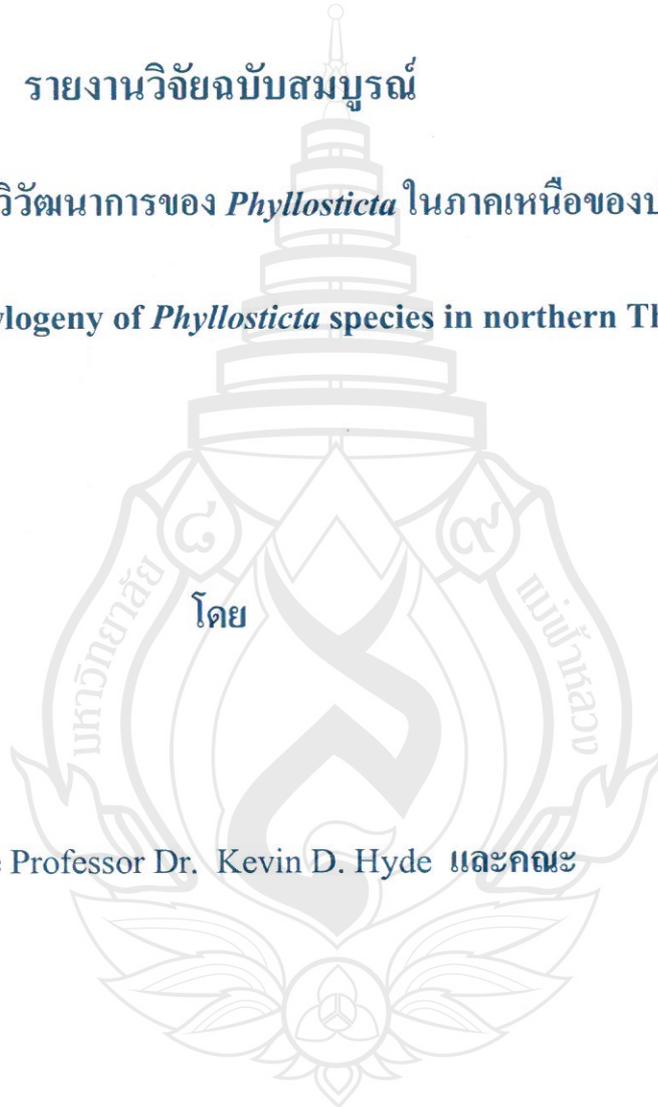




รายงานวิจัยฉบับสมบูรณ์

อนุกรมวิธาน และวงศ์วานวิวัฒนาการของ *Phyllosticta* ในภาคเหนือของประเทศไทย

Taxonomy and phylogeny of *Phyllosticta* species in northern Thailand



Associate Professor Dr. Kevin D. Hyde และคณะ

งานวิจัยนี้ได้รับทุนสนับสนุนจากมหาวิทยาลัยแม่ฟ้าหลวง

ประจำปี พ.ศ. 2553

Acknowledgement

Mae Fah Luang University is thanked for the award of grant No 53101020017 to study the genus *Phyllosticta* in northern Thailand and also for providing laboratory facilities through STIC.



Taxonomy and phylogeny of *Phyllosticta* species in northern Thailand

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Abstract

The genus *Phyllosticta* (*Guignardia* its teleomorph) cause economically significant diseases of important crops and horticultural plants such as banana, citrus, grape, orchids and palms. Species concepts in *Phyllosticta* and *Guignardia* are however ambiguous as there are more than 3,000 names. Of these, ~190 species of *phyllosticta* are officially named and accepted. Besides, it should be noted that only a few characteristic can be used to differentiate at species level. It is therefore important that species concepts of this genus must be revised and clarified so that plant pathologists can readily identify species. Correct identification can help implement disease control management strategies. This project aims to study *Phyllosticta* taxonomy especially those that are of great importance in plant pathology although saprobes and endophytes will also be included. Their morphological and cultural characters as well as the phylogenetic relationships of *Phyllosticta* species isolated from various hosts (e.g., on banana, citrus, grapes, orchids, palms) will be studied. Their *Guignardia* teleomorphs, if available, will also be investigated. Relationships will be elucidated using morphological and cultural characters and phylogenetic interpretation of gene sequences. This project would provide a clear understanding of the taxonomy of *Phyllosticta* species in Thailand, particularly in the

north. We also expect to identify the important species that cause disease and reduce yield and quality of plant products.

Keywords: *Phyllosticta*, *Guignardia*

Introduction

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

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

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

Of the diseases caused by *Phyllosticta*, those on Citrus have been relatively well researched (Wulanderi *et al.*, 2009), however few other species have been well

researched and our knowledge of the genus *Phyllosticta* in Thailand is poor. A few species of *Phyllosticta* causing leaf spot diseases have been reported in Thailand, such as *Phyllosticta* sp. on pear (Visarathanonth, http://www.actahort.org/members/showpdf?booknrarnr=279_67) and a *Phyllosticta* sp causing spots on Soybean leaves (Nachaiwiang *et al.*, 2001). *Phyllosticta* endophytes have been isolated from banana and *Amomum* leaves (Photita *et al.*, 2001; Bussaban *et al.*, 2001) and a *Phyllosticta* sp. is known to cause post harvest disease of Durian (Poeltz, 2003). However a search on the topic reveals that very little is known concerning *Phyllosticta* species in Thailand and most taxa are named as *Phyllosticta* sp. There is obviously much work required to establish the diversity and importance of the genus in Thailand.

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

Objectives

- (1) To clarify the species of *Phyllosticta* associated with disease in a range of hosts in northern Thailand.
- (2) To understand the relationship between morphology and phylogeny characters of *Phyllosticta* species and their relationships with hosts.

Research Methodology

(1) Collection of the samples

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

(2) Morphological examination

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

(3) Phylogenetic study

DNA will be extracted from any morphologically different taxa (isolates) and PCR will be carried out to amplify rDNA ITS region by using primers ITS 4 and ITS 5 and partial β -tubulin gene by using Bt2A and Bt2B. Sequencing will be carried out for the respective region. Phylogenetic analysis of the sequences either from ITS region or β -tubulin gene will be carried out by using Maximum parsimony analysis from Paup[@].

Expected results

- (1) Accurate identification of *Phyllosticta* species from diseases of a range of hosts
- (2) Understanding of the phylogenetic relationship between *Phyllosticta* species with regards to host and morphological characters.

Research Timetable

Research activities	Duration	Time schedule
Collection of samples	9 months	October 2009 – June, 2010
Morphological characterization	11 months	October 2009 – August, 2010

DNA extraction, PCR and Sequencing	3 months	May – July, 2010
Data analysis (morphology data analysis and phylogenetic analysis)	1 months	August, 2010
Writing paper(s)	2 months	August - September, 2010

Budget for October 2009-September 2010

Item	Amount (Baht)
Student Remuneration (5 students) (700 x 12 x 5)	42,000
Collection of samples	
❖ Traveling costs for sample collection	40,000
Other expenses cost	
❖ Information research cost	5,000
Morphological experiments	
❖ Media (Water agar/ PDA/ MEA)	20,000
❖ Equipment needed for microscopic work (Petridishes, glass slides, cover slips, fine forceps)	15,000
❖ Chemical Reagent	15,000
❖ Analysis of sample	20,000
DNA extraction, PCR and Sequencing	
❖ DNA extraction kits (approximately 60 extractions)	73,000
❖ Reagents for PCR (buffer, dNTPs, primers, Taq polymerase, PCR	

purification kit, etc.) (approximately 60 reactions)

❖ Sequencing service cost (to HKU) (approximately 30 reactions)

Total budget 230,000

Expected Outputs and Outcomes

We would expect one or two publications from this project in SCI journals.

The publications and data will form the basis for a larger proposal to TGIST and NRCT.

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เงินอุดหนุนโครงการวิจัย จาก มหาวิทยาลัยแม่ฟ้าหลวง ประจำปีงบประมาณ พ.ศ. 2553

ครั้งที่ 1 ตั้งแต่เดือน ตุลาคม พ.ศ. 2553 ถึง เดือน มีนาคม พ.ศ. 2554

1. ชื่อโครงการ: อนุกรมวิธาน และวงศ์วานวิวัฒนาการของ *Phyllosticta* ในภาคเหนือของประเทศไทย/
Taxonomy and phylogeny of *Phyllosticta* species in northern Thailand
2. ชื่อหัวหน้าโครงการ/ สำนักวิชา Associate Professor Dr. Kevin D. Hyde, School of Science
รายชื่อผู้ร่วมวิจัย / สำนักวิชา Dr. Ekachai Chukeatirote, Assistant Professor, School of Science
หน่วยงานภายนอกที่ร่วมทำวิจัย (ถ้ามี)
Dr. Somsak Sivichai, Research Officer, BIOTEC, Bangkok
Professor Pedro Crous, Director, CBS, Utrecht, Netherlands
3. ความสำคัญ และที่มาของปัญหาที่ทำการวิจัย (โดยย่อ)

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

4. วัตถุประสงค์ของโครงการวิจัย

- (1) To clarify the species of *Phyllosticta* associated with disease in a range of hosts in northern Thailand.
- (2) To understand the relationship between morphology and phylogeny characters of *Phyllosticta* species and their relationships with hosts.

5. ขอบเขตของโครงการวิจัย

To observe and collect the plant diseases caused by *Phyllosticta* sp. in Northern Thailand. Then all isolates will be identified by their morphology and molecular.

6. ระเบียบวิธีวิจัย/ วิธีดำเนินการวิจัย

(1) Collection of the samples

Phyllosticta isolates will be collected from the leaf spots and diseased fruits of various hosts in northern Thailand.

(2) Morphological examination

Morphological characters of selected isolates collected

(3) Phylogenetic study

Research Timetable

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❖ information research cost	5,000
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❖ Media (Water agar/ PDA/ MEA)	20,000
❖ Equipment needed for microscopic work (Petridishes, glass slides, cover slips, fine forceps)	15,000
❖ Chemical Reagent	15,000
❖ Analysis of sample	20,000
DNA extraction, PCR and Sequencing	
❖ DNA extraction kits (approximately 60 extractions)	73,000
❖ Reagents for PCR (buffer, dNTPs, primers, Taq polymerase, PCR purification kit, etc.) (approximately 60 reactions)	
❖ Sequencing service cost (to HKU) (approximately 30 reactions)	
Total budget	230,000

7. ผลการดำเนินงานวิจัย อภิปรายผล

During one year of collecting of the genus *Phyllosticta* in northern Thailand (Chiangrai, Chiangmai, Pa-Yao and Lampang) we have isolated more than 50 cultures and have herbarium material for more than 100 collections.

8. ผลการวิจัย ณ ปัจจุบัน บรรลุผลสำเร็จตามวัตถุประสงค์ข้อใดบ้าง

The study has clarified some species of *Phyllosticta* associated with disease in a range of hosts in northern Thailand and established the relationships between morphology and phylogeny characters among *Phyllosticta* species and their relationships with hosts.

9. เปรียบเทียบผลการดำเนินงาน ณ ช่วงที่รายงาน กับแผนงานวิจัยทั้งโครงการ

Activities	Month											
	1	2	3	4	5	6	7	8	9	10	11	12
Collection of samples	←→											
Morphological characterization			←→									
DNA extraction, PCR and Sequencing					←→							
Data analysis (morphology data analysis and phylogenetic analysis)								←→				
Writing report											←→	

10. อุปสรรค/ปัญหาที่เกิดขึ้นระหว่างดำเนินการวิจัย พบในการดำเนินงาน(ถ้ามี)และแนวทางแก้ไข

11. การเผยแพร่ผลงานวิจัย ระหว่างการดำเนินงานวิจัย (ถ้ามีให้แนบเอกสารประกอบด้วย)

1. Wulandari N. F, To-Anun C, McKenzie E. H. C and Hyde K. D *Guignardia bispora* and *G. ellipsoidea* spp. nov. and other *Guignardia* species from palms (Arecaceae). Mycosphere 2(2), 115–128.

