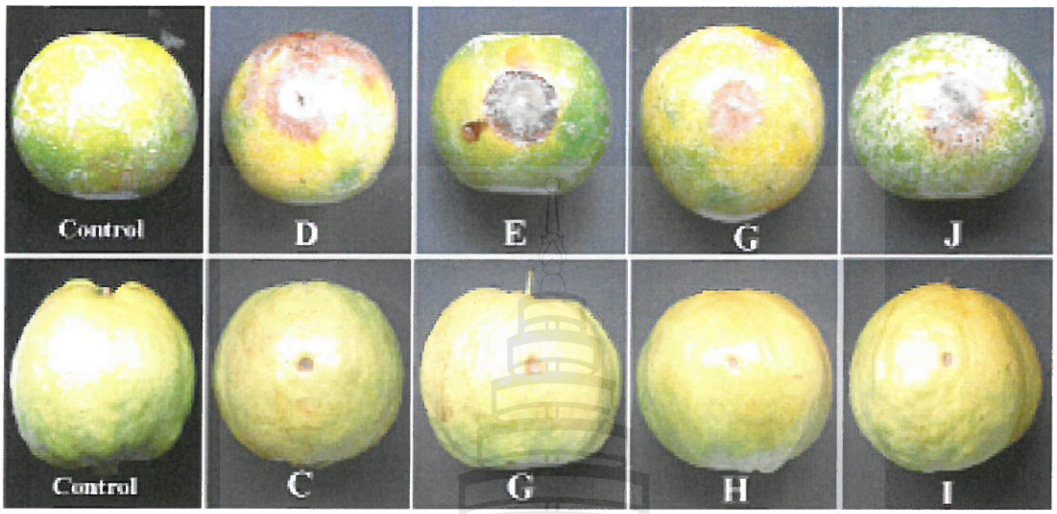

Fig. 5 – Anthracnose symptoms on selected orange (i.e. D, E, G, J and control) and guava (i.e. C, G, H, I and control) 7 days after inoculation: **D** *C. fructicola* from coffee berries; **E** *C. fructicola* from papaya fruit; **G** *C. siamense* from coffee berries; **J** *C. simmondsii* from papaya fruit; **C** *C. cordylinicola* from rose apple fruit; **G** *C. siamense* from coffee berries; **H** *C. siamense* from chili fruit; **I** *C. simmondsii* from papaya fruit.

guava, mango, papaya, rose apple and strawberry (Phoulivong et al. 2010a, Table 2).

In Table 1 we list the species used in this study and their potential to infect various hosts, where species were identified based on molecular data. Strains of *Colletotrichum asianum* infected chili, mango and rose apple host and strains of *C. fructicola* infected chili, citrus, rose apple, and papaya. *Colletotrichum cordylinicola* was specific to *Cordyline fruticosa* leaves. It is therefore apparent that *C. asianum*, *C. fructicola*, *C. siamense* and *C. simmondsii* have wide host ranges, while *C. cordylinicola* has a narrow host range. This is important for understanding the ability of *Colletotrichum* species to infect different hosts (Stankova et al. 2011).

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TABLE 2 - *Colletotrichum* species known from *Hemerocallis* spp.

Species	Host	Country	Strain	Reference
Colletotrichum dematium	*Hemerocallis* sp.	Zimbabwe	Unknown	Farr & Rossman (2011)
C. gloeosporioides/	*H. citrina*	China	Unknown	Gu et al. (2007)
Glomerella cingulata		China	CDLG1	This study
		Brunei Darussalam	Unknown	Farr & Rossman (2011)'
	H. fulva	China	CDLG4	This study
C. hemerocallidis	*H. fulva* var. *kwanso*	China	CDLG5, CDLN6	This study
	H. fulva	China	CDLN7	This study
	H. fulva	Canada	CBS 105338*	Damm et al. (2009)
C. liliacearum"	*Hemerocallis* sp.,	USA	Unknown	Farr & Rossman (2011)
	H. fulva var. *kwanso*	China	Unknown	Jiang et al. (1993)
C. lilii	*Hemerocallis* sp.	USA	Unknown	Farr & Rossman (2011)
C. liriopes	*H. fulva*	China	CDLG3	This study
C. spaethianum	*H. citrina*	New Zealand	CBS 101631"	Damm et al. (2009)
		China	CDLL1, CDLL2	This study
	H. fulva	China	CDLG2	This study

Note: *, a synonym of *Colletotrichum spaethianum* according to Damm et al. (2009); ", previously reported as *C. capsici* (CBS 101631), *, cited as *Colletotrichum* sp. 2 by Damm et al. (2009)

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Colletotrichum species associated with cultivated citrus in China

F. Huang · G. Q. Chen · X. Hou · Y. S. Fu · L. Cai · K. D. Hyde · H. Y. Li

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Abstract There have been considerable advances in the understanding of species concepts in the genus *Colletotrichum*. This has lead to the need to carry out fresh surveys of *Colletotrichum* species associated with important hosts. *Colletotrichum* species are associated with *Citrus* plants as saprobes, important pre-harvest and post-harvest pathogens, as well as endophytes. In this study, a total of 312 *Colletotrichum* strains were isolated from leaves, shoots and fruits of cultivated *Citrus* and *Fortunella* species with or without disease symptoms across the main citrus production areas in China. The morphology of all strains were studied and multilocus (ACT, TUB2, CAL, GAPDH, GS, ITS) phylogeny established. Strains were from four important species complexes of *Colletotrichum*, namely *C. gloeosporioides* species complex, *C. boninense* species complex, *C. acutatum* species complex and a final group including *C. truncatum*, which was rare on *Citrus* species. The species belonging to the *C. gloeosporioides* species complex comprised *C. gloeosporioides* and *C. fructicola*, the *C. boninense* complex comprised *C. karsii* and a new species *C. citricola* and the *C. acutatum* complex included a new species, *C. cori*. The ability of strains to cause anthracnose on citrus fruits was tested by inoculation and strains of *Colletotrichum gloeosporioides*, *C. fructicola* and *C. truncatum* were pathogenic.

Keywords Citrus industry · Citrus diseases · Anthracnose · *Colletotrichum gloeosporioides* · *Colletotrichum acutatum* · *Colletotrichum boninense* · Morphology · Phylogenetic analysis

Introduction

Colletotrichum is among the most economically important genera of plant pathogenic fungi worldwide (Sutton 1992; Cai et al. 2009; Phoulivong 2011). Many species of *Colletotrichum* cause diseases of a wide range of important crops commonly known as anthracnose (Sutton 1992; Hyde et al. 2009a). In addition, many *Colletotrichum* species are latent plant pathogens, species essentially being endophytes, epiphytes or saprobes, switching to a pathogenic lifestyle when host plants are stressed or in postharvest storage (Hyde et al. 2009b).

The history of the naming of *Colletotrichum* species has recently been reviewed in several important papers (Cannon et al. 2008; Hyde et al. 2009a; Weir et al. 2012) and recent protocols for the identification of new species were outlined in Cai et al. (2009). Following adoption of these polyphasic protocols for studying the genus *Colletotrichum*, especially the use of multi-gene phylogenetic analysis, the classification and species concepts in *Colletotrichum* changed significantly (Cai et al. 2009; Cannon et al. 2012; Damm et al. 2012a, b; Weir et al. 2012). A systematic study of nearly all acknowledged species, revealed at least nine clades in this genus (Cannon et al. 2012); many species previously known as a single species proved to be polyphyletic taxa (Cannon et al. 2012). *Colletotrichum gloeosporioides* (Cannon et al. 2008; Phoulivong et al. 2010b; Weir et al. 2012), *C.*

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F. Huang · G. Q. Chen · X. Hou · Y. S. Fu · H. Y. Li (✉)
Institute of Biotechnology, Zhejiang University, Hangzhou 310058, China
e-mail: hyli@zju.edu.cn

L. Cai
State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China

K. D. Hyde
Institute of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand

K. D. Hyde
School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

species cause *Citrus* leaf anthracnose. *Colletotrichum citricola* is a new species isolated from leaf anthracnose in the northwestern *Citrus* cultivation areas of China. *Colletotrichum truncatum* may also be a potential pathogen of *Citrus* species as shown in the pathogenicity tests. This species occurs in herbaceous plants (Damm et al. 2009), but this is the first report on *Citrus* spp.. *Colletotrichum citri*, a new species with fusiform conidia in the *C. acutatum* species complex (Damm et al. 2012a), was closest to *C. nymphaeae* (Van der Aa 1978).