2. Wulandari N. F, To-Anun C, Cai L, Abd-Elsalam K. A, Hyde K. D, 2010. *Guignardia/Phyllosticta* species on banana. *Cryptogamie Mycologie* 31, 403-418.
3. Wulandari N. F, To-Anun C, Hyde K. D *Guignardia morindae* frog eye leaf spotting disease of *Morinda citrifolia* (Rubiaceae) *Mycosphere* 1(4), 325–331
4. *Phyllosticta – perspective and challenges* - Fungal Diversity
5. *Phyllosticta ophiopogonis* sp. nov. from *Ophiopogon japonicus* (Liliaceae) - *Cryptogamie Mycologie* (in press)

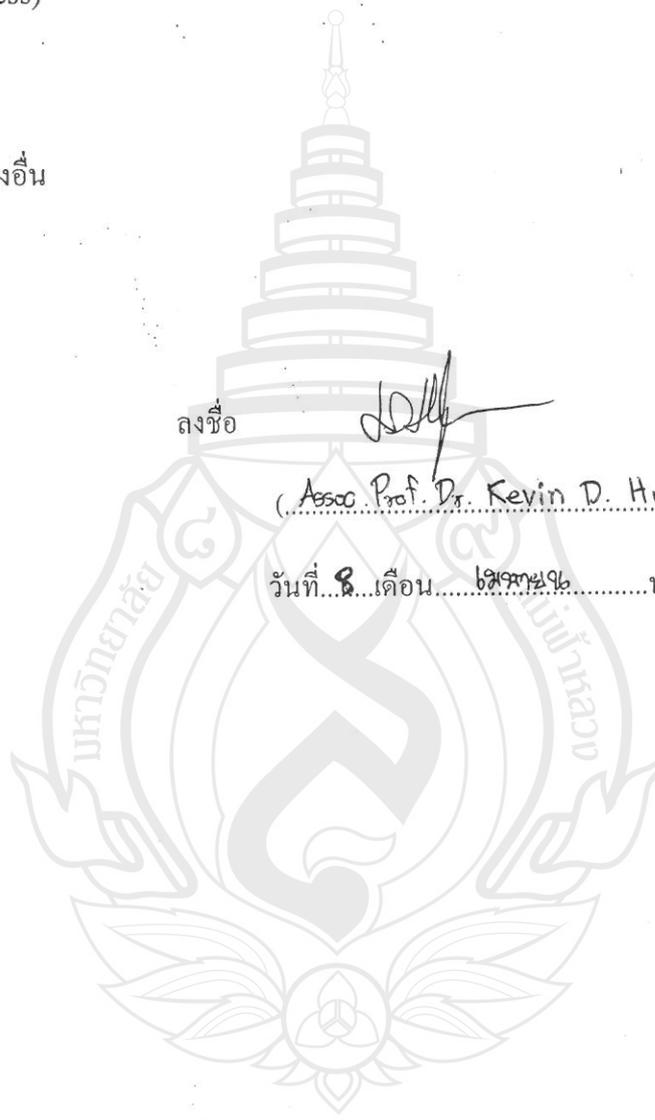
12. ข้อคิดเห็นและข้อเสนอแนะอย่างอื่น

ลงชื่อ



(Assoc. Prof. Dr. Kevin D. Hyde)

วันที่ 8 เดือน สิงหาคม พ.ศ. 2554



อนุกรมวิธานและวงศ์วานวิวัฒนาการของเชื้อราสกุล *Phyllosticta* ในภาคเหนือของประเทศไทย

Hyde, K. D.¹, Chukeatirote, E.¹, Sivichai, S.², Crous, P.³

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³ CBS, Utrecht, Netherlands

บทคัดย่อ

เชื้อราสกุล *Phyllosticta* (ระยะสืบพันธุ์แบบอาศัยเพศ *Guignardia*) สร้างความเสียหายต่อพืชเศรษฐกิจสำคัญหลายชนิด เช่น กล้วย ส้ม องุ่น กล้วยไม้ และ พืชตระกูลปาล์ม ปัจจุบันเชื้อราสกุล *Phyllosticta* และ *Guignardia* ที่ถูกเสนอชื่อมีประมาณ 3000 ชื่อแต่มีเพียง *Phyllosticta* ประมาณ 190 สปีชีส์เท่านั้นที่ได้รับการยอมรับ นอกจากนั้นความแตกต่างระหว่างสปีชีส์ เป็นสิ่งสำคัญที่นักโรคพืชวิทยาจะต้องใช้ในการจำแนกและระบุถึงระดับสปีชีส์อย่างแม่นยำ ซึ่งการจำแนกได้อย่างถูกต้องแม่นยำนั้นสามารถนำไปใช้ในการควบคุมโรคและการจัดการโรคพืช งานวิจัยนี้มีจุดมุ่งหมายที่จะศึกษาอนุกรมวิธานของเชื้อราสกุล *Phyllosticta* รวมทั้งการศึกษาบทบาทด้านความเป็นราโรคพืช ร่ายย่อยสลาย และ ราเอนโดไฟต์ รวมถึงการศึกษาด้านสัณฐานวิทยา ลักษณะเฉพาะบนอาหารเลี้ยงเชื้อ อีกทั้ง วงศ์วานวิวัฒนาการและความสัมพันธ์ของ *phyllosticta* บนพืชเจ้าบ้าน เช่น กล้วย ส้ม องุ่น กล้วยไม้ และ พืชตระกูลปาล์ม รวมไปถึงการศึกษา *Guignardia* ซึ่งเป็นระยะที่ใช้การสืบพันธุ์แบบอาศัยเพศ ความสัมพันธ์ระหว่างระยะอาศัยเพศและไม่อาศัยเพศ จะอาศัยการศึกษาด้านสัณฐานวิทยา ลักษณะเฉพาะบนอาหารเลี้ยงเชื้อ ตลอดจนการเปรียบเทียบลำดับของ DNA และยีนส์บางกลุ่ม เข้าใจถึงวงศ์วานวิวัฒนาการและงานวิจัยนี้จะทำให้การศึกษาด้านอนุกรมวิธานของ *Phyllosticta* เป็นระบบและสามารถใช้เป็นเอกสารอ้างอิงในการศึกษาด้านความหลากหลายของเชื้อรากลุ่มนี้โดยเฉพาะในประเทศไทย นอกจากนี้ ความหลากหลายและการระบุชนิดของเชื้อราในระดับสปีชีส์จะมีส่วนช่วยในงานด้านโรคพืชอีกด้วย

คำสำคัญ *Phyllosticta*, *Guignardia*

Taxonomy and phylogeny of *Phyllosticta* species in northern Thailand

Hyde, K. D.¹, Chukeatirote, E.¹, Sivichai, S.², Crous, P.³

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Abstract

The genus *Phyllosticta* (*Guignardia* its teleomorph) cause economically significant diseases of important crops and horticultural plants such as banana, citrus, grape, orchids and palms. Species concepts in *Phyllosticta* and *Guignardia* are however ambiguous as there are more than 3,000 names. Of these, ~190 species of *phyllosticta* are officially named and accepted. Besides, it should be noted that only a few characteristic can be used to differentiate at species level. It is therefore important that species concepts of this genus must be revised and clarified so that plant pathologists can readily identify species. Correct identification can help implement disease control management strategies. This project aims to study *Phyllosticta* taxonomy especially those that are of great importance in plant pathology although saprobes and endophytes will also be included. Their morphological and cultural characters as well as the phylogenetic relationships of *Phyllosticta* species isolated from various hosts (e.g., on banana, citrus, grapes, orchids, palms) will be studied. Their *Guignardia* teleomorphs, if available, will also be investigated. Relationships will be elucidated using morphological and cultural characters and phylogenetic interpretation of gene sequences. This project would provide a clear understanding of the taxonomy of *Phyllosticta* species in Thailand, particularly in the north. We also expect to identify the important species that cause disease and reduce yield and quality of plant products.

Keywords: *Phyllosticta*, *Guignardia*

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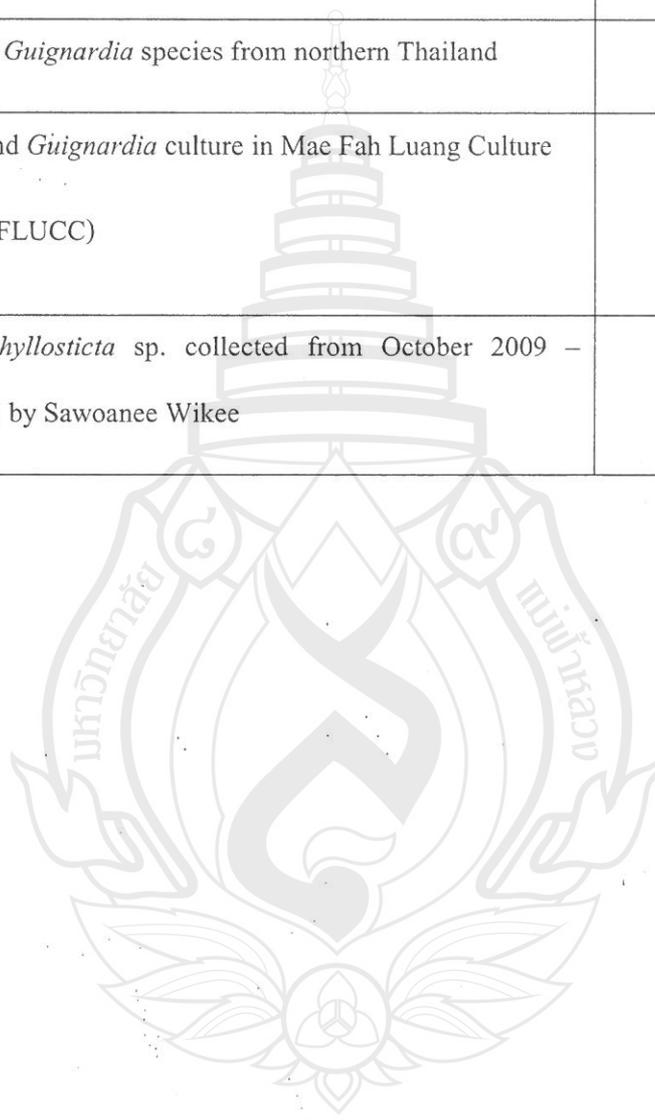


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Abbreviations

%	=	Percent
°C	=	Degree Celsius
µm	=	micrometer
cm	=	centimeter
g	=	gram
g/l	=	gram per liter
hr	=	hour
ml	=	milliliter
mm	=	millimeter
No.	=	Number
PDA	=	Potato Dextrose Agar
sp.	=	species
Temp.	=	Temperature



CHAPTER 1

Introduction

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

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

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

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

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

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

Objectives

- (1) To clarify the species of *Phyllosticta* associated with disease in a range of hosts in northern Thailand.
- (2) To understand the relationship between morphology and phylogeny characters of *Phyllosticta* species and their relationships with hosts.

Expected results

- (1) Accurate identification of *Phyllosticta* species from diseases of a range of hosts
- (2) Understanding of the phylogenetic relationship between *Phyllosticta* species with regards to host and morphological characters.

CHAPTER 2

Review of Related Literature

Species of *Phyllosticta* are pathogens, endophytes and saprobes worldwide and are found on a large number of hosts such as apple, banana, and Citrus (Gardner et al., 1923; Wulandari et al., 2009). *Phyllosticta* and its sexual state *Guignardia* cause leaf spots on a large number of plants resulting in economic losses in Asia and Europe (Nelson 1971; van der Aa 1973; Baayen, Bonants et al. 2002; Paul, Van Jaarsveld et al. 2005; Wulandari, To-Anun et al. 2009). Species are also important biocontrol agents and have been recorded as producers of novel bioactive compounds. *Phomopsis* (and its sexual *Guignardia* state) is therefore an important genus requiring further study and is the topic of this review.

The classification of *Phyllosticta* species is complicated by the fact that there are few morphological characters to differentiate species as well as by the practices of some earlier mycologists, who defined new species based on fungus/host relationships with little or no consideration of morphology of previously described species (van der Aa & Vanev 2002). Accurate naming of *Phyllosticta* species is important for establishing host ranges, geographic distribution, and quarantine of species and in using species in biocontrol (Bailey et al., 1992). Traditionally, *Phyllosticta* species have been identified based on morphological characters; which include the size and shape of conidia; teleomorph state and culture characters such as colony color, growth rate and occurrence on a host. These criteria are however, not enough for reliable differentiation among *Phyllosticta* species due to variation in morphology and phenotype due to environmental influences. Presently about 200 species are accepted in *Phyllosticta* (van der Aa and Vanev 2002). Therefore, future studies should use molecular diagnostic tools along with traditional morphological techniques as an appropriate for classifying *Phyllosticta* species (Wulandari et al. 2009).

History

Phyllosticta was originally established by Persoon (1818) as *Phyllosticta* Pers. with *Phyllosticta convallariae* Pers. as the type species (van der Aa and Vanev 2002). The teleomorph

state *Guignardia* was introduced by Donk (1968). Most species of *Phyllosticta* have been described based on host association and therefore the classification is very confusing and the relation between host range and disease are poorly understood (van der Aa and Vanev 2002).

Phyllosticta* versus *Guignardia

Phyllosticta and *Guignardia* are asexual and sexual states of the same genus but species have been given separate names because of the dual classification system used by mycologists over several decades (Shenoy et al. 2007, 2010). For instance *Phyllosticta musarum* (Cooke) Aa and *Guignardia musae* Racib. are the same biology species but have different names, *P. musarum* being the asexual state and *Guignardia musae* being the sexual state (Cooke, 1880, Racib, 1909, Van der Aa, 1973). In the past it has often been difficult or impossible to link these asexual and sexual states unless both one state could be isolated from single spores and produce the other state or both states in culture (Shenoy et al. 2007, 2010). However with the use of molecular data it is now possible to link the asexual and asexual states and the use of the binomial system of classification in fungi is becoming redundant. Therefore a single name should be adopted and there are various views as to which names should be followed, i.e. the oldest, the sexual state name, the most important name, and there is a view maintaining the two names (Hyde et al 2011). Our view is that we should generally adopt the oldest name, but also taking into account which is most important. *Phyllosticta* (1818) is a much older name than *Guignardia* (1892) and generally *Phyllosticta* species cause important diseases. There are exceptions, for example *Guignardia candeloflamma* is only known in its teleomorph state, while banana freckle is caused by both states. Because *Phyllosticta* is the oldest name and generally more important as the causal agent of disease we name and chose to adopt this name and treat all *Guignardia* species as synonyms of *Phyllosticta*. Because of this decision we use the name *Phyllosticta* throughout this review unless we specifically refer to a *Guignardia* species.

CHAPTER 3

Research Methodology

(1) Collection of *Phyllosticta* strains

Samples of black spot or black spot-like symptoms were collected from various hosts, including *Arthocarpus heterophyllus*, *Dioscorea penthapylla*, *Caryota* sp., *Citrus maxima*, *Dendrobium* sp., *Dioscorea bulbiferae*, *Dracaena sanderiana*, *Jasminum sambac*, *Ficus benjamina* L. var. *variegata*, *Musa acuminata*, *Musa paradisiacal*, *Ophiopogon japonicus*, *Pandanus amaryllifolia*, *Shorea* sp., *Sphatolobus suberectus*, *Vanda* sp. (Table 3-1), during 2010 to February 2011. Collecting sites are listed in Table 3b. Strains were isolated from lesions of infected fruits or leaves. A small piece of tissue (5 × 5 mm) was taken from the margin of infected tissues, surface sterilized by immersing in 70% ethanol solution for 1 minute, 1% sodium hypochlorite solution for 1 minute, rinsed three times with sterilized water and finally dried in sterilized tissue paper. Samples were placed on PDA amended with 100 µg/ml streptomycin and 100 µg/ml ampicillin and incubated at 25 °C until sporulation (about 12 days). Single spore subcultures were obtained for each *Phyllosticta* isolate using the procedure described by Goh (1999). Cultures were maintained on PDA slants at 4°C.

Table 3-1. Plant host species examined in this study

Serial No.	Host Name	Collection Places	Province
1	<i>Sphatolobus suberectus</i>	TV	Chiang Mai
2	<i>Arthocarpus heterophyllus</i>	MRC	Chiang Mai
3	<i>Dioscorea bulbiferae</i>	15M	Chiang Mai
4	<i>Dioscorea penthapylla</i>	15M	Chiang Mai
5	<i>Dioscorea bulbiferae</i>	TMRC	Chiang Mai
6	<i>Thunbergia grandiflora</i>	TMRC	Chiang Mai
7	<i>Artocarpus heterophyllus</i>	MRC	Chiang Mai

8	<i>Dioscorea penthapylla</i>	TMRC	Chiang Mai
9	<i>Dioscorea bulbiferae</i>	MRC	Chiang Mai
10	<i>Dioscorea bulbiferae</i>	TMRC	Chiang Mai
11	<i>Dioscorea penthapylla</i>	SN	Chiang Mai
12	<i>Cassia agness</i>	CHC	Chiang Mai
13	<i>Dioscorea penthapylla</i>	MRC	Chiang Mai
14	<i>Dioscorea bulbiferae</i>	MRC	Chiang Mai
15	<i>Dioscorea bulbiferae</i>	MRC	Chiang Mai
16	<i>Dioscorea bulbiferae</i>	15M	Chiang Mai
17	<i>Dioscorea bulbiferae</i>	TMRC	Chiang Mai
18	<i>Dioscorea penthapylla</i>	MRC	Chiang Mai
19	<i>Vanda</i> sp.	ML	Chiang Mai
20	<i>Caryota</i> sp.	MFS3	Chiang Rai
21	<i>Citrus maxima</i> (Pumello) fruit	TMY	Chiang Rai
22	<i>Vanda</i> sp.	CGH	Chiang Mai
23	<i>Citrus maxima</i> (Pumello) fruit	TMY	Chiang Rai
24	<i>Citrus maxima</i> (Pumello) fruit	CDC	Chiang Mai
25	<i>Samanea saman</i>	MFS	Chiang Rai
26	<i>Pandanus amaryllifolia</i>	RD	Chiang Rai
27	<i>Cordyline</i> sp. (red)	KKW	Chiang Rai
28	<i>Cordyline</i> sp. (red)	NHMW	Chiang Rai
29	<i>Dracontomelon</i> sp.	NHMW	Chiang Rai
30	<i>Caryota</i> sp.	MFS3	Chiang Rai
31	<i>Dioscorea bulbiferae</i>	KDH	Chiang Rai
32	<i>Dendrobium</i> sp.	KDH	Chiang Rai
33	<i>Cordyline</i> sp. (red)	KDH	Chiang Rai
34	<i>Jasminum sambac</i>	KDH	Chiang Rai

35	<i>Caryota</i> sp.	MFG	Chiang Rai
36	<i>Cordyline</i> sp. (red)	PWN	Chiang Rai
37	<i>Cordyline</i> sp. (Small)	CT	Chiang Rai
38	<i>Pandanus amaryllifolia</i>	RD	Chiang Rai
39	<i>Aglaonema</i> sp.	CGH	Chiang Mai
40	<i>Cordyline</i> sp. (red)	MRC	Chiang Mai
41	<i>Vanda</i> sp.	NRT	Chiang Rai
42	<i>Morinda citrifolia</i>	CIA	Chiang Rai
43	<i>Aglaonema</i> sp.	CGH	Chiang Mai
44	<i>Shorea</i> sp.	CGH	Chiang Mai
45	<i>Amaryllis</i> sp.	KDH	Chiang Rai
46	<i>Nephrolepis</i> sp.	NHMW	Chiang Rai
47	<i>Cordyline</i> sp. (Green)	NHMW	Chiang Rai
48	<i>Cordyline</i> sp. (Red)	NHMW	Chiang Rai
49	<i>Cordyline</i> sp. (Small)	CT	Chiang Rai
50	<i>Dracontomelon</i> sp.	NHMW	Chiang Rai
51	<i>Alpinia purpurata</i>	MFS3	Chiang Rai
52	<i>Dendrobium</i> sp.	RD	Chiang Rai
53	<i>Areca</i> sp.	MM	Chiang Mai
54	<i>Pterocarpus</i> sp.	MFG	Chiang Rai
55	<i>Morinda citrifolia</i>	CIA	Chiang Rai
56	<i>Bischofia javanica</i>	KKW	Chiang Rai
57	<i>Cordyline</i> sp. (red)	PPG	Chiang Mai
58	<i>Areca</i> sp	PPG	Chiang Mai
59	<i>Bauhinia</i> sp.	PPG	Chiang Mai
60	<i>Cordyline</i> sp. (Small)	DT	Chiang Rai
61	<i>Pterocarpus</i> sp.	MFG	Chiang Rai
62	<i>Caryota</i> sp.	MFS3	Chiang Rai
63	<i>Dendrobium</i> sp.	RD	Chiang Rai

64	<i>Raphis</i> sp.	MFPH	Chiang Rai
65	<i>Spathiphyllum</i> sp.	KDH	Chiang Rai
66	<i>Pothos aureus</i> .	MFPH	Chiang Rai
67	<i>Caryota</i> sp.	MFG	Chiang Rai
68	<i>Caryota</i> sp.	MFS3	Chiang Rai
69	<i>Ophiopogon japonicus</i>	MFFC	Chiang Rai
70	<i>Ficus elastica</i>	MFS3	Chiang Rai
71	<i>Ophiopogon japonicus</i>	MFFC	Chiang Rai
72	<i>Amaryllis</i> sp.	MFPH	Chiang Rai
73	<i>Areca</i> sp.	MFPH	Chiang Rai
74	<i>Dioscorea pentaphylla</i>	MFG	Chiang Rai
75	<i>Ficus elastica</i>	MFS3	Chiang Rai
76	<i>Dracaena</i> sp.	RD	Chiang Rai
77	<i>Syzygium</i> sp.	CIO	Chiang Rai
78	<i>Asplenium</i> sp.	MFFC	Chiang Rai
79	<i>Asplenium</i> sp.	MFFC	Chiang Rai
80	<i>Ophiopogon japonicus</i>	TC	Chiang Rai
81	<i>Bischofia javanica</i>	MFPH	Chiang Rai
82	<i>Ophiopogon japonicus</i>	TC	Chiang Rai
83	<i>Sterculia monosperma</i>	PWN	Chiang Rai
84	<i>Sterculia monosperma</i>	RD	Chiang Rai
85	<i>Sterculia monosperma</i>	RD	Chiang Rai
86	<i>Musa paradisiaca</i>	MRC	Chiang Mai
87	<i>Musa nanae</i>	OP	Chiang Mai
88	<i>Musa paradisiaca</i>	CM	Chiang Mai
89	<i>Musa paradisiaca</i>	CH	Chiang Mai
90	<i>Musa paradisiaca</i>	MRC	Chiang Mai
91	<i>Musa paradisiaca</i>	CGH	Chiang Mai
92	<i>Musa paradisiaca</i>	MRC	Chiang Mai

93	<i>Musa paradisiaca</i>	CGH	Chiang Mai
94	<i>Musa paradisiaca</i>	TMRC	Chiang Mai
95	<i>Musa paradisiaca</i>	MRC	Chiang Mai
96	<i>Musa paradisiaca</i>	MRC	Chiang Mai
97	<i>Musa paradisiaca</i>	PVL	Chiang Mai
98	<i>Musa paradisiaca</i>	MRC	Chiang Mai
99	<i>Musa acuminata</i>	TJV	Chiang Mai
100	<i>Musa acuminata</i>	MRC	Chiang Mai
101	<i>Musa paradisiaca</i>	MRC	Chiang Mai
102	<i>Musa paradisiaca</i>	MRC	Chiang Mai
103	<i>Musa paradisiaca</i>	CDPG	Chiang Mai
104	<i>Musa paradisiaca</i>	CGH	Chiang Mai
105	<i>Musa paradisiaca</i>	CS	Chiang Mai
106	<i>Musa paradisiaca</i>	CS	Chiang Mai
107	<i>Musa paradisiaca</i>	PPG	Chiang Mai
108	<i>Musa paradisiaca</i>	CGH	Chiang Mai
109	<i>Musa paradisiaca</i>	MFG	Chiang Rai
110	<i>Musa paradisiaca</i>	CS	Chiang Mai
111	<i>Musa paradisiaca</i>	CGH	Chiang Mai
112	<i>Musa paradisiaca</i>	CS	Chiang Mai
113	<i>Musa paradisiaca</i>	RD	Chiang Rai
114	<i>Musa paradisiaca</i>	PWN	Chiang Rai
115	<i>Musa paradisiaca</i>	PV	Chiang Rai
116	<i>Musa paradisiaca</i>	RD	Chiang Rai
117	<i>Musa acuminata</i>	KKW	Chiang Rai
118	<i>Musa acuminata</i>	NHMW	Chiang Rai
119	<i>Musa acuminata</i>	TP3	Chiang Rai
120	<i>Musa paradisiaca</i>	MFG	Chiang Rai
121	<i>Musa paradisiaca</i>	RD	Chiang Rai

122	<i>Musa paradisiaca</i>	MRC	Chiang Mai
123	<i>Musa acuminata</i>	TLC	Chiang Rai
124	<i>Musa acuminata</i>	MCR	Chiang Rai
125	<i>Musa acuminata</i>	PVR	Chiang Rai
126	<i>Musa paradisiaca</i>	PPR	Chiang Rai
127	<i>Musa acuminata</i>	NHMW	Chiang Rai
128	<i>Unknown</i>	MJPU	Prae
129	<i>Punica granatum L.</i>	MFUC	Chiang Rai
130	<i>Scheffera venulosa Harms.</i>	MFG	Chiang Rai
131	<i>Saccharum</i>	MKT	Chiang Rai
132	<i>Arecaceae</i>	PWN	Chiang Rai
133	<i>Ophiopogon japonicus</i>	KKW	Chiang Rai
134	<i>Ficus benjamina L. var. variegata</i>	PWN	Chiang Rai
135	<i>Ophiopogon japonicus</i>	HMSW	Chiang Rai
136	<i>Orchidaceae</i>	PWN	Chiang Rai
137	<i>Dracaena sanderiana</i>	PWN	Chiang Rai
138	<i>Ophiopogon japonicus</i>	PWN	Chiang Rai
139	<i>Orchidaceae</i>	PWN	Chiang Rai
140	<i>Cordyline fruticosa</i>	PWN	Chiang Rai

Table 3-2 Collection sites

Conserved rainforest/Waterfall

1. Srilanna Park, Chiang Mai (SN).
2. Mae Lod, Royal Project, Chiang Mai (ML).
3. Chiang Dao Cave, Chiang Mai (CDC).
4. Medicinal Plant Garden CMU, Chiang Mai (PPG).
5. Ob Luang Park, Chiang Mai (OP).
6. Kun Khon Waterfall, Chiang Rai (KKW).

122	<i>Musa paradisiaca</i>	MRC	Chiang Mai
123	<i>Musa acuminata</i>	TLC	Chiang Rai
124	<i>Musa acuminata</i>	MCR	Chiang Rai
125	<i>Musa acuminata</i>	PVR	Chiang Rai
126	<i>Musa paradisiaca</i>	PPR	Chiang Rai
127	<i>Musa acuminata</i>	NHMW	Chiang Rai
128	<i>Unknown</i>	MJPU	Prae
129	<i>Punica granatum L.</i>	MFUC	Chiang Rai
130	<i>Scheffera venulosa Harms.</i>	MFG	Chiang Rai
131	<i>Saccharum</i>	MKT	Chiang Rai
132	<i>Arecaceae</i>	PWN	Chiang Rai
133	<i>Ophiopogon japonicus</i>	KKW	Chiang Rai
134	<i>Ficus benjamina L. var. variegata</i>	PWN	Chiang Rai
135	<i>Ophiopogon japonicus</i>	HMSW	Chiang Rai
136	<i>Orchidaceae</i>	PWN	Chiang Rai
137	<i>Dracaena sanderiana</i>	PWN	Chiang Rai
138	<i>Ophiopogon japonicus</i>	PWN	Chiang Rai
139	<i>Orchidaceae</i>	PWN	Chiang Rai
140	<i>Cordyline fruticosa</i>	PWN	Chiang Rai

Table 3-2 Collection sites

Conserved rainforest/Waterfall

1. Srilanna Park, Chiang Mai (SN).
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5. Ob Luang Park, Chiang Mai (OP).
6. Kun Khon Waterfall, Chiang Rai (KKW).

7. Nam Tok Huey Maesak Forest Park, Chiang Rai (NHMW).
8. Doi Tung, Chiang Rai (DOI)
9. Hoi Mae Sak Waterfall, Wieng Chiangrung, Chiangrai(HMSW)
10. Pong Prabat, Nang lae, Chiangrai (PPN)

Disturbed rainforest

1. Takam Village, Chiang Mai (TV).
2. Pha Dheng Village, Chiang Mai (PVL).
3. Tung Joaw Village, Chiang Mai (TJV).
4. Chiang Daow Pumello Garden, Chiang Mai (CDPG).
5. Mushroom Research Centre, Chiang Mai (MRC).
6. 15 Marker on the way to MRC, Chiang Mai (15M).
7. Pathumikkaram temple, near MRC, Chiang Mai (TMRC).
8. Tambon Muang, Yai, Ampheur district, Viangkan, Chiang Rai (TMY)
9. Phan District, Chiang Rai (PV).
10. Tad Sai Rung Waterfall Forest Park (P3), Chiang Rung, Chiang Rai (TP3).
11. Tham Luang Cave, Chiang Rai (TLC).
12. Phlu Village, Chiang Rai (PVR).
13. Phrathat Pangao, Chiang Rai (PPR).