Colletotrichum comprises species that can infect several host genera, such as *C. siamense* (Prihastuti et al. 2009; Yang et al. 2009; Wikee et al. 2011). On the other hand, a single host can harbor several species of *Colletotrichum* (Peng et al. 2012; Peng et al. 2013). Some species of *Colletotrichum*, however, appear to be specific to a single host species or genus (Prihastuti et al. 2010; Liu et al. 2011; Su et al. 2011)

Colletotrichum gloeosporioides was previously thought to be a common pathogen of numerous crops and most tropical fruits (Sutton 1992; Cannon et al. 2008). However, epitypification of this species by Cannon et al. (2008) has allowed for accurate identification of the taxon using analysis of multi-locus molecular data. Subsequent studies by Phoulivong et al. (2010a) have shown that *C. gloeosporioides* is not the common tropical pathogen as once believed (Phoulivong et al. 2010a). Since multi-locus analysis of *Colletotrichum* disease showed this species had been common on *Citrus* where it causes anthracnose, but rare on most other hosts (Prihastuti et al. 2009; Yang et al. 2009; Wikee et al. 2011; Weir et al. 2012; Peng et al. 2013).

In this study we have determined the species of *Colletotrichum* that cause disease of *Citrus* in the main growing areas of China. The most important causal agent is *C. gloeosporioides*. *C. acutatum* has not been found in China, probably because we did not survey in flowering season, and the collected strains were from the infected petals. In this study pathogenicity testing was only carried out on mature *Citrus* fruits by wound inoculation. The virulence and potential threat of these species to cause disease of leaves, shoots and petals needs to be clarified (Ko Ko et al. 2011a; Ko Ko et al. 2011b).

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Five *Colletotrichum* species are responsible for mango anthracnose in northeastern Brazil

Nelson B. Lima · Marcus Vinicius de A. Batista · Marcos A. De Morais Jr · Maria A. G. Barbosa · Sami J. Michereff · Kevin D. Hyde · Marcos P. S. Câmara

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Abstract *Colletotrichum* species are the most important and widespread form of decay affecting mango fruit worldwide. In this study, *Colletotrichum* species associated with fruit anthracnose isolated from mango in northeastern Brazil were subject to molecular and morphological analyses. The partial sequences of the glyceraldehyde-3-phosphate dehydrogenase gene of 143 *Colletotrichum* isolates was amplified, as an initial measure of genetic diversity. A subset of 47 isolates, selected to represent the range of genetic diversity and geographic origin, were further sequenced using the partial actin, β-tubulin, calmodulin, glutamine synthetase genes and rDNA-ITS region. The multilocus sequence analysis, together with a critical examination of the phenotypic characters, revealed four previously described species (*Colletotrichum asianum*, *Colletotrichum fructicola*, *Colletotrichum siamense* and *Colletotrichum karstii*) and one new species. The new species is introduced as *Colletotrichum alienum* and formally described, illustrated and compared with similar taxa. Only *C. asianum* and *C. karstii* have previously been reported from mango, while the other species represent the first report associated with the mango fruits worldwide. All species are reported for the first time associated with the mango fruits in Brazil.

Keywords Multilocus phylogeny · Morphology · New species · Plant disease

Introduction

The mango (*Mangifera indica* L.) is an important fruit crop in Brazil and other tropical and subtropical countries of the world (Evans and Mendoza 2009). In Brazil, the main areas of cultivation are in the northeastern region and in these areas are mainly produced for export. In 2010, Brazil exported 124,694 tons of mangoes worth approximately US$ 119,929 million (Anuário Brasileiro de Fruticultura 2012). Mango is affected by a number of diseases at all stages of its development, from seedlings in the nursery, to the fruits in storage or transit (Ploetz 2003; Prakash 2004). Anthracnose, caused by species of *Colletotrichum*, is the most important disease of mango in Brazil; the disease limits productivity and reduces fruit quality, thus directly affecting fruit export (Santos Filho and Matos 2003). In India, losses due to anthracnose have been estimated to be 2–39 % (Prakash 2004).

The genus *Colletotrichum* includes a number of plant pathogens of major importance, causing diseases of a wide variety of woody and herbaceous plants (Hyde et al. 2009; Wikee et al. 2011; Cannon et al. 2012). Identification of *Colletotrichum* species was previously based on morphology and to a lesser extend host association (Hyde et al. 2009). A polyphasic approach, using combined sequence analysis of multiple loci, coupled with morphological data, is now recommended for accurate species identification in the genus (Cai et al. 2009, 2011). Ko Ko et al. (2011) noted the need to resurvey plant

N. B. Lima · S. J. Michereff · M. P. S. Câmara (✉)
Departamento de Agronomia, Universidade Federal Rural de Pernambuco, 52171-900 Recife, Brazil
e-mail: mpsc@depa.ufrpe.br

M. V. de A. Batista · M. A. De Morais Jr
Departamento de Genética, Universidade Federal de Pernambuco, 50732-970 Recife, Brazil

M. A. G. Barbosa
Embrapa Semiárido, 56302-970 Petrolina, Brazil

K. D. Hyde
Institute of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand

K. D. Hyde
School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

to mango fruits. Symptoms development may vary considerably with factors such as variety and condition of the fruit, humidity and temperature, and the concentration of inoculum (Simmonds 1965; Freeman et al. 1998). This result may not accurately reflect the true virulence potential of these species. Additional research should be conducted to determine the virulence potential of *Colletotrichum* species according to natural infections rather than artificial inoculations.

The *Colletotrichum* isolates from mango analyzed in this study showed high variability based on GPDH gene and the morphological characteristics. Five species were identified, with the majority of the species had more than one haplotype and a high number of morphotypes. The greater the genetic diversity of a population, greater evolutionary potential and hence the more likely it to adapt to changing environmental conditions (McDonald and Linde 2002). That is, the greater the diversity, the greater the chance there is an individual that is adapted to certain restrictive condition covering the population. Accordingly, such information is relevant because it can assist in the implementation of disease control measures more effectively.

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Endophytic *Colletotrichum* from tropical grasses with a new species *C. endophytica*

Dimuthu S. Manamgoda · Dhanushka Udayanga · Lei Cai · Ekachai Chukeatirote · Kevin D. Hyde

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Abstract Endophytic fungi are a diverse and important group of microorganisms. We investigated the occurrence of *Colletotrichum* species as endophytes in two common tropical grass species, *Pennisetum purpureum* (dwarf napier) and *Cymbopogon citratus* (lemon grass) in Thailand. Combined phylogenetic analysis of ITS, partial sequences of actin (ACT), calmodulin (CAL) and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) gene regions and morphology were used to characterize the species. This is the first report of an association as endophytes of *Colletotrichum fructicola*, *C. tropicale* and *C. siamense* with *Pennisetum purpureum*, and *C. fructicola* and *C. siamense* with *Cymbopogon citratus*. *Colletotrichum endophytica* sp. nov. associated with *Pennisetum purpureum*, is introduced based on multi-locus phylogenetic analysis with descriptions and illustrations. The potential hyperdiversity of the endophytic *Colletotrichum* species associated with tropical grasses is discussed with an emphasis on future research.

Keywords Phylogeny · Poaceae · Taxonomy · *Pennisetum purpureum* · *Cymbopogon citratus*

Introduction

Colletotrichum is an important pathogenic genus causing anthracnose of various plant hosts including grasses and cereals worldwide (Crouch et al. 2009a, b; Prihastuti et al. 2010; Hyde et al. 2009a, b). *Colletotrichum* species have been recorded as endophytes in almost all major groups of angiosperms (Rivera-Orduña et al. 2011; Holstetter et al. 2012; Tadych et al. 2012), conifers (Daman et al. 2012; Cannon et al. 2012), ferns (McKenzie et al. 2009) and lichens (Petrini et al. 1990). The genus has been recorded in association with more than 42 genera in the family *Poaceae* (Crouch and Beirn 2009). Most graminicolous *Colletotrichum* species generally produce falcate conidia (Sutton 1980; Shenoy et al. 2007; Crouch et al. 2009a). Some exceptional cases of *Colletotrichum* species producing elliptic conidia have been reported as pathogens on *Sorghum* sp. and rye grass (*Lolium perenne* L.) (Neill 1940; Mathur et al. 2002). Molecular data are essential for the accurate identification of the species in *Colletotrichum* and the current number of accepted species supported with molecular data is likely to rise with future studies (Hyde et al. 2009a; Cai et al. 2011a, b; Weir et al. 2012; Cannon et al. 2012). *Colletotrichum graminicola* (Ces.) G.W. Wilson and *C. sublineola* Henn. ex Sacc. & Trotter are important graminicolous species responsible for leaf anthracnose disease of *Zea mays* L. (Sutton 1980) and *Colletotrichum graminicola* has been used as a model organism for studying genetics and pathogenicity (Crouch and Beirn 2009).