City

1. City Hall, Chiang Mai (CHC).
2. Chiang Mai University Greenhouse, Chiang Mai (CGH).
3. Mae Mae Lai Market, Chiang Mai (MM).
4. Champeuq mosque, Chiang Mai (CM).
5. Chiang Mai Isra Guest House, Chiang Mai (CH).
6. Chiang Mai University Shop, Chiang Mai (CS).
7. S3-317 Building, Mae Fah Luang University, Chiang Rai (MFS3).
8. Sung Teaw Parking Area, MFLU, Chiang Rai (MFS).
9. Ratchana Dormitory, Chiang Rai (RD).
10. Prof. Kevin D. Hyde residential house, Chiang Rai (KDH).
11. Phasang Wiwat, Nanglae, Mueng, Chiang Rai (PWN).

12. Mae Fah Luang University, Chiang Rai (MFG).
13. Huey Pui Temple, Chiang Rai (CT).
14. Nursery to Chiang Rai, Chiang Rai (NRT).
15. Chiang Rai International Airport, Chiang Rai (CIA).
16. Mae Fah Luang University Presidential House, Chiang Rai (MFPH).
17. Mae Fah Luang University Food Court, Chiang Rai (MFFC).
18. Mae Fah Luang University Chinese Center (MFUC)
19. Mae Jo-Prae University, Prae (MJPU)
20. Mae Khaw Tom, Thasud, Chiangrai (MKT)

DNA extraction

Isolates were grown on PDA at 25°C for 10 days. Mycelia were scrapped using a sterile blade and placed in centrifuge tube. Genomic DNA was extracted using a Biospin Fungus Genomic DNA Extraction Kit (Bio-Flux, Bioer technology Co., Ltd, China) according to the instructions of the manufacturer provided, and suspended in 1 × TE buffer and stored at -20°C.

PCR amplification and DNA sequencing

The primer pairs ITS4 /ITS5 (White et al, 1990) were used to amplify the internal transcribed spacer region of the nuclear ribosomal RNA operon, including the 3' end of the 18S rRNA, the first internal transcribed spacer region, the 5.8S rRNA gene, the second internal transcribed spacer region and the 5' end of the 28S rRNA gene. The primer pairs EF1-728F/EF1-986R (Carbone and Kohn, 1999) were used to amplify partial translation elongation factor 1- α gene (TEF1). The primer pairs ACT-512F/ACT-783R (Carbone and Kohn, 1999) were used to amplify the partial actin gene (ACT). Amplification conditions followed Prihastuti et al. (2009). Amplification were carried out in S1000TM Thermal Cycler (Bio-Rad Laboratories, Inc. Germany), with the cycling parameters as previously described (Prihastuti et al, 2009). The PCR products were verified by staining with gelview on 1% agarose electrophoresis gel, then purified and cloned into pMD18-T vector, and followed by the transformation into *E. coli* (DH5 α). The positive clones were sent for sequencing (Invitrogen by

Life Technologies, Shanghai, China) using universal forward and reverse primers and the results were manually checked. Isolates selected for sequencing are listed in Table 2.

Phylogenetic analysis

The complete ITS, partial actin (ACT) and the translation elongation factor 1- α gene (TEF1) of representative isolates were aligned in Mega 4.0 (Tamura et al, 2007). Alignments were manually adjusted to allow maximum alignment and maximum sequence similarity. Gaps were treated as missing data. Phylogenetic analyses were performed using Mega 4.0 (Tamura et al, 2007) with neighbor-joining method. All positions containing gaps and missing data were eliminated from the dataset. Clade stability of the trees resulting from the parsimony analyses were assessed by bootstrap with 1000 replicates. To obtain more genetic information, ITS, ACT and TEF1 gene sequences were assembled to construct combined phylogenetic trees. *Botryosphaeria obtusa* (ITS=AY972105, TEF1=DQ280419, ACT=AY972111) was used as outgroup in the construction of phylogenetic trees.

Morphology of *Phyllosticta* species

Microscopic structures were obtained from isolates sporulating on 2% water agar with sterile pine needles as the substratum (WAP) (Crous et al., 2006; Wulandari et al. 2009) at 25°C with a 12/12 h photoperiod for 30 days. Microscopic structures were observed under differential interference contrast microscopy (Nikon, Eclipse 80i, Jpan), and photographed using a digital camera. About 30 measurements were made of each structure. Ninety five percent confidence levels were determined.

To observe the cultural characteristics of representative strains of each species, mycelial discs (7 mm diameter) were obtained from the actively growing edge of colony cultivated for 10 days on PDA, and then transferred to the centre of fresh PDA, malt extract agar (MEA), oat agar (OA), and cornmeal agar (CMA), followed by 14-d incubation at 25°C in darkness. The cultural characteristics of upper and reverse sides of colony, growth rate, and optimal temperature requirements were determined as described by Wulandari et al. (2009).

Species identification of *Phyllosticta* associated with disease in northern Thailand

The *Phyllosticta* isolates collected from various hosts will be subjected to PCR identification using species-specific primer pairs, followed by checking morphological and cultural characters.



CHAPTER 4

Results

Phyllosticta spp. was collected throughout Northern of Thailand from agricultural fields, waterfalls, national parks and house gardens (Tables 4-1, 4-2). To date we have collected more than 100 specimens belonging to at least 25 species. *Phyllosticta* species cause spots on living leaves and are also saprobes on dead leaves, but the pathogenic species are generally different from those on fallen leaves. Normally, pycnidia develop as black spots and black hyphae on leaf lesions. The *Guignardia* teleomorph and *Phyllosticta* asexual state are often found in the same leaf lesion. Fresh material of plant infected by *Phyllosticta* or *Guignardia* was isolated by endophyte technique, hyphal tip and single spore isolation. Conidia are typically small to medium sized, 5–10 μm in diam, hyaline, one-celled, have a thin and flexible sheath, are smooth-walled with an apical appendage. Often in dried specimens the appendage could not be observed. Characteristics and morphology have been examined in pure culture, where colonies form irregularly folded crusts and have dark mycelium. Some species produce white tendril of mycelium on the upper surface of the colony, which after 2 weeks on PDA is 2-3 cm in diam.

The species recorded and isolates found are listed in Tables 4-1 to 4-3, while descriptions of the 15 selected species follow.

Table 4-1. Collections of *Guignardia* species from northern Thailand

No	Original code	Species	Site
1	NCC 001	<i>Guignardia</i> sp.	Nam Tok Huey Mesak Forest Park, Chiang Rai, Thailand
2	NCC 002	<i>Guignardia</i> sp.	Nam Tok Huey Mesak Forest Park, Chiang Rai, Thailand
3	NCC 003	<i>Guignardia</i> sp.	Ratchana Dorm, Chiang Rai, Thailand
4	NCC 004	<i>Guignardia</i> sp.	Temple near Nam Tok Huey Mesak Forest Park, Chiang Rai, Thailand
5	NCC 005	<i>Guignardia</i> sp.	Nam Tok Huey Mesak Forest Park, Chiang Rai, Thailand
6	NCC 006	<i>Guignardia</i> sp.	Chiang Mai University, Chiang Mai, Thailand
7	NCC 007	<i>Guignardia</i> sp.	Nam Tok Huey Mesak Forest Park, Chiang Rai, Thailand
8	NCC 008	<i>Guignardia</i> sp.	Mae Fah Luang Garden, Chiang Rai, Thailand
9	NCC 009	<i>Guignardia</i> sp.	Ratchana Dorm, Chiang Rai, Thailand
10	NCC 010	<i>Guignardia</i> sp.	Ratchana Dorm, Chiang Rai, Thailand
11	NCC 011	<i>Guignardia</i> sp.	Nam Tok Huey Mesak Forest Park, Chiang Rai, Thailand
12	NCC 012	<i>Guignardia</i> sp.	Ratchana Dorm, Chiang Rai, Thailand
13	NCC 013	<i>Guignardia</i> sp.	Mae Fah Luang Garden, Chiang Rai, Thailand
14	NCC 014	<i>Guignardia</i> sp.	Mae Fah Luang Garden, Chiang Rai, Thailand
15	NCC 015	<i>Guignardia</i> sp.	Mae Fah Luang Garden, Chiang Rai, Thailand
16	NCC 016	<i>Guignardia</i> sp.	Mae Fah Luang Garden, Chiang Rai, Thailand
17	NCC 017	<i>Guignardia</i> sp.	Mae Fah Luang Garden, Chiang Rai, Thailand
18	NCC 018	<i>Guignardia</i> sp.	Mae Fah Luang Garden, Chiang Rai, Thailand

No	Original code	Species	Site
19	NCC 019	<i>Guignardia</i> sp.	Mae Fah Luang Garden, Chiang Rai, Thailand
20	NCC 020	<i>Guignardia</i> sp.	Chiang Mai herb Garden, Chiang Mai, Thailand
21	NCC 021	<i>Guignardia</i> sp.	Dr Hyde House, Chiang Rai, Thailand
22	NCC 022	<i>Guignardia</i> sp.	Mushroom Research Centre, Chiang Mai, Thailand
23	NCC 023	<i>Guignardia</i> sp.	Chiang Mai herb Garden, Chiang Mai, Thailand
24	NCC 024	<i>Guignardia</i> sp.	Chiang Mai herb Garden, Chiang Mai, Thailand
25	NCC 025	<i>Guignardia</i> sp.	Chiang Mai herb Garden, Chiang Mai, Thailand
26	NCC 026	<i>Guignardia</i> sp.	Chiang Mai herb Garden, Chiang Mai, Thailand
27	NCC 027	<i>Guignardia</i> sp.	Mae Fah Luang Garden, Chiang Rai, Thailand
28	NCC 028	<i>Guignardia</i> sp.	Chiang Rai, Thailand
29	NCC 029	<i>Guignardia</i> sp.	Chiang Rai, Thailand
30	NCC 030	<i>Guignardia</i> sp.	Chiang Rai, Thailand
31	NCC 031	<i>Guignardia</i> sp.	Chiang Rai, Thailand
32	NCC 032	<i>Guignardia</i> sp.	Chiang Rai, Thailand
33	NCC 033	<i>Guignardia</i> sp.	Chiang Rai, Thailand
34	NCC 034	<i>Guignardia</i> sp.	Chiang Rai, Thailand
35	NCC 035	<i>Guignardia</i> sp.	Chiang Rai, Thailand
36	NCC 036	<i>Guignardia</i> sp.	Chiang Rai, Thailand
37	NCC 037	<i>Guignardia</i> sp.	Temple, Chiang Rai, Thailand

No	Original code	Species	Site
38	WK013	<i>Guignardia sp.</i>	Pasang, Nang lae, Chiangrai
39	WK023	<i>Guignardia sp.</i>	Pasang, Nang lae, Chiangrai

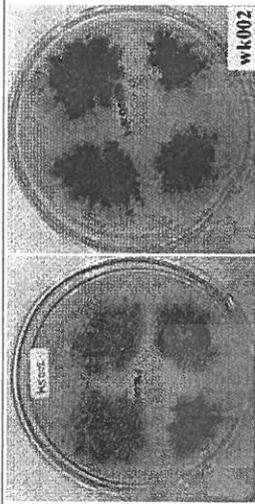
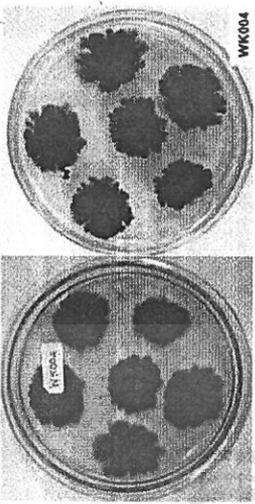
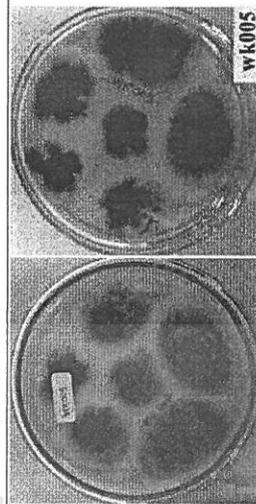


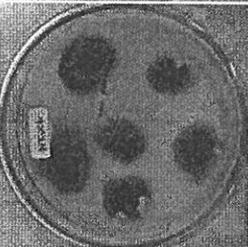
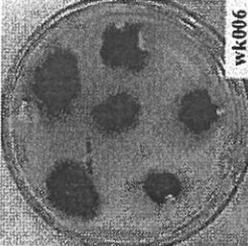
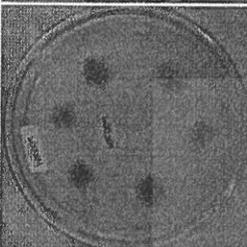
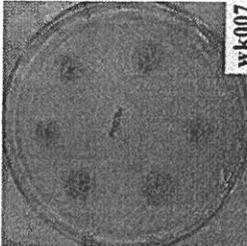
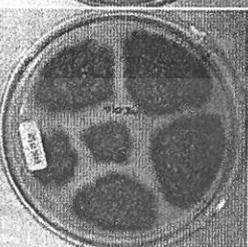
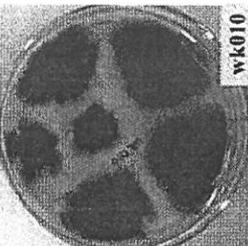
Table 4-2 *Phyllosticta* and *Guignardia* culture in Mae Fah Luang Culture Collection (MFLUCC)

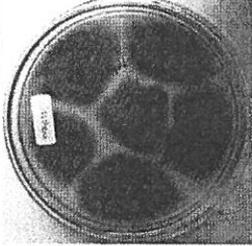
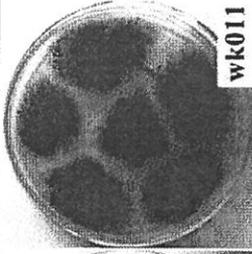
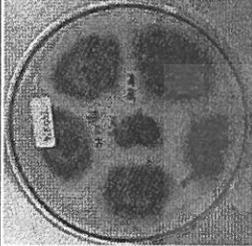
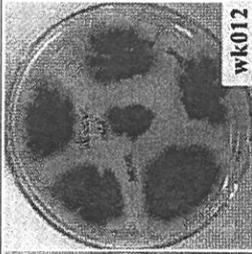
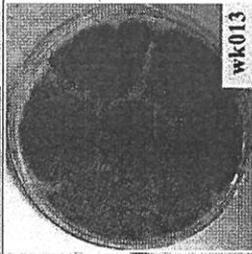
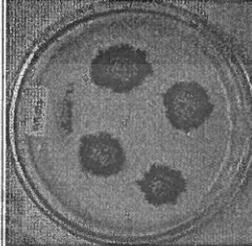
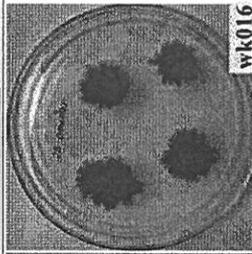
No	MFLUCC code	Original code	Species
1	10-0306	NCC 001	<i>Guignardia</i> sp.
2	10-0307	NCC 002	<i>Guignardia</i> sp.
3	10-0308	NCC 003	<i>Guignardia</i> sp.
4	10-0309	NCC 004	<i>Guignardia</i> sp.
5	10-0310	NCC 005	<i>Guignardia</i> sp.
6	10-0311	NCC 006	<i>Guignardia</i> sp.
7	10-0312	NCC 007	<i>Guignardia</i> sp.
8	10-0313	NCC 008	<i>Guignardia</i> sp.
9	10-0314	NCC 009	<i>Guignardia</i> sp.
10	10-0315	NCC 010	<i>Guignardia</i> sp.
11	10-0316	NCC 011	<i>Guignardia</i> sp.
12	10-0317	NCC 012	<i>Guignardia</i> sp.
13	10-0318	NCC 013	<i>Guignardia</i> sp.
14	10-0319	NCC 014	<i>Guignardia</i> sp.
15	10-0320	NCC 015	<i>Guignardia</i> sp.
16	10-0321	NCC 016	<i>Guignardia</i> sp.
17	10-0322	NCC 017	<i>Guignardia</i> sp.
18	10-0323	NCC 018	<i>Guignardia</i> sp.
19	10-0324	NCC 019	<i>Guignardia</i> sp.
20	10-0325	NCC 020	<i>Guignardia</i> sp.
21	10-0326	NCC 021	<i>Guignardia</i> sp.
22	10-0327	NCC 022	<i>Guignardia</i> sp.
23	10-0328	NCC 023	<i>Guignardia</i> sp.
24	10-0329	NCC 024	<i>Guignardia</i> sp.
25	10-0330	NCC 025	<i>Guignardia</i> sp.

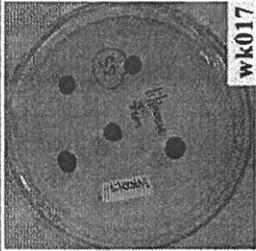
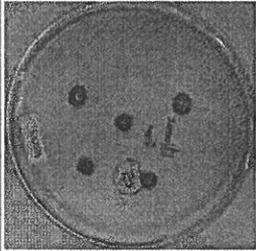
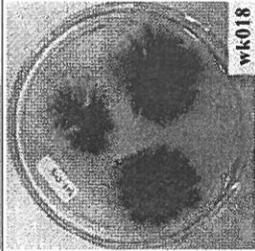
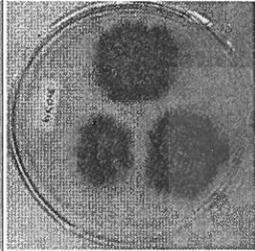
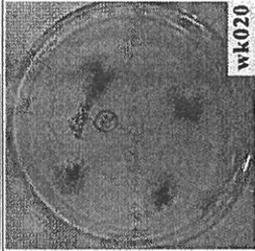
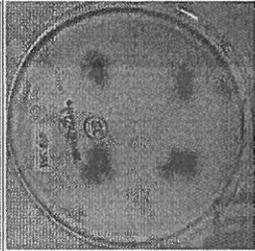
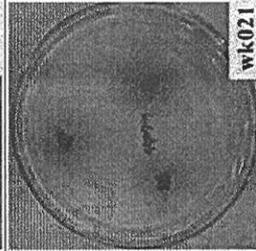
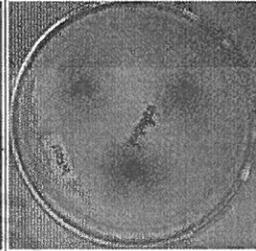
26	10-0331	NCC 026	<i>Guignardia</i> sp.
27	10-0332	NCC 027	<i>Guignardia</i> sp.
28	10-0333	NCC 028	<i>Guignardia</i> sp.
29	10-0334	NCC 029	<i>Guignardia</i> sp.
30	10-0335	NCC 030	<i>Guignardia</i> sp.
No	MFLUCC code	Original code	Species
31	10-0336	NCC 031	<i>Guignardia</i> sp.
32	10-0337	NCC 032	<i>Guignardia</i> sp.
33	10-0338	NCC 033	<i>Guignardia</i> sp.
34	10-0339	NCC 034	<i>Guignardia</i> sp.
35	10-0340	NCC 035	<i>Guignardia</i> sp.
36	10-0341	NCC 036	<i>Guignardia</i> sp.
37	10-0342	NCC 037	<i>Guignardia</i> sp.
38	11-0051	WK002	<i>Phyllosticta</i> sp.
39	11-0053	WK004	<i>Phyllosticta</i> sp.
40	11-0054	WK005	<i>Phyllosticta</i> sp.
41	11-0055	WK006	<i>Phyllosticta</i> sp.
42	11-0056	WK007	<i>Phyllosticta</i> sp.
43	11-0057	WK010	<i>Phyllosticta</i> sp.
44	11-0058	WK011	<i>Phyllosticta</i> sp.
45	11-0059	WK012	<i>Phyllosticta</i> sp.
46	11-0060	WK013	<i>Guignardia</i> sp.
47	11-0062	WK016	<i>Phyllosticta</i> sp.
48	11-0063	WK017	<i>Phyllosticta</i> sp.
49	11-0064	WK018	<i>Phyllosticta</i> sp.
50	11-0066	WK020	<i>Phyllosticta</i> sp.
51	11-0067	WK021	<i>Phyllosticta</i> sp.
52	11-0068	WK022	<i>Phyllosticta</i> sp.
53	11-0069	WK023	<i>Guignardia</i> sp.

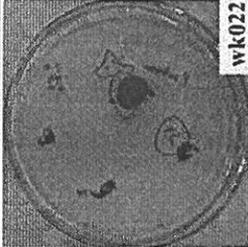
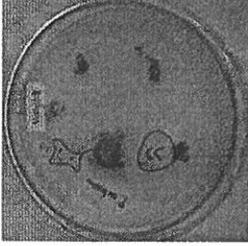
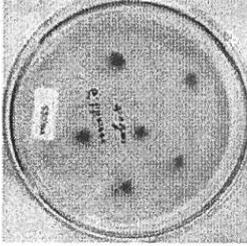
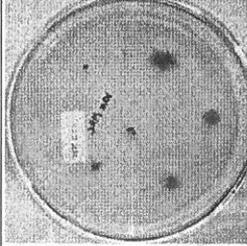
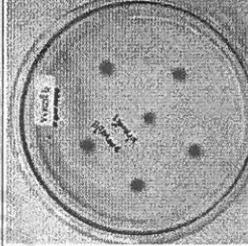
Table 4-3 Culture of *Phyllosticta* sp. collected from October 2009 – February 2011 by Sawoanee Wikee

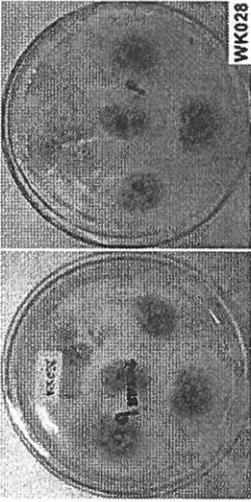
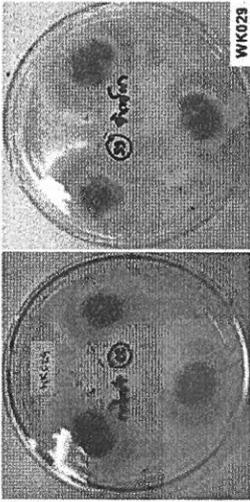
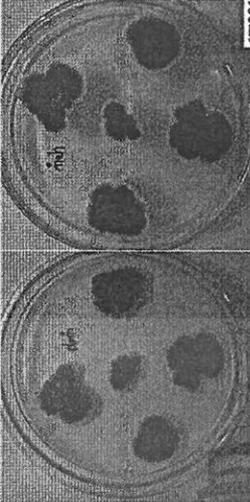
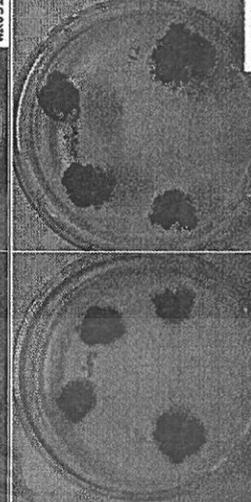
No.	Original Code	Species Name	Habitat	Host	Collection Site	Photo
1	WK002	<i>Phyllosticta</i> sp.	Living leave	unknown	Mae Jo-Prae University, Prae	
2	WK004	<i>Phyllosticta</i> sp.	Living leave	<i>Punica granatum</i> L.	Chinese center, Mae Fah Luang University, Nang lae, Chiangrai	
3	WK005	<i>Phyllosticta</i> sp.	Living leave	<i>Scheffera venulosa</i> Harms.	Mae Fah Luang University, Nang lae, Chiangrai	

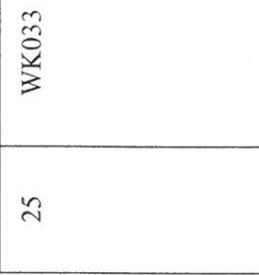
4	WK006	<i>Phyllosticta</i> sp.	Living leaf	Saccharum	Mae khaw tom, Nang lae, Chiangrai	 
5	WK007	<i>Phyllosticta</i> sp.	Living leaf	Areaceae	Pasang, Nang lae, Chiangrai	 
6	WK008	<i>Phyllosticta</i> sp.	Living leaf	<i>Stereulia monosperma</i>	Pa-Kha, Phan, Chiangrai	-
7	WK009	<i>Phyllosticta</i> sp.	Living leaf	<i>Hedera helix</i>	Pa-Kha, Phan, Chiangrai	-
8	WK010	<i>Phyllosticta</i> sp.	Living leaf	Liliaceae	Khunkom waterfall	 

9	WK011	<i>Phyllosticta</i> sp.	Living leave	<i>Ficus benjamina</i> L. var. <i>variegata</i>	Pasang, Nang lae, Chiangrai	 
10	WK012	<i>Phyllosticta</i> sp.	Living leave	Liliaceae	Hoi Mae sak waterfall, Wieng Chiangrung, Chiangrai	 
11	WK013	<i>Guignardia</i> sp.	Living leave	Orchidaceae	Pasang, Nang lae, Chiangrai	 
12	WK016	<i>Phyllosticta</i> sp.	Dried leave	<i>Dracaena sanderiana</i>	Pasang, Nang lae, Chiangrai	 

13	WK017	<i>Phyllosticta</i> sp.	Living leave	Liliaceae	Pasang, Nang lac, Chiangrai	 
14	WK018	<i>Phyllosticta</i> sp.	Living leave	Orchidaceae	Pasang, Nang lac, Chiangrai	 
15	WK020	<i>Phyllosticta</i> sp.	Living leave	<i>Cordyline</i> sp.	Pong Prabat, Nang lac, Chiangrai	 
16	WK021	<i>Phyllosticta</i> sp.	Living leave	Arecaceae	Pasang, Nang lac, Chiangrai	 

17	WK022	<i>Phyllosticta</i> sp.	Living leave	unknown	Doi inthanon, Chiangmai	 
18	WK023	<i>Guignardia</i> sp.	Living leave	Liliaceae	Pasang, Nang lae, Chiangrai	 
19	WK024	<i>Phyllosticta</i> sp.	Living leave	<i>Cordyline</i> sp.	MRC, Chiangmai	 
20	WK026	<i>Phyllosticta</i> sp.	Living leave	Liliaceae	Wiengkan, Chiangrai	 

21	WK028	<i>Phyllosticta</i> sp.	Living leave	<i>Morus alba</i> L.	Pasang, Nang lae, Chiangrai	
22	WK029	<i>Phyllosticta</i> sp.	Living leave	<i>Dracaena loureiri</i> Gagnep	Pasang, Nang lae, Chiangrai	
23	WK031	<i>Phyllosticta</i> sp.	Living leave	Magnoliaceae	MaeChan, Chiangrai	
24	WK032	<i>Phyllosticta</i> sp.	Living leave	Mangifera	MaeChan, Chiangrai	

25	WK033	<i>Phyllosticta</i> sp.	Living leave	Euphorbiaceae	Pasang, Nang lae, Chiangrai	
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Species of *Phyllosticta* collected during this study.

1. *Phyllosticta sterculiicola* Traverso, *Annlis mycol.* 1(1): 3 (1903)

Teleomorph: *Guignardia* Viala & Ravaz, *Bull. Soc. mycol. Fr.* 8: 63 (1892)

Host: *Sterculia nobilis*

Symptom: Black spot

Habitat: Living leaf

Collecting Site: Phasang, Nang Lae, Chiangrai

Pycnidia epiphyllous, circular, black, 0.2 mm diam, wall composed of 1-layers, 134–146 μm long μm , 120–133 wide, 14–22 thick, black. *Conidiogenous cells* lining wall of pycnidium, phialidic, cylindrical, hyaline, *Conidia* ellipsoidal, hyaline, 1-celled, 7–9 \times 3.5–5 μm , smooth-walled, surrounded by mucilaginous sheath, bearing single apical appendage, usually 4–5 μm long.

Colonies is black, fimbriate, black in reverse, 3–4 cm in diam. after 7 days of incubation on half – PDA. After 1 month on PDA, mycelium formed a mass of hyphae.