Several endophytic species of *Colletotrichum* have been isolated from a single host in recent studies. For example, 17 different *Colletotrichum* species, including seven new species have been reported from *Blattia ochracea* Schltr. (Chinese Butterfly Hardy ground orchid) (Tao et al. 2013). In another study, 39 *Colletotrichum* isolates of *C. gloeosporioides* (Penz.) Penz. & Sacc. *sensu lato*, *C. boninense* Moriwaki, and *C. simmondsii* R.G. Shivas & Y.P. Tan have been isolated from *Schinus terebinthifolius* Raddi (Brazilian pepper tree) (Lima et al. 2012). There have been several studies on grass endophytes (Sánchez Márquez et al. 2007, 2010; Ghimire et al. 2011), but the diversity of *Colletotrichum* species found as endophytes is still poorly understood. Many endophytic

D. S. Manamgoda · D. Udayanga · L. Cai (✉)
State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, No 3 1st West Beichen Road, Chaoyang District, Beijing 100101, People's Republic of China
e-mail: mcaules@gmail.com

D. S. Manamgoda · D. Udayanga · E. Chukeatirote · K. D. Hyde
Institute of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand

D. S. Manamgoda · D. Udayanga · E. Chukeatirote · K. D. Hyde
School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

to cause disease of grass species. A recent study focusing on the relationship between airborne spores and allergen exposure found that air borne spores of fungal genera such as *Cladosporium* and *Fusarium* are common grass endophytes (Vázquez de Aldana et al. 2013). Endophytic *Colletotrichum* species commonly found on grasses may also become pathogens or saprobes and be responsible for airborne allergies in humans. The endophytic isolates from cacao in Panama were thought to comprise part of the background endophytic community in the local forest ecosystems (Rojas et al. 2010). The occurrence of multiple species from the two tropical grass species in this study shows the potential hyperdiversity of endophytic *Colletotrichum*. Further investigations are required to clarify the ecological relationships of the pathogenic and endophytic *Colletotrichum* species on crops, and tropical wild grasses.

The presence of *Colletotrichum* species as endophytes adds an extra dimension to the understanding of host specificity in the genus (Rojas et al. 2010; Cannon et al. 2012). Common temperate grass associated *Colletotrichum* species are falcate-spored *C. anonypoli* Crouch, B.B. Clarke, J.F. White & B.L. Hillman, *C. caudatum* (Peck ex Sacc.) Peck, *C. cereale* Manns, *C. falcatum* Went, *C. graminicola*, *C. hanaui* Crouch, B.B. Clarke, J.F. White & B.L. Hillman, *C. jacksonii* Crouch, B.B. Clarke, J.F. White & B.L. Hillman, *C. nicholsonii* Crouch, B.B. Clarke, J.F. White & B.L. Hillman, *C. purpurii* Crouch, B.B. Clarke, J.F. White & B.L. Hillman, *C. sublineolum* Henn. ex Sacc. & Trotter and *C. sutchuenchinae* J.A. Crouch & Tomaso-Peterson (Crouch et al. 2009b; Crouch and Tomaso-Peterson 2012). There are a few *Colletotrichum* species reported from grasses as endophytes. For example, *C. cereale*, *C. graminicola* and *C. phyllachoroides* have previously been reported from the cool season grasses, *Panicum virgatum* and *Sasaella fruticosa* respectively (Fisher and Petrini 1987; Crouch et al. 2009a; b; Ghimire et al. 2011). An unidentified *Colletotrichum* species has been isolated from *Dactylis glomerata* and *Holcus lanatus* (Sánchez Márquez et al. 2007; 2010). In this study, *C. endophytica*, *C. fructicola*, *C. siamense* and *C. tropicale* were isolated from *Cymbopogon citratus* and *Pennisetum purpureum* in Thailand. This is the first report of latter four species associated with tropical grasses. Future studies are needed on endophytic *Colletotrichum* species on a wide range of grass hosts and locations in the tropics to assess the occurrence and host range of species in grasses and to reveal their ecology and biology related to host distribution and life modes.

In this study combined ACT, CAL, GAPDH, and ITS analysis enabled us to identify most of the isolates to species level, with the exception of two isolates. A large number of type sequences are available in GenBank for taxa in the *C. gloeosporioides* species complex, and therefore we could resolve most species accurately and with confidence using combined ACT, CAL, GAPDH, and ITS gene sequence data. According to Weir et al. (2012), ITS, GAPDH, CAL and ACT is unable to distinguish *C. aeschynomenes* B.S. Weir & P.R. Johnst. and *C. tropicale* from all other species. Recent studies have shown that apn2/mat1/2 IGS (intergenic spacer bridging the DNA lyase and mating type locus) provides greater resolution in the *C. gloeosporioides* species complex. Combined analysis of ITS, *apn2*, *tub2* and *ApMat* gene-markers were reported to give a better resolution of cryptic *C. gloeosporioides* species (Doyle et al. 2013; Sharma et al. 2013). *Colletotrichum siamense sensu lato* also appears to be a species complex and was the most common grass endophyte isolated in this study. Combined analysis of the apn2/mat1/2 gene has revealed several different lineages within *Colletotrichum siamense sensu lato*, including *C. hymenocallidis*, *C. jasmini-sambac*, *C. siamense sensu stricto*, *C. melanocaulon* and three undescribed clades which are probably new species (Doyle et al. 2013; Sharma et al. 2013; Udayanga et al. 2013). Generally, in future studies of *Colletotrichum* endophytes, we expect that most of species can be accurately identified using available molecular markers and the type sequence data available in public databases.

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A distributed third-party annotation effort of fungal ITS sequences from plant pathogenic fungi

R. Henrik Nilsson[1], Kevin D. Hyde[x], S.A. Alias[x], Artur Alves[x], Cajsa-Lisa Andersson[x], Mohammad Bahram[x], Anna Berlin[x], Sara Branco[x], Ekachai Chukeatirote[x], Gerald Cobian[x], Asha Dissanayake[x], Anna Friberg[x], Martin Hartmann[x], Beatrice Henricot[x], Fredrik Heyman[x], Ottmar Holdenrieder[x], Ruvishika Jayawardena[x], Håvard Kauserud[x], Tomasz Kulik[x], Daniel Lindner[x], Jian-Kui Liu[x], Sajeewa Maharachchikumbura[x], Dimuthu Manamgoda[x], Svante Martinsson[x], Maria Alice Neves[x], Nookia Nookiaja[x], Julia Pawłowska[x], Valentin Queloz[x], Martin Ryberg[x], Filipe de Sousa[x], Emil Stefańczyk[x], Mariusz Tadych[x], Susumu Takamatsu[x], Qing Tian[x], Dhanushka Udayanga[x], Martin Untersehr[x], Yong Wang[x], Zheng Wang[x], Saowanee Wikee[x], Jy Yan[x], Urmas Kõljalg[x], Kessy Abarenkov[x,*]

[1] Department of Biological and Environmental Sciences, University of Gothenburg, Box 461, 405 30 Gothenburg, Sweden

[x] Department of Organismal Biology, Uppsala University, Norbyvägen 18D, 75236 Uppsala, Sweden

[x] Institute of Ecology and Earth Sciences, University of Tartu, Tartu, Estonia

[x] Natural History Museum, University of Tartu, Tartu, Estonia

[*] To whom correspondence should be addressed. E-mail: kessy@ut.ee

Co-authors 3-X contributed equally to the project and are given in alphabetical order.

Abstract

Plant pathogenic fungi form a large and very diverse assemblage of eukaryotes with substantial repercussion on human endeavour. These fungi often have a poorly understood life cycle, and the lack or sparsity of observable, discriminatory morphological structures and

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Full paper

Colletotrichum species on grape in Guizhou and Yunnan provinces, China

Li-Juan Peng [a,b], Tao Sun [c], You-Lian Yang [d], Lei Cai [e], Kevin D. Hyde [f], Ali H. Bahkali [g], Zuo-Yi Liu [h,i,*]

[a] The Provincial Key Laboratory for Agricultural Pest Management of Mountainous Region, Guizhou University, Guiyang, Guizhou 550025, PR China

[b] Agricultural College of Guizhou University, Guiyang, Guizhou 550025, PR China

[c] Chongqing Entry-Exit Inspection and Quarantine Bureau, Chongqing 400020, PR China

[d] Department of Biology and Geography, Liupanshui Normal University, Shuicheng, Guizhou 553006, PR China

[e] Key Laboratory of Systematic Mycology and Lichenology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100190, PR China

[f] Institute of Excellence in Fungal Research, School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

[g] King Saud University, College of Science, Botany and Microbiology Department, Riyadh, Saudi Arabia

[h] Guizhou Academy of Agricultural Sciences, Guiyang, Guizhou 550006, PR China

[i] Guizhou Key Laboratory of Agricultural Biotechnology, Guiyang, Guizhou 550006, PR China

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ABSTRACT

Twenty six strains representing three species of *Colletotrichum* were isolated from leaf and fruit lesions of vitaceous plants in Guizhou and Yunnan provinces, China. The strains were characterized by morphology and phylogenetic analyses of actin, β-tubulin, calmodulin, glutamine synthetase, glyceraldehyde-3-phosphate dehydrogenase and rDNA internal transcribed spacer gene sequences. The combined dataset showed that 20 of 26 strains represented a novel species, the rest being *Colletotrichum fructicola* (four strains) and *Colletotrichum gloeosporioides* (two strains). The new species is described herein as *Colletotrichum viniferum*. Its conidia, compared with similar *Colletotrichum* species, are cylindrical and 12–16 μm long. Based on pathogenicity tests, *C. viniferum* caused leaf spots and anthracnose of table grape but was not host-specific.

1. Introduction

Grape (*Vitis* spp.) is one of the most widely planted fruit trees worldwide (Sung et al. 2008) and the table grape (*Vitis vinifera*) is one of the most important grapevines in China. In recent years, there has been a rapid increase in areas planted with *V. vinifera* throughout China. In 2005, the total area with grape vines was 421,000 hm^2 with a yield of 817,600 tons. Due to the warm and rainy climate in southern China, yield losses of up to 50% have been reported due to disease and insects (Liu 2005). Ripe rot of grape caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. and *Colletotrichum acutatum* J.H. Simmonds ex J.H. Simmonds were considered to be serious diseases occurring in most vineyards, and caused big loss and deterioration of grape vines (Sung et al. 2008).

* Corresponding author. Guizhou Academy of Agricultural Sciences, Guiyang, Guizhou 550006, PR China.
E-mail address: liuzuoyi@yahoo.com.cn (Z.-Y. Liu).

4. Discussion

Grape ripe rot is a disease effecting grapes at or near harvest. The fruits infected turn dark brown and then on the skin produced pink or orange spore masses. As infected fruits mature, tiny black fruiting bodies (acervuli) develop within the lesion in a circular arrangement (Figs. 2 and 3). The symptoms of disease on grape fruits caused by all three species isolated in this study could not be distinguished.

Several *Colletotrichum* species have been reported from *V. vinifera* (grape) including *C. acutatum* (Shirasishi et al. 2007; Whitelaw-Weckert et al. 2007; Greer et al. 2011), *Colletotrichum crassipes* (Speg.) Arx (Hyde et al. 2009), *C. gloeosporioides* (Sung et al. 2008; Greer et al. 2011) and *C. vitis* Istv. (Saccardo 1913) and *Colletotrichum* sp. (Gonzalez and Tello 2011). The morphological data of *Colletotrichum* species on grape are listed in Table 5. *C. crassipes* originally isolated from fruit of *V. vinifera* (grape) from Conegliano, Italy (Hyde et al. 2009) has wider, longer conidia and deeply lobed appressoria, which is different from *C. gloeosporioides* (Sutton 1992). Conidia in *C. crassipes* are 14–28 × 5–7 μm and larger than those of *C. viniferum* (12–16 × 4.5–6 μm). The average length of conidia in *C. viniferum* ($\bar{x}$ = 13.79 ± 0.98 μm) is shorter than that of *C. gloeosporioides* ($\bar{x}$ = 14.4 μm). Conidia in *C. vitis* are 21–25 × 2.5 μm, smaller than those of *C viniferum* although the former species is hardly known. The name of *C. vitis* was not on the list of 66 *Colletotrichum* names in common use (Hyde et al. 2009).

Rojas et al. (2010) described two new species, *Colletotrichum tropicale* Rojas, Rehner & Samuels and *Colletotrichum ignotum* Rojas, Rehner & Samuels, that were frequent asymptomatic associates of cacao and other neotropical plant species, and epitypified *Colletotrichum theobromicola* Delacr. & Bull. Soc., which was associated with foliar and fruit anthracnose lesions of cacao in Panama among 77 *C. gloeosporioides* strains and different from *C. viniferum* in phylogeny with ITS gene (Fig. 2S). The gene loci that Rojas et al. (2010) used were different from those used in this study. The ITS gene tree shows *C. viniferum*, the new species we identified is different from *C. ignotum*, *C. theobromicola* and *C. tropicale*.

Among the 26 strains isolated from vitaceous plants, five strains were isolated from leaf lesions with one strain of *C. gloeosporioides* from a leaf lesion of *Ampelopsis* sp., two of *C. fructicola* from the same lesions and the other two of *C. viniferum*, one from an *Ampelopsis* sp. leaf lesion and the other from a *Cayratia japonica* (Japanese cayratia herb) leaf lesion. The remaining 21 strains, eighteen of *C. viniferum*, two of *C. fructicola* and one of *C. gloeosporioides* were isolated from anthracnose lesions on *V. vinifera* (grape) fruits. This was the first report of *C. fructicola* from *Ampelopsis* sp. and *V. vinifera* (grape) that indicated the wide range host. *C. fructicola* had already been isolated from coffee berries in northern Thailand (Prihastuti et al. 2009) and fruits lesions of papaya and longan at Chiang Mai, Thailand (Phoulivong et al. 2010a). *C. gloeosporioides* was previously listed as infective of many fruits, especially in the tropics (Paull et al. 1997; Freeman et al. 1998; Afanador-Kafuri et al. 2003; Jeffries et al. 2004; Gangopadhyay and caused anthracnose of tropical fruits and is not a common pathogen in the tropics (Prihastuti et al. 2009; Phoulivong et al. 2010a; Rojas et al. 2010; Wilcox et al. 2011).

The main objectives of this study were to identify the species of *Colletotrichum* from Vitaceae in Guizhou and Yunnan provinces with combined morphological and molecular data. The ITS region is the most widely used region in fungi identification (Moriwaki et al. 2002; Jeewon et al. 2003; Phillips et al. 2007; Yang et al. 2009). The ITS support for nodes was low and the current ITS tree could not well-defined certain *Colletotrichum* species (Moriwaki et al. 2002; Yang et al. 2009), such as *C. crassipes* and *C. kahawae*, *Colletotrichum jasmini-sambac* and *C. tropicale*, *C. fructicola* and *C. theobromicola* as this paper revealed (Fig. 2S). While through multigene analyses, *C. fructicola*, *C. jasmini-sambac* and *C. kahawae* represented three clades with bootstrap value of 97%, 77% and 100% respectively (Fig. 1). The phylograms based on ITS and six genes showed that the isolates of *C. asianum*, *C. gloeosporioides*, *C. horii*, *C. musae*, *C. siamense*, *C. simmondsii* and *C. viniferum* were a monophyletic lineage in our study (Figs. 1 and 2S).