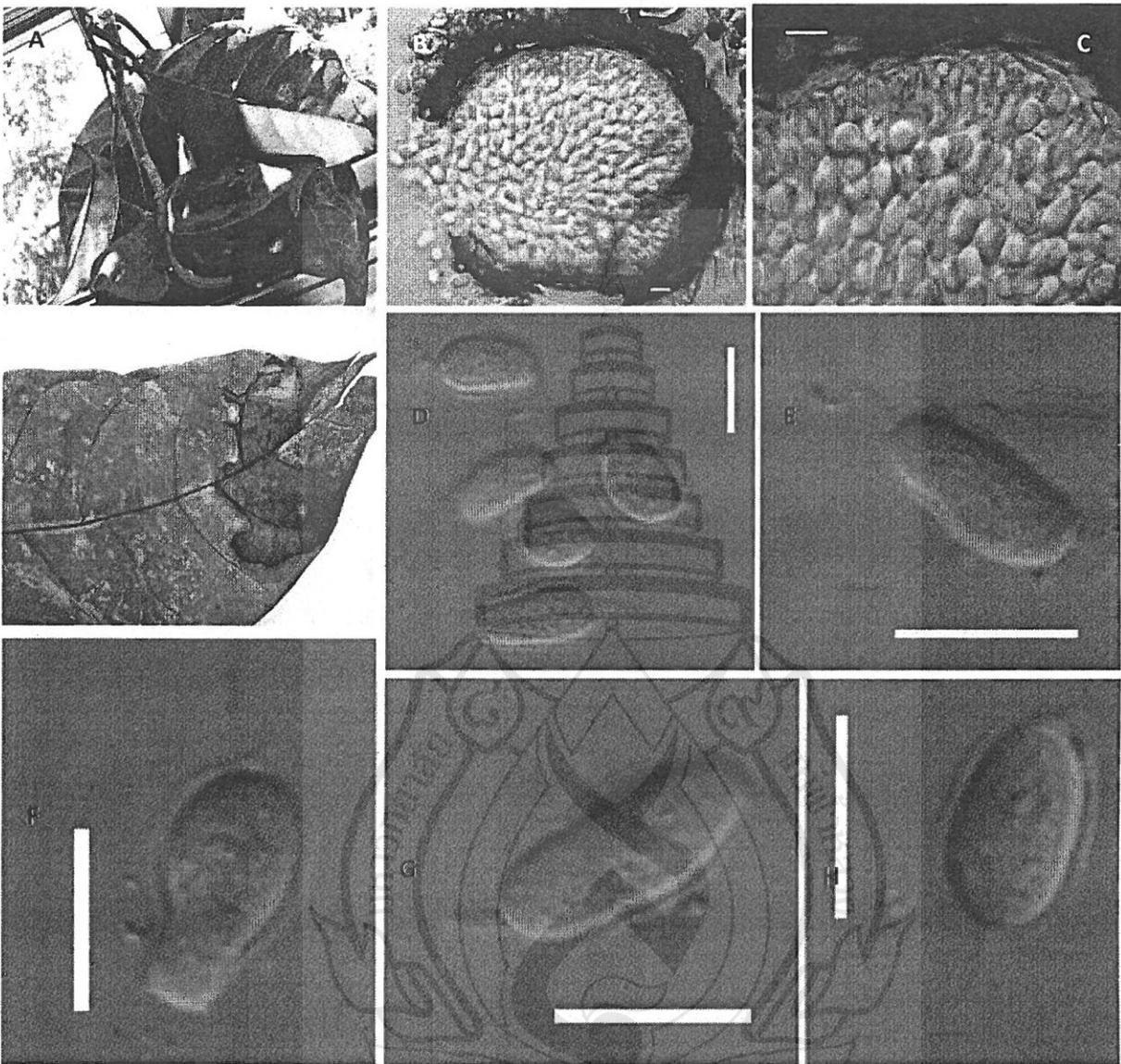


Fig. 4-1 (A) Spots on leaf of *Sterculia nobilis* (B-C) Pycnidia growing on infected *Sterculia* leaf ;
(D-H) Conidia; scale bar= 10 μ m.

2. *Phyllosticta* on *Artocarpus heterophyllus*

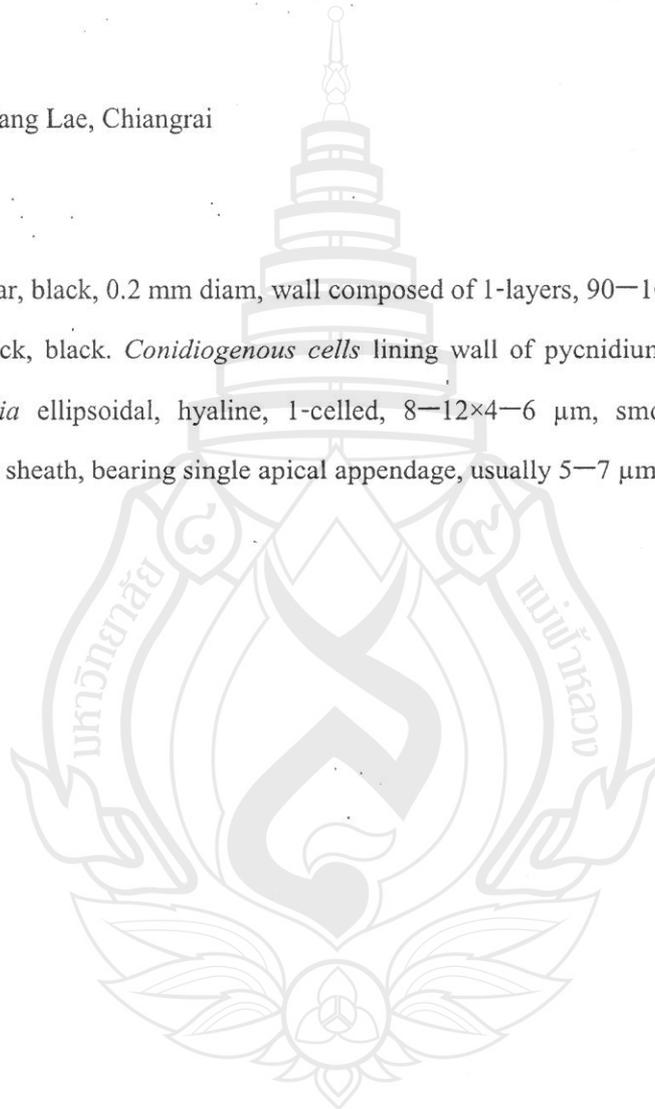
Host: *Artocarpus heterophyllus*

Symptom: Black spot

Habitat: Living leaf

Collecting Site: Phasang, Nang Lae, Chiangrai

Pycnidia epiphyllous, circular, black, 0.2 mm diam, wall composed of 1-layers, 90–100 μm long, 60–70 μm wide, 8–16 thick, black. *Conidiogenous cells* lining wall of pycnidium, phialidic, cylindrical, hyaline, *Conidia* ellipsoidal, hyaline, 1-celled, 8–12 \times 4–6 μm , smooth-walled, surrounded by mucilaginous sheath, bearing single apical appendage, usually 5–7 μm long.



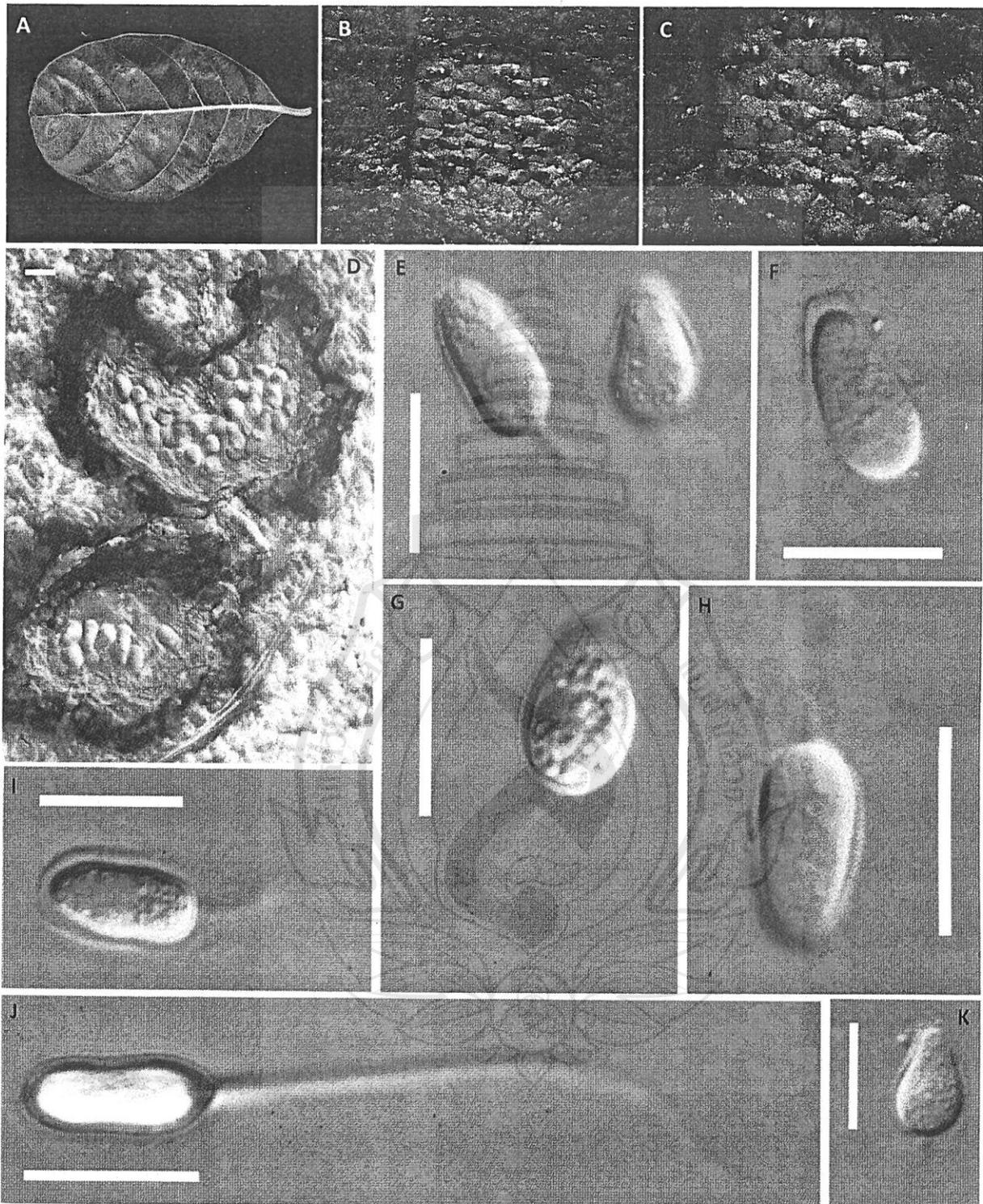


Fig. 4-2 (A) Spots on leaf of jackfruit (B-C) Pycnidia growing on infected leaf; *Artocarpus heterophyllus* (D) Pycnidia (E-K) Conidia; scale bar= 10 μ m.

3. *Phyllosticta* on *Schefflera arbicola*

Host: *Schefflera arbicola*

Symptom: Black spot

Habitat: Living leaf

Collecting Site: Botany Garden, Thasud, MFU, Chiangrai

Pycnidia epiphyllous, circular, black, 0.1 mm diam., wall composed of 1-layers, 80–100 μm long, 70–80 μm wide, 11–18 μm thick, brown, inside consisting, *Conidiogenous cells* lining wall of pycnidium, phialidic, cylindrical, hyaline, 2–2.5 \times 3–5 μm . *Conidia* ellipsoidal, hyaline, 1-celled, smooth-walled, 9–11 \times 5–6 μm , surrounded by mucilaginous sheath, bearing single apical appendage, usually 5–7 μm long.

Colonies black, fimbriat, black in reverse, 5–8 cm in diam. after 14 days of incubation on half – PDA.

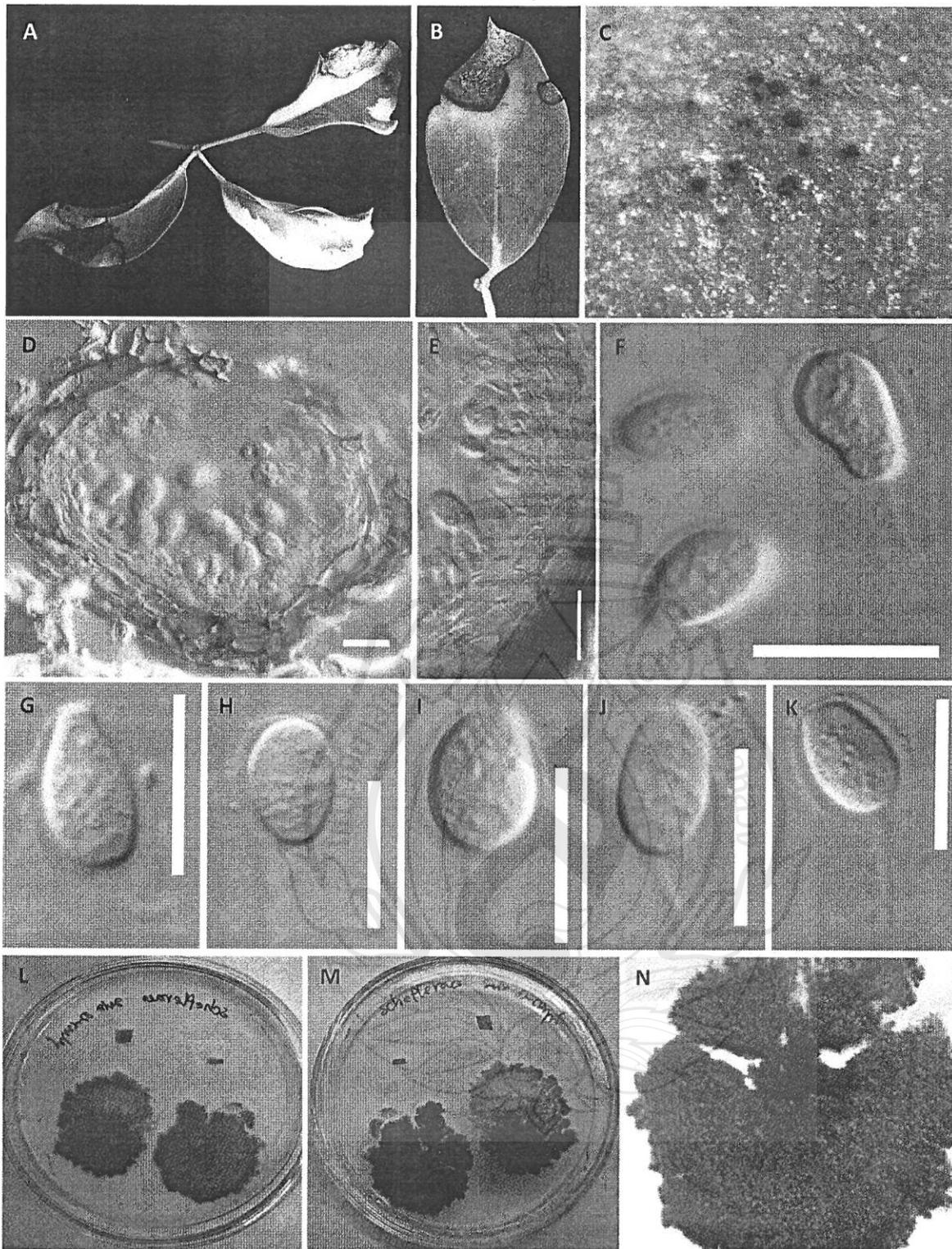


Fig. 4-3 (A-C) Pycnidia growing on infected leaf of *Schefflera arbicola* (D-E) Cross section through pycnidia showing part of the pycnidial wall with developing conidia (F-K) conidia (L-M) Colonies of *Phyllosticta* sp. on half-PDA supplement with antibiotic. Upper and reverse of cultures after 2 weeks (N) Fimbriat colony, black mycelia ; scale bar= 10 μ m.