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The *ApMat* marker can resolve *Colletotrichum* species: a case study with *Mangifera indica*

Gunjan Sharma · Navinder Kumar · Bevan S. Weir · Kevin D. Hyde · Belle Damodara Shenoy

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Abstract Anthracnose disease caused by the *Colletotrichum gloeosporioides* species complex is a major problem worldwide. In this study, we investigated the phylogenetic diversity of 207 Indian *Colletotrichum* isolates, associated with symptomatic and asymptomatic tissues of mango, belonging to this species complex. Phylogenetic analyses were performed based on a 6-gene dataset (*act*, *cal*, *chs1*, *gapdh*, ITS and *tub2*), followed by *ApMat* sequence analysis. The *ApMat*-based phylogeny was found to be superior as it provided finer resolution in most of the species-level clades. Importantly, the *ApMat* marker identified seven lineages within *C. siamense sensu lato*, including *C. javanense-sambae*, *C. hymenocallidis*, *C. melanocaulon*, *C. siamense sensu stricto* and three undesignated, potentially novel lineages. In this study, *C. fragariae sensu stricto*, *C. fructicola*, *C. javanense-sambae*, *C. melanocaulon* and five undesignated, potentially novel lineages were found to be associated with mango tissues. There is a need to develop a consensus among mycologists as to which genes should be used to define and delimit a *Colletotrichum* species and in the mean time mycologists should voluntarily restrain from describing new species based on inadequate datasets.

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G. Sharma · N. Kumar
Microbial Type Culture Collection and Gene Bank, CSIR-Institute of Microbial Technology, Sector 39-A, Chandigarh 160036, India

B. S. Weir
Landcare Research, Private Bag 92170, Auckland, New Zealand

K. D. Hyde
Institute of Excellence in Fungal Research and School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

B. D. Shenoy (✉)
CSIR-National Institute of Oceanography, Dona Paula, Goa 403004, India
e-mail: shenoynio@gmail.com

Key words Anthracnose · Identification · Phylogeny · Polyphasic taxonomy · Secondary barcode · Systematics

Introduction

Mango (*Mangifera indica* L., Anacardiaceae) is native to India where it is an important fruit crop and is now grown in most tropical and subtropical regions (Sarkiyayi et al. 2013). India is the largest producer, consumer and exporter of mango, with approximately 40 % of the total world production (Ferrier et al. 2012). Production of mango in the fiscal year 2010–11 was 15.2 million tons (Kumar et al. 2011) with India exporting 0.06 million tons of fresh mangoes to more than 30 countries worldwide and earning USD 38.2 million as revenue in the fiscal year 2011–2012 (DCCIS Annual Report 2012).

The temperature range and high humid conditions of tropical regions are favourable for anthracnose disease development, previously reported to be caused by *Colletotrichum gloeosporioides sensu lato* (Waller 1992; Freeman et al. 1998; Kumar et al. 2007; Lasani et al. 2011). *Colletotrichum gloeosporioides sensu lato* has been reported from various tropical fruits as endophytes or pathogens and also as agents of postharvest fruit rots (Bailey and Jeger 1992; Freeman et al. 1998; Cai et al. 2009; Hyde et al. 2009a, b). Anthracnose disease mainly affects the aerial plant parts such as leaves, twigs, fruits and inflorescences, and causes post-harvest fruit rots, yield and revenue loss (Swamy 2012). In addition to economic losses, issues pertaining to biosecurity of *C. gloeosporioides sensu lato* are also important.

Mango anthracnose is characterized by the formation of dark brown spots on fruits and leaves (Chadha 2009). Farr and Rossman (2013) listed 1481 fungal taxa associated with mango worldwide, including *C. acutatum* J.H. Simmonds, *C. asianum* Prihast. L. et al., *C. capsici* (Syd. & P. Syd.) E.J. Butler & Bisby (= *C. truncatum* (Schwein.) Andrus & W.D. Moore), *C. floriniae* (Marcelino & Gouli) R.G. Shivas & Y.P.

representative isolates are capable of producing anthracnose symptoms on mango fruits (Fig. 5). The cross pathogenicity tests conducted using chilli fruits suggest that these isolates are not host-specific to *Mangifera indica*.

We have also gained an insight about host and geographical distribution of various species within the *C. gloeosporioides* species complex by extensive analysis involving 221 *ApMat* sequences (Fig. 4) as well as from other recent studies (Rojas et al. 2010; Silva et al. 2012a, b; Doyle et al. 2013). A few *Colletotrichum* species are restricted to a particular host, while others are limited to a particular geographical location. For example, among the major clades obtained in *ApMat* analysis: *C. hymenocallidis* is limited to China, *C. aotearoa* is limited to New Zealand; *C. theobromicola sensu stricto* is limited to Panama region; *C. fructivorum*, *C. nupharicola* and *C. rheiae* are limited to North America. Details about other minor clades recovered in the *ApMat* analysis are provided in Table 4. Generally, members of the *C. gloeosporioides* complex are known to possess a broad host range, with only a few exceptions such as *C. horii* (*Diospyros* sp.) and *C. musae* (*Musa* sp.) (Table 4).

Recognition of cryptic species in a species complex with the incorporation of gene sequence-data is not limited to the *C. gloeosporioides* species complex and other species complexes of *Colletotrichum*. Many phytopathogenic genera such as *Botryosphaeria* (Pavlic et al. 2009; Liu et al. 2012), *Diaporthe* (Udayanga et al. 2011, 2012), *Fusarium* (Gräfenhan et al. 2011; Summerell and Leslie 2011; Summerell et al. 2011), and *Mycosphaerella* (Schoch et al. 2009; Hunter et al. 2011) include cryptic species that could not be diagnosed based solely on classical morpho-taxonomic characters. This warrants updating of existing checklists and fungal databases with correct taxonomic information about phytopathogenic fungal taxa to facilitate accurate plant quarantine decisions (Cai et al. 2011). In conclusion, this study has revealed the rich fungal diversity associated with different mango varieties of India. Future studies are needed to reveal cryptic species of *Colletotrichum* associated with other tropical fruits and also native plants. Nevertheless, consensus among mycologists as to which genes or taxonomic characters should be used while describing a fungal species will play a significant role in satisfying the needs of taxonomic end-users.

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What are the common anthracnose pathogens of tropical fruits?

Dhanushka Udayanga · Dimuthu S. Manamgoda · Xingzhong Liu · Ekachai Chukeatirote · Kevin D. Hyde

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Abstract Species of *Colletotrichum* are associated with anthracnose of a wide range of host plants including cultivated and wild tropical fruits. The genetic and ecological diversity of species associated with wild fruits are poorly explored, as compared to those associated with pre and postharvest diseases of cultivated fruits. In the present study, isolates of *Colletotrichum* were obtained from commercially available cultivated fruits, wild fruits (from native trees in natural habitats) and a few herbaceous hosts collected in northern Thailand. These isolates were initially characterized based on analysis of complete sequences of nuclear ribosomal internal transcribed spacer (ITS), into the genetically defined species complexes of *Colletotrichum gloeosporioides*, *C. acutatum*, *C. boninense* and *C. truncatum*. The isolates were primarily identified in the *C. gloeosporioides* species complex, based on a strongly supported clade within the ITS gene tree and were further characterized using multi-gene phylogenetic analyses and morphology. Phylogenetic analyses of ITS, partial sequences of actin (ACT), calmodulin (CAL), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), glutamine synthetase (GS) and β-tubulin (TUB2) genetic markers were performed individually and in combination. *Colletotrichum gloeosporioides sensu stricto* was identified from lime (*Citrus aurantiifolia*) and rose apple (*Syzygium samarangense*). *Colletotrichum fructicola* was isolated from dragon fruit (*Hylocereus undatus*) and jujube (*Ziziphus* sp.). *Colletotrichum endophytica* was found only from an unknown wild fruit. We observed a considerable genetic and host diversity of species occurring on tropical fruits within the clade previously known as *Colletotrichum siamense sensu lato*. The clade consists of isolates identified as pre and postharvest pathogens on a wide range of fruits, including coffee (*Coffea arabica*), custard apple (*Annona reticulata*), *Cerbera* sp., figs (*Ficus racemosa*) mango (*Mangifera indica*), neem (*Azadirachta indica*) and papaya (*Carica papaya*) and was the dominant group of species among most wild fruits studied. With the exception of one isolate from banana, which grouped in the *C. siamense* clade, all the other isolates were identified as *Colletotrichum musae*. A new species, *Colletotrichum syzygicola*, associated with *Syzygium samarangense* in Thailand, is introduced with descriptions and illustrations. This study highlights the need to re-assess the evolutionary relationships of *Colletotrichum* species occurring on cultivated and wild fruits with emphasis on their ecology and cryptic diversification including sampling at regional and global scales.

Key words *Colletotrichum gloeosporioides* · Multi-gene phylogeny · Postharvest diseases · Quarantine · Systematics · Species complex · Tropical Asia · Wild fruits

D. Udayanga · D. S. Manamgoda · X. Liu (✉)
State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, No 3 1st West Beichen Road, Chaoyang District, Beijing 100101, People's Republic of China
e-mail: liuxzmicro@gmail.com

D. Udayanga · D. S. Manamgoda · E. Chukeatirote · K. D. Hyde
Institute of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand

D. Udayanga · D. S. Manamgoda · E. Chukeatirote · K. D. Hyde
School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

Introduction

Colletotrichum Corda, is an important plant pathogenic genus causing anthracnose of a wide range of fruits, vegetables, cereals, grasses and ornamental plants in tropical and temperate regions (Mills et al. 1992; Johnston and Jones 1997; Freeman et al. 2001; Chung et al. 2006; Yang et al. 2009; Rojas et al. 2010). Fruit production is mostly affected in both high value crops and wild fruits in natural habitats. However,

rheadae Ellis and Everh. from *Rheadia virginica* are recently epitypified based on fresh collections (Su et al. 2011; Doyle et al. 2013). Some authors have introduced novel species only when the taxonomic entity cannot be reliably linked to earlier names (Crouch and Tamaso-Peterson 2012; Lima et al. 2013; Peng et al. 2013). However, the description of novel taxa should be well supported by adequate molecular datasets and state- of- the-art analysis with the inclusion of frequent and abundant fresh collections (Hyde et al. 2013; Sharma et al. 2013).

Tropical Asia is an economically and physiographically rich region with a unique, hyperdiverse flora and fungi (Hyde 2003; Hawksworth 2004; Mueller and Schmit 2007; Karunarathna et al. 2012). Post-harvest management of fruit and vegetables in most developing countries in the Asian region is, however, far from satisfactory (Report of International Centre for Science and High Technology, United Nations Industrial Development Organization, 2012). Major constraints include inefficient handling and transportation and loss due to fungal and bacterial diseases due to lack of implementation of postharvest technologies (Adikaram 1986; Aidoo 1993; Choudhury 2006). Cultivated fruits in Southeast Asia suffer from great yield losses due to factors including fungal disease encouraged by favourable environmental factors (Li 1970; Yaacob and Subhadrabandhu 1995; Rolle 2006). Phytosanitary standards maintained by some developed countries limit the exports of fruits from developing countries which often impact on agricultural trade (Romberg and Roberts 2008). The species associated with the diseases of commercial fruits are critical to re- regulating movement of pathogens and establish effective disease control measures. Therefore, it is important to integrate the dynamic changes taking place in classification and nomenclature of pathogenic fungi with the broad applications in biosecurity, quarantine and disease control (Rossman and Palm-Hernández 2008; Cai et al. 2011; Zhang et al. 2013). Incorporation of molecular data derived from the isolates in regional and global sampling of *Colletotrichum* species, improves the knowledge of diversity, population structure, extent of host and geographic distribution.

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Diverse species of *Colletotrichum* associated with grapevine anthracnose in China

J-Ye Yan • M.M.R.S. Jayawardena • Yong Wang • Wei Zhang • Mei Liu • Hong Yan • Jin-Bao Huang • Zhong-Yue Wang • Jing-Jing Zhang • You-Liang Peng • Ali Bahkali • Kevin D. Hyde • Xing-Hong Li

Abstract Grapevine anthracnose is an important disease in the field, responsible for mild to severe yields losses in grape production, and is also an important post harvest disease. The disease was studied in vineyards in six provinces in China, and with 34 isolates was obtained from diseased grapes. Multi-gene (ACT, ITS, GAPDH, TUB2, SOD, CHS and AP2/MAT) analysis coupled with morphology showed that *Colletotrichum aenigma*, *C. hebeiense* sp. nov. and *C. viniferum* were associated with grapevine anthracnose in China. *Colletotrichum aenigma* is reported for the first time as associated with grapevine anthracnose. *Colletotrichum hebeiense* is a new species introduced here; it was associated with grapevine anthracnose in Hebei Province. Pathogenicity testing showed that all species can infect grapes causing anthracnose, however virulence of species and isolates showed great variation. Phylogenetic analysis showed that *C. viniferum* is a species complex and its species need to be resolved in the future.

Keywords: Grapevine anthracnose; *Colletotrichum viniferum*; *C. aenigma*; *C. hebeiense*

J.Y. Yan • M.M.R.S. Jayawardena • W. Zhang • M. Liu • H. Yan • J.B. Huang • J.J. Zhang • X.H. Li (Corresponding author)

Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, People's Republic of China

M.M.R.S. Jayawardena • Kevin D. Hyde (Corresponding author)

Institute of Excellence in Fungal Research, School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

Y. Wang

plant disease

First Report of Papaya Fruit Anthracnose Caused by Colletotrichum brevisporum in Brazil

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Keywords:	Fungi and oomycetes < Causal Agent, tree fruits < Fruit < Crop Type, Etiology < Subject Areas

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Principal Investigator(PI)

Name: ภาษาไทย: นายเควิน ดี ไฮด์

ภาษาอังกฤษ: **Dr. Kevin D Hyde**

Date of Birth: May 5, 1955

ID no: 761264856

Position: Associate Professor

Office Address School of Science, Mae Fah Luang University, 333 M. 1 T.Tasud, Muang District, Chiang Rai 57100

Phone: 0861148549 Fax.: 053 916776

E-mail Address: kdhyde1@gmail.com

Education Background

- Doctor of Science, University of Wales, 2001
  DISSERTATION: *Biodiversity and Biology of Tropical Microfungi*
- Doctor of Philosophy, University of Portsmouth, UK, 1987
  DISSERTATION: *Marine Mycology*
- Master of Science, University of Portsmouth, UK, 1981
  DISSERTATION: *Biodeterioration*
- Postgraduate Certificate of Education, Bristol University, UK, 1980
- Bacholer of Science, University of Wales, Cardiff, 1979 (Zoology)

Field of Specialization Skill

Mycology, Plant Pathology

- Project Title: Diversity, molecular taxonomy of nematophagous fungi and characterisation of microbial enzymes associated with nematode infection (awarded ****)

Principal Investigator: Dr. KD Hyde, Principal investigator.

Investigator: Dr. R Jeewon, Co-Investigator

Project Status: Completed

Project Objective: The main objectives of this project are:

- To survey nematophagous fungi in Hong Kong.

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

- To extract and purify extracellular enzymes from virulent microbes involved innematode infection.

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

- Evaluate the expressed proteins in nematode biocontrol. Resulted in 3 SCI publications

- Project Title: Evolutionary relationships of loculoascomycetes (fungi) based on a phylogenetic Approach (awarded *****)

Principal Investigator: Dr. KD Hyde.

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

Project Status: Completed

Project Objective:

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

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

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

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

- Resulted in 5 SCI publications

- Project Title: Molecular evolution of genes for phylogenetic analysis of the class Sordariomycetes (Mycota) – (awarded ****)

Principal Investigator: Dr. KD Hyde.

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

Project Status: Completed.

Project Objective:

This resulted in 3 SCI publications

Selected publications:

1. **Hyde, K.D.** (2003). Mycology and its future in the Asia region. Fungal Diversity 13: 59-68. Impact Factor = 3.59 (Journal Citation Reports 2007).

2. Whitton, S.R., McKenzie, E.H.C. and **Hyde, K.D.** (2003). Microfungi on the Pandanaceae: Zygosporium, a review of the genus and two new species. Fungal Diversity 12: 207-222. Impact Factor = 3.59 (Journal Citation Reports 2007).

3. Tsui, C.K.M., Goh, T.K., and **Hyde, K.D.** (2003). Reflections on the genus Vanakripa, with a description of V. ellipsoidea sp. nov. Mycologia 95: 124-127. Impact Factor = 1.808 (Journal Citation Reports 2007).