4. *Phyllosticta* on *Punica granatum* L.

Host: *Punica granatum* L.

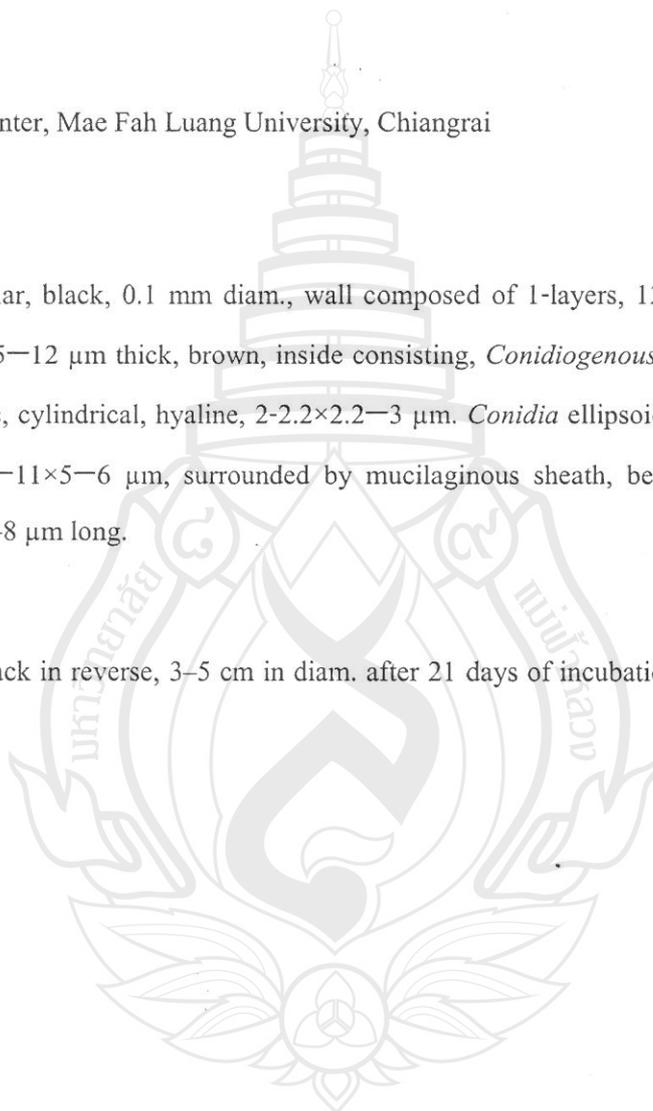
Symptom: Black spot

Habitat: Living leaf

Collecting Site: Chinese Center, Mae Fah Luang University, Chiangrai

Pycnidia epiphyllous, circular, black, 0.1 mm diam., wall composed of 1-layers, 120–125 μm long, 136–140 μm wide, 15–12 μm thick, brown, inside consisting, *Conidiogenous cells* lining wall of pycnidium, phialidic, cylindrical, hyaline, 2-2.2 \times 2.2–3 μm . *Conidia* ellipsoidal, hyaline, 1-celled, smooth-walled, 8–11 \times 5–6 μm , surrounded by mucilaginous sheath, bearing single apical appendage, usually 5–8 μm long.

Colonies black, fimbriat, black in reverse, 3–5 cm in diam. after 21 days of incubation on half – PDA.



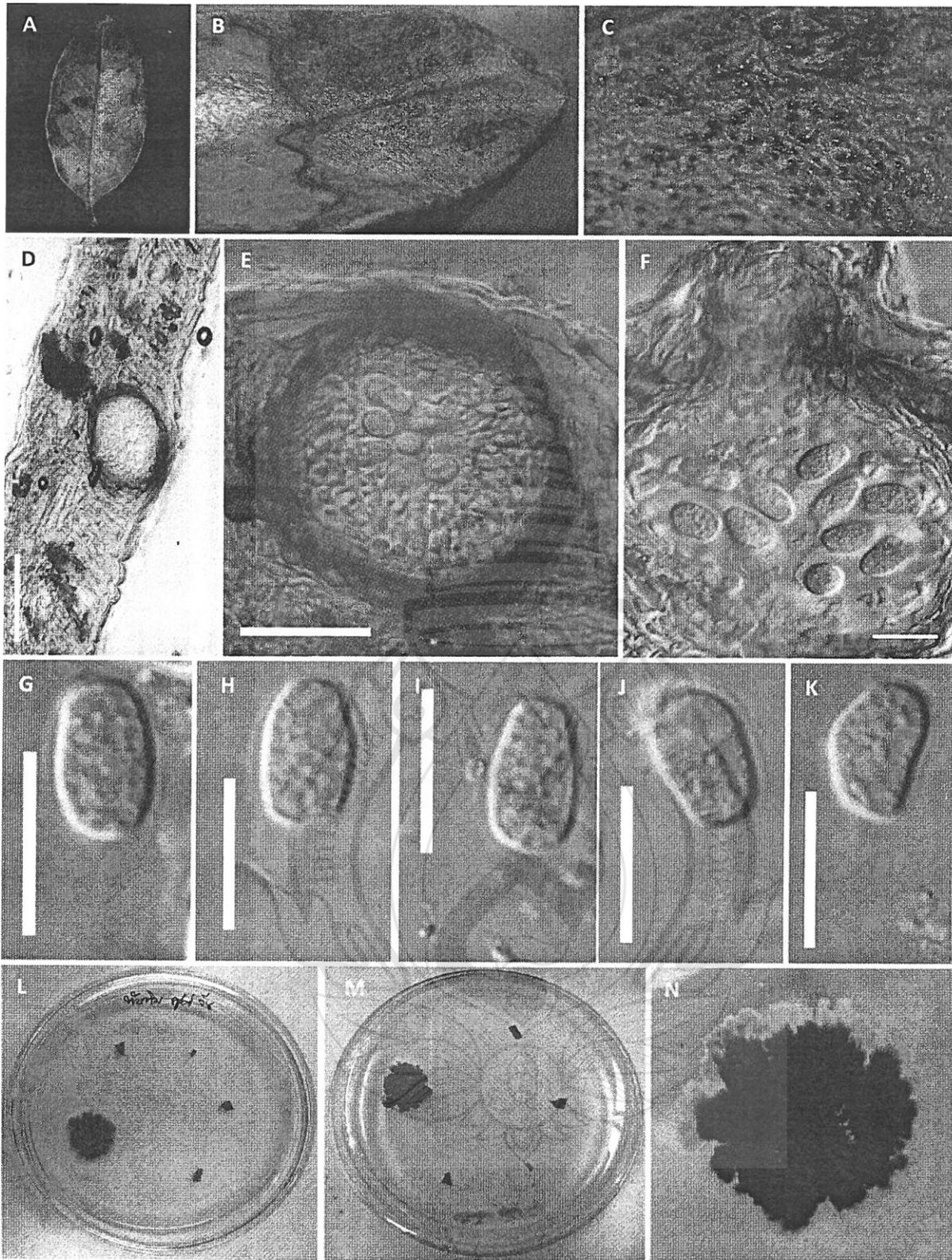


Fig. 4-4 Pycnidia growing on infected leaf of *Punica* (A-C) Cross section through pycnidia showing part of the pycnidia wall with developing conidia(D-F); scale bar = 50 μ m. conidia (G-K); scale bar = 10 μ m. Upper and reverse of cultures after 3 weeks.

5. *Phyllosticta* on *Ophiopogon japonica*

Host: *Ophiopogon japonica*

Symptom: Black spot

Habitat: Living leaf

Collecting Site: Khun Korn Waterfall, Mae Lao, Chiangrai

Pycnidia epiphyllous, circular, black, 0.1 mm diam., wall composed of 1-layers, *Conidiogenous cells* lining wall of pycnidium, phialidic, cylindrical, hyaline, 2–3×6–8 µm. *Conidia* ellipsoidal, hyaline, 1-celled, smooth-walled, 9–12×5–6 µm, surrounded by mucilaginous sheath, bearing single apical appendage, usually 6–9 µm long. *Leptodothiorella* state, 120–126 µm long, 45–50 µm wide, 12–20 µm thick, *Spermatia* are produced from spermatogenous cells, cylindrical and globose at two ends 6.5–8×2.0–2.3 µm.

Colonies black, fimbriat, black in reverse, 3–5 cm in diam. after 21 days of incubation on half – PDA.

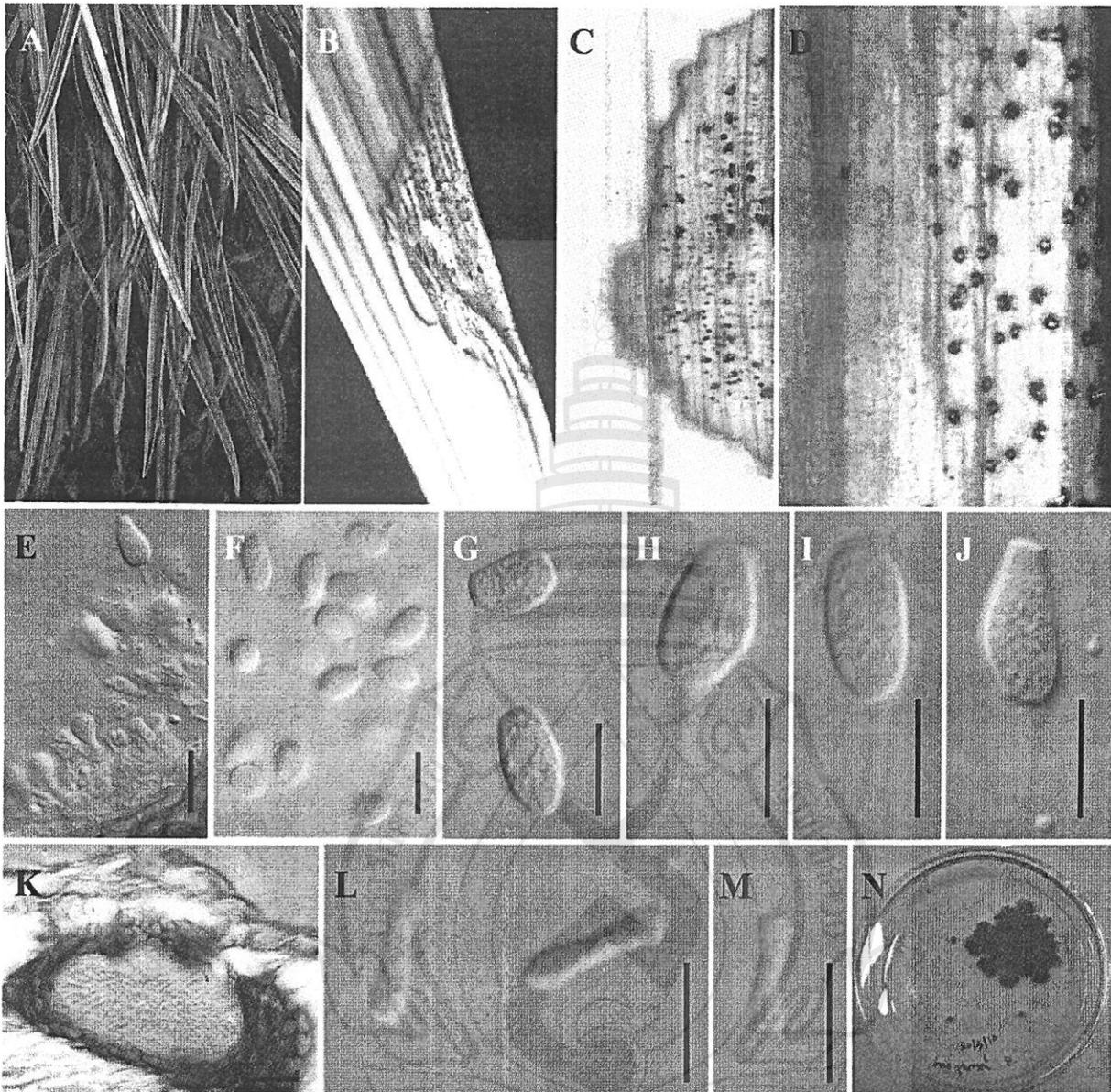


Fig.4-5 Symptom of disease (A) Pycnidia growing on infected leaf of *Ophiopogon jaburan* (B–D) Cross section through pycnidia showing part of the pycnidial wall with developing conidia (E) Conidiogenous cell (E) conidia (F–J) Cross section through *Leptodothiorella* state (K) scale bar= 50μm Spermatia are produced from spermatogenous cells (L) Spermatia (M) Upper of cultures after 5 weeks (N); scale bar = 10 μm.