4. Tang, A., **Hyde, K.D.** and Corlett, R.T.C. (2003). Diversity of fungi on wild fruits in Hong Kong. Fungal Diversity 14: 165-185. Impact Factor = 3.59 (Journal Citation Reports 2007).

5. Paulus, B., Gadek, P. and **Hyde, K.D.** (2003). Estimation of microfungal diversity in tropical rain forest leaf litter using particle filtration: the effects of leaf storage and surface treatment. Mycological Research 107: 748-756. Impact Factor = 1.86 (Journal Citation Reports 2007).

6. Pointing, S.B., Parungao, M.M. and **Hyde K.D.** (2003). Production of wood-decay enzymes, mass loss and lignin solubilization in wood by tropical Xylariaceae. Mycological Research 107: 231-235. Impact Factor = 1.86 (Journal Citation Reports 2007).

7. Guo, L.D., Huang, G.R., Wang, Y., He, W.H., Zheng, W.H. and **Hyde, K.D.** (2003). Molecular identification of white morphotype strains of endophytic fungi from Pinus tabulaeformis. Mycological Research 107: 680-688. Impact Factor = 1.86 (Journal Citation Reports 2007).

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11. Pinnoi, A., McKenzie, E.H.C., Jones, E.B.G. and **Hyde, K.D.** (2003). Palm fungi from Thailand. Custingophora undulatistipes sp. nov. and Vanakripa minutellipsoidea sp. nov. Nova Hedwigia 77: 213-219. Impact Factor = 0.77 (Journal Citation Reports 2007).

12. Tsui, C.K.M., Hodgkiss, I.J. and **Hyde, K.D.** (2003). Three new species of Aquaticola (Ascomycetes) from tropical freshwater habitats. Nova Hedwigia 77: 161-168. Impact Factor = 0.77 (Journal Citation Reports 2007).

13. Lam, D.M., Lumyong, S., **Hyde, K.D.** and Jeewon, J. (2004). Emarcea castanopsicola gen. et sp. nov. from Thailand, a new xylariaceous taxon based on morphology and DNA sequences. Studies in Mycology 50: 253-260. Impact Factor = 5.9 (Journal Citation Reports 2007).

14. 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 marine ascomycetes and their anamorphs. Fungal Diversity 15: 1-14. Impact Factor = 3.59 (Journal Citation Reports 2007).

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17. Luo, J., Yin, J.F., Cai, L., Zhang, K. and **Hyde, K.D.** (2004). Freshwater fungi in Lake Dianchi, a heavily polluted lake in Yunnan, China. Fungal Diversity 16: 93-112. Impact Factor = 3.59 (Journal Citation Reports 2007).

18. Lee, S.W., Ho, W.H. and **Hyde, K.D.** (2004). Ultrastructure of the asci and ascospores of Torrentispora fibrosa. Fungal Diversity 16: 87-91. Impact Factor = 3.59 (Journal Citation Reports 2007).

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20. Photita, W., Lumyong. S., Lumyong, P., McKenzie, E.H.C. and **Hyde, K.D.** (2004). Are some endophytes of Musa acuminata latent pathogens? Fungal Diversity 16: 131-140. Impact Factor = 3.59 (Journal Citation Reports 2007).

21. Fryar, S.C., Booth, W., Davies, J., Hodgkiss, I.J. and **Hyde, K.D.** (2004). Distribution of fungi on wood in the Tutong River, Brunei. Fungal Diversity 17: 17-38. Impact Factor = 3.59 (Journal Citation Reports 2007).

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submerged in freshwater. Nova Hedwigia 78: 439-445. Impact Factor = 0.77 (Journal Citation Reports 2007).

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37. Kumar, D.S.S., Lau, C.S., Wan, J.M.F., Yang, D. and **Hyde K.D.** (2005). Immunomodulatory compounds from Pestalotiopsis leucothës (HKUCC 10197), an endophytic fungus of Tripterygium wilfordii. Life Sciences 78: 147-156. Impact Factor = 2.257 (Journal Citation Reports 2007).

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39. Tang, A.M.C., Jeewon, R. and **Hyde, K.D.** (2005). Successional patterns of microfungi in fallen leaves of Castanopsis fissa (Fagaceae) in Hong Kong forest. Canadian Journal of Microbiology 51: 967-974. Impact Factor = 1.286 (Journal Citation Reports 2007).

40. Fryar, S.C., Booth, W., Davies, J., Hodgkiss, I.J. and **Hyde, K.D.** (2005). Evidence of in situ competition between fungi in freshwater. Fungal Diversity 18: 59-71.

41. Cai, L., Jeewon, R. and **Hyde, K.D.** (2005). Phylogenetic evaluation and taxonomic revision of Schizothecium based on ribosomal DNA and protein coding genes. Fungal Diversity 19: 1-21.

42. Promputtha, I., Jeewon, R., Lumyong, S., McKenzie, E.H.C. and **Hyde, K.D.** (2005). Ribosomal DNA fingerprinting in the identification of non sporulating endophytes from Magnolia liliifera (Magnoliaceae). Fungal Diversity 20: 167-186.

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45. Bussaban, B., Lumyong, S., Lumyong, P., Seelanan, T., Park, D.C., McKenzie, E.H.C. and **Hyde, K.D.** (2005). Molecular and morphological characterization of Pyricularia and allied genera. Mycologia 97: 1002-1011.

46. Li, Y., **Hyde, K.D.**, Jeewon, R., Cai, L., Vijaykrishna D. and Zhang, K.Q. (2005). Phylogenetics and evolution of nematode trapping fungi (Orbiliales) estimated from nuclear and protein coding genes. Mycologia 97: 1034-1046.

47. Crous, P.W., Groenewald, J.Z., Risède, J.M., Simoneau, P. and **Hyde, K.D.** (2006). Calonectria speces and their Cylindrocladium anamorphs: species with clavate vesicles. Studies in Mycology 55: 213-226.

48. Paulus, B., Gadek, P. and **Hyde, K.D.** (2006). Successional patterns of microfungi in fallen leaves of Ficus pleurocarpa (Moraceae) in an Australian tropical rainforest. Biotropica 38: 42-51. Impact Factor = 1.7 (Journal Citation Reports 2007).

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50. Vijaykrishna, D. and **Hyde, K.D.** (2006). Inter and intra stream variation of lignicolous freshwater fungi in tropical Australia. Fungal Diversity 21: 203-224.

51. Pinnoi, A., Lumyong, S., **Hyde, K.D.** and Jones, E.B.G. (2006). Biodiversity of fungi on the palm Eleiodoxa conferta in Sirindhorn peat swamp forest, Narathiwat, Thailand. Fungal Diversity 22: 205-218.

52. Kodsueb, R., Jeewon, R., Dhanasekaran, V., McKenzie, E.H.C., Lumyong, P., Lumyong, S. and **Hyde, K.D.**, (2006). Systematic revision of Tubeufiaceae based on morphological and molecular data. Fungal Diversity 21: 105-130.

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54. Duong, L.M., Jeewon, R., Lumyong, S. and **Hyde, K.D** (2006). DGGE coupled with ribosomal DNA phylogenies reveal uncharacterized fungal phylotypes on living leaves of Magnolia liliifera. Fungal Diversity 23: 121-138.

55. Vijaykrishna, D., Jeewon, R. and **Hyde, K.D.** (2006). Molecular taxonomy, origins and evolution of freshwater ascomycetes. Fungal Diversity 23: 367-406.

56. El-Morsy, E.M., El-Dohlob, S.M. and **Hyde, K.D.** (2006). Diversity of Alternaria alternata a common destructive pathogen of Eichhornia crassipes in Egypt and its potential use in biological control. Fungal Diversity 23: 139-158.

57. Hidayat, I., Jeewon, R., To-anun, C. and **Hyde, K.D.** (2006). The genus Oxydothis: New palmicolous taxa and phylogenetic relationships within Xylariales. Fungal Diversity 23: 159-179.

58. Kodsueb, R., Jeewon, R., Vijaykrishna, D., Lumyong, S., McKenzie, E.H.C. and **Hyde, K.D.** (2006). The family Pleosporaceae: intergeneric relationships and phylogenetic perspectives based on sequence analyses of partial 28S rDNA. Mycologia 98: 571-583.

59. 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.

60. Paulus, B., Kanowski, J., Gadek, P. and **Hyde, K.D.** (2006). Diversity and distribution of saprobic microfungi in leaf litter of an Australian tropical rainforest. Mycological Research 110: 1441-1454.

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63. Cai, L., Jeewon, R. and **Hyde, K.D.** (2006). Molecular systematics of Zopfiella and allied genera: evidence from multiple gene sequence analysis. Mycological Research 110: 359-368.

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(Liliopsida-Arecales) along the Tutong River, Brunei. Indian Journal of Marine Sciences 35: 297-307. 0.3

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73. 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.

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Co-investigator

1. **Name: Ekachai Chukeatirote**

Department of Biotechnology, School of Science, Mae Fah Luang University, Chiang Rai 57100 Thailand

Tel.: 66-53-916210; Fax: 66-53-916112 E-mail: ekachai@mfu.ac.th

ID No: 3 2096 000 52 70 3

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^{CAG} genes from various *Candida* species and expression in *Saccharomyces cerevisiae*" with Prof. Mick Tuite

1990-1994 BSc (First Class Hons.) in Biology, Department of Biology, Faculty of Science, Chiang Mai Univeristy, Thailand; Project title "Lactic acid

production by starch-utilising lactic acid bacteria" with Assoc. Prof. Dr. Saisamorn Lumyong.

Awards and Scholarships:

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

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

1994 Prof. Dr. Dhab Nelanithi Foundation Award

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

Work experiences:

2000-present Lecturer, Mae Fah Luang University

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

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

1995-1999 Teaching Assistance in the following undergraduate practical:

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

Membership of Learning Societies:

- Editorial Board, Research Journal of Microbiology (2005 – present)
- Member of Thai Society of Biotechnology (2004 – present)
- Member of the Science Advisory Board (2004 – present)
- Member of CRN Microbiology (2004 – present)
- Member of Society of General Microbiology (1995 – 1999)

- Member of Researcher Panel of the IRPUS Project, Thailand (2005 – present)
- Member of the Thai-UK Alumni and Professional Network (2004 – present)

Selected publications:

1. 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.

2. 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.

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4. **Chukeatirote E**, Hanpattanakit P, Kaprom A and Tovaranonte J (2007) Antimicrobial Activity of *Senna spectabilis* and *S. tora*. *J Plant Sci* 2: 123-126.

5. 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.

6. **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.

7. **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.

8. Wisitrasamewong K and **Chukeatirote E** (2005) *in silico* PCR-RFLP. Thailand Innovation Awards 2005, pp. 53-54.

9. Sakai K, Mori M, Fujii A, Iwami Y, **Chukeatirote E** and Shirai Y (2004) Fluorescent *in situ* hybridization analysis of open lactic acid fermentation of kitchen refuse using rRNA-targeted oligonucleotide probes. *J Biosci Bioeng* 98: 48-56.

10. **Chukeatirote E**, Bankluay K, Kaprom A, Sampanvejsobha S and Winyayong P (2004) Microbiological quality of some tea products in Chiang Rai Province, Thailand. *Chiang Mai J Sci* 31: 185-189.

2. Name: Dr. Rampai Kodsueb

ID No: 350080005 5 284

Date of Birth: June 13, 1979

Position: Lecturer (15 October 2007-present)

Office Address: Biology Programme, Faculty of Science and Technology, Pibulsongkram Rajabhat University, 156 Moo 5, Plaichoomphol, Muang, Phitsanulok, 65000, Thailand

Phone: 66-55-267105 Fax: 66-55-267106

Email Address: kodsueb@yahoo.com

Education Background

- B.Sc. in Agriculture (Plant Pathology) Hons. Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand. March, 2001
- Ph.D. in Biodiversity and Ethnobiology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand. October, 2007

Field of Specialization Skill

Mycology, Plant Pathology

Scholarships

- Centre for Research in Fungal Diversity (2002–2005)
- The Commission on Higher Education (2005–2007)

Awards

- Aventis CropScience Award 2000- The 3rd Place Winning Team of "Innovative Rice Production Technology" Essay Contest.
- The Excellence Dissertation Award Academic Year 2007 on Thesis Entitled "Biodiversity of Saprobic Fungi on Woody Litter" from Chiang Mai University Graduate School.

Selected publications:

1. **Kodsueb, R.**, McKenzie, E.H.C., Lumyong, S. and Hyde, K.D. (2008). Diversity of saprobic fungi on *Magnoliaceae*. *Fungal Diversity* 30: 37-53.

2. **Kodsueb, R.**, McKenzie, E.H.C., Lumyong, S. and Hyde, K.D. (2008). Fungal succession on woody litter of *Magnolia liliifera* (*Magnoliaceae*). Fungal Diversity 30: 55-72.

3. **Kodsueb R.,** Jeewon R., Hyde K.D., McKenzie E.H.C., Ho W.H. and Lumyong S. (2007). Molecular phylogeny of new synnematous hyphomycete taxon from Thailand and its teleomorphic affinities to Massarinaceae (Pleosporales). *Botanical Journal of the Linnean Society* 155: 283–296.

4. **Kodsueb R.**, McKenzie, E.H.C., Ho, W.H., Hyde K.D., Lumyong P. and Lumyong S. (2007). New anamorphic fungi from decaying woody litter of. *Michelia baillonii* (Magnoliaceae) in northern Thailand. *Cryptogamie Mycologie* 28: 237–245.

5. **Kodsueb, R.**, Jeewon, R., Vijaykrishna, D., McKenzie, E.H.C., Lumyong, P., Lumyong, S. and Hyde, K.D. (2006). Systematic revision of *Tubeufiaceae* based on morphological and molecular data. *Fungal Diversity* 21: 105–130.

6. **Kodsueb R.,** Lumyong S., Hyde K.D., Lumyong P. and McKenzie E.H.C. (2006). *Acrodictys micheliae* and *Dictyosporium manglietiae*, two new anamorphic fungi from woody litter of Magnoliaceae in northern Thailand. *Cryptogamie Mycologie* 27: 111–119.

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10. Hyde K.D., Jeewon R, Bahl J., Bhilabut B., Bussaban B., Cai L., Damodar S.B., **Kodsueb R.**, Lam C.W.H., Lam D.M., Photita W., Promputtha I.,

Tang A.M.C., Thongkantha S., Vijaykrishna D., Yeung S.Y. (2004). Fungal studies at the University of Hong Kong. Annual Meeting of Mycological Society of America, Asheville, North Carolina, USA, 2004.

3. **Name: Dr Matchima Naradisorn**

ID No: 3 5014 00 697 30 1

Contact address: School of Agro-Industry, Mae Fah Luang University, 333 Moo 1, Tasood, Muang, Chiang Rai, THAILAND 57100

Tel.: 66-5391-6753, Fax: 66-5391-673

E-mail: matchima@mfu.ac.th

Education:

- 2003 – 2007 PhD (Postharvest Pathology), The University of Adelaide (Australia)
- 1996 – 1997 M.Agr. (Plant Pathology), The University of Sydney (Australia)
- 1991 – 1994 B.Sc. (Agriculture), Chiangmai University (Thailand)

Publications and proceedings:

1. **Naradisorn,** M., Able, A. J., Scott, E., Klieber, A. and Sedgley, M. 2005. Effect of preharvest calcium application on grey mould development and postharvest quality in strawberries. Acta Hort. 2005; 708:147-150.
2. **Naradisorn**, M., Sedgley, M., Scott, E. and Able, A.J. Effect of calcium lactate on grey mould development in strawberry. Poster presentation at Australasian Postharvest Horticulture Conference, Rotorua, New Zealand (27-30 September 2005) and received attendee spot prize.
3. Raksaboon, N, Ruenkum, A. and **Naradisorn**, M. Control of anthracnose in mango using n-propyl dihydrojasmonate. Proceedings of Industrial and Research Projects for Undergraduate Student Symposium (IRPUS-2009). March 26-29, 2009. Siam Paragon, Bangkok.

4. **Naradisorn**, M. and Ruenkum, A. Preliminary study on antimicrobial activity of crude extracts of pomelo albedo against Colletotrichum gloeosporioides. Proceedings of International Symposium "Go…Organic 2009". August 19-21, 2009. Bangkok, Thailand.

Current projects:

- Antimicrobial activity of pomelo albedo tissues against postharvest fungal pathogens

Postharvest handling of cut anthuriums