6. *Phyllosticta* on *Crinum asiaticum*

Teleomorph stage: *Guignardia* sp.

Host: *Crinum asiaticum* L.

Symptom: Black spot

Habitat: Living leaf

Collecting Site: Mae Jo, Chiangmai

Ascomata epiphyllous, circular, black, 0.2 mm diam., 90–100 × 120–130 µm, wall composed of 1-layers, 10–12 µm thick, black. *Asci* clavate, 6-spored, 33–56 µm long, 10–12 µm wide, bitunicate, wall 2.1–2.3 µm thick. *Ascospores* cylindrical but swollen in the middle, hyaline, 1-celled, smooth-walled, surrounded by mucilaginous sheath, 3–4 × 12–14 µm, overlapping biseriate. *Pycnidia* epiphyllous, associated with ascomata, circular, black, 0.1 mm diam., wall composed of 1-layers, black, inside consisting *Conidiogenous cells* lining wall of pycnidium, phialidic, cylindrical, hyaline. *Conidia* ellipsoidal, hyaline, 1-celled, smooth-walled, 9–10 × 4–5 µm surrounded by mucilaginous sheath 1.1–1.4 µm thick bearing single apical appendage, usually 2.3–4 µm long.

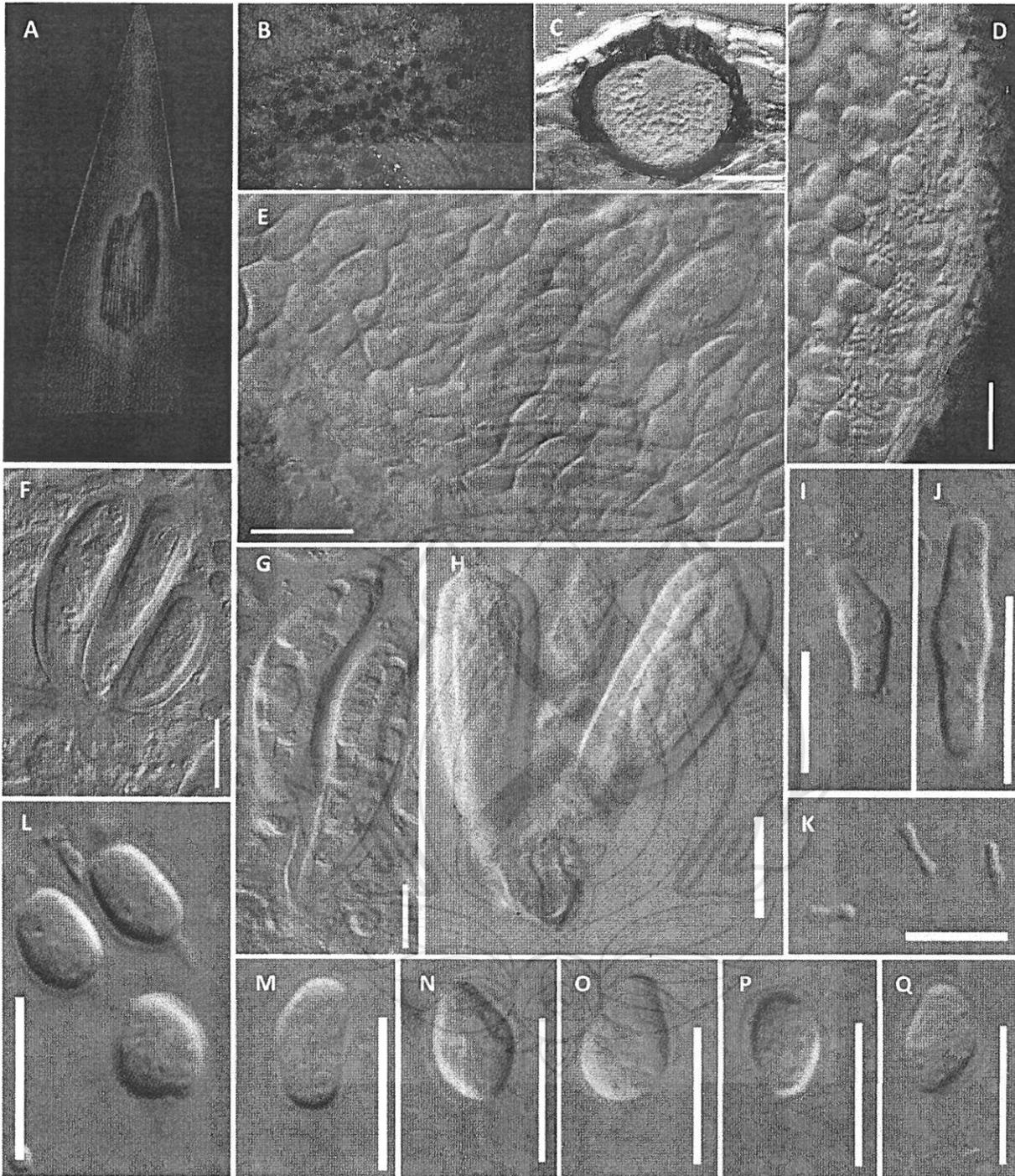


Fig.4-6 Ascomata growing on infected *Crinum asiaticum* L. (A-B) Cross section through ascomata (C); scale bar= 10 μ m , Conidiophore (D-E) asci (F-H) ascospores (I-J) Spermatia (K) conidia (L-Q); scale bar = 10 μ m.

7. *Guignardia on Dracaena lourieri*

Anamorph stage: *Phyllosticta* sp.

Host: *Dracaena lourieri*

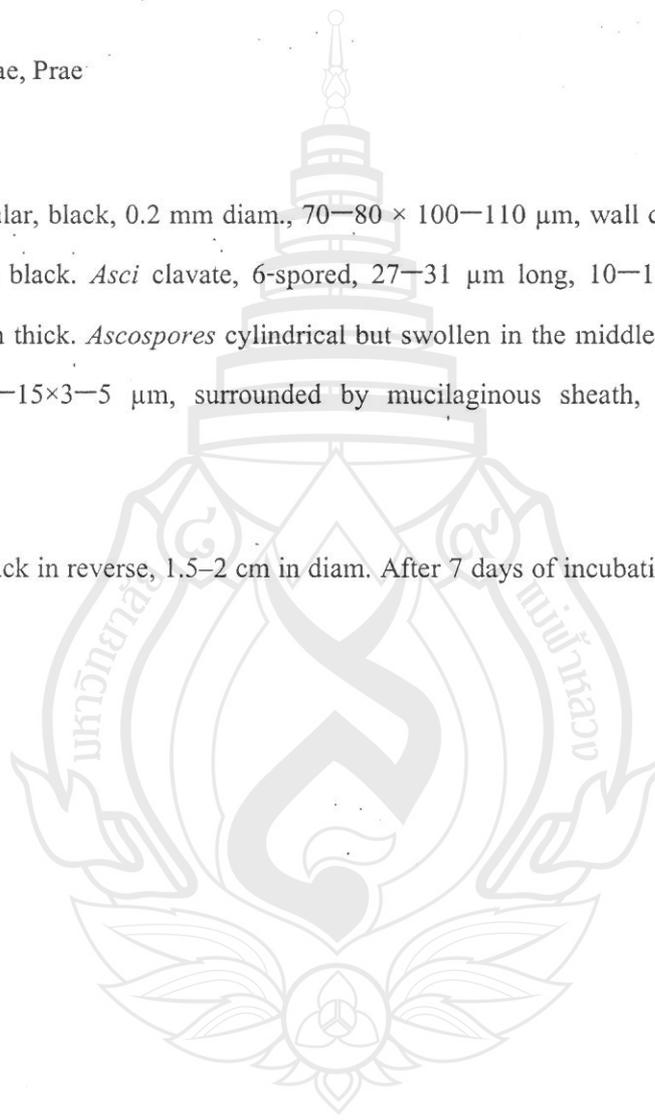
Symptom: Black spot

Habitat: Living leaf

Collecting Site: Mae Jo—Prae, Prae

Ascomata epiphyllous, circular, black, 0.2 mm diam., 70–80 × 100–110 μm, wall composed of 1-layers, 10–12 μm thick, black. *Asci* clavate, 6-spored, 27–31 μm long, 10–12 μm wide, bitunicate, wall 1.2–2.2 μm thick. *Ascospores* cylindrical but swollen in the middle, hyaline, 1-celled, smooth-walled, 13–15 × 3–5 μm, surrounded by mucilaginous sheath, overlapping biseriate.

Colonies black, fimbriat, black in reverse, 1.5–2 cm in diam. After 7 days of incubation on half – PDA.



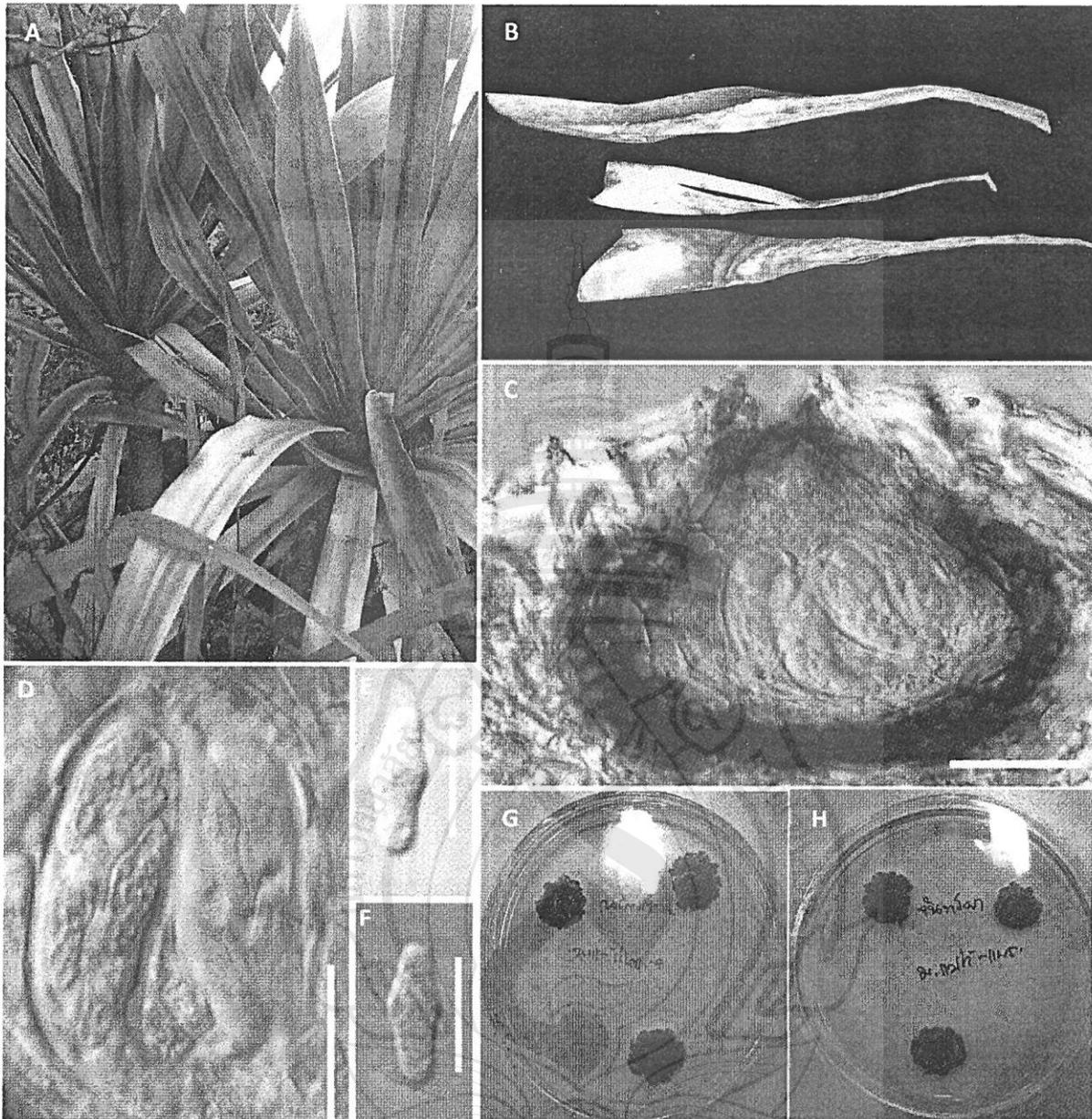


Fig. 4-7 Ascomata growing on infected *Dracaena lourieri* (A-B) Cross section through ascomata (C); scale bar = 20 μm , asci (D) ascospore (E-F) Upper and reverse of cultures after 1 week (G-H); scale bar = 10 μm .

8. *Guignardia on Spathiphyllum wallisei*

Anamorph stage: *Phyllosticta* sp.

Host: *Spathiphyllum wallisei*

Symptom: Black spot

Habitat: Living leaf

Collecting Site: Pa Kha, Phan, Chiangrai

Ascomata epiphyllous, circular, black, 0.2 mm diam., *Asci* clavate, 6-spored, 44–48 μm long, 9–12 μm wide, bitunicate, wall 1.4–2 μm thick. *Ascospores* cylindrical but swollen in the middle, hyaline, 1-celled, smooth-walled, surrounded by mucilaginous sheath, 11–13 \times 2.6–3.5 μm , overlapping biseriate. *Pycnidia* epiphyllous, associated with ascomata, circular, black, 0.1 mm diam., wall composed of 1-layers, 100–110 \times 65–70 μm 13–15 μm thick, black, inside consisting *Conidiogenous cells* lining wall of pycnidium, phialidic, cylindrical, hyaline. *Conidia* ellipsoidal, hyaline, 1-celled, smooth-walled, 9–11 \times 4–6.5 μm , surrounded by mucilaginous sheath 0.7–1.2 μm thick bearing single apical appendage, usually 3–5 μm long.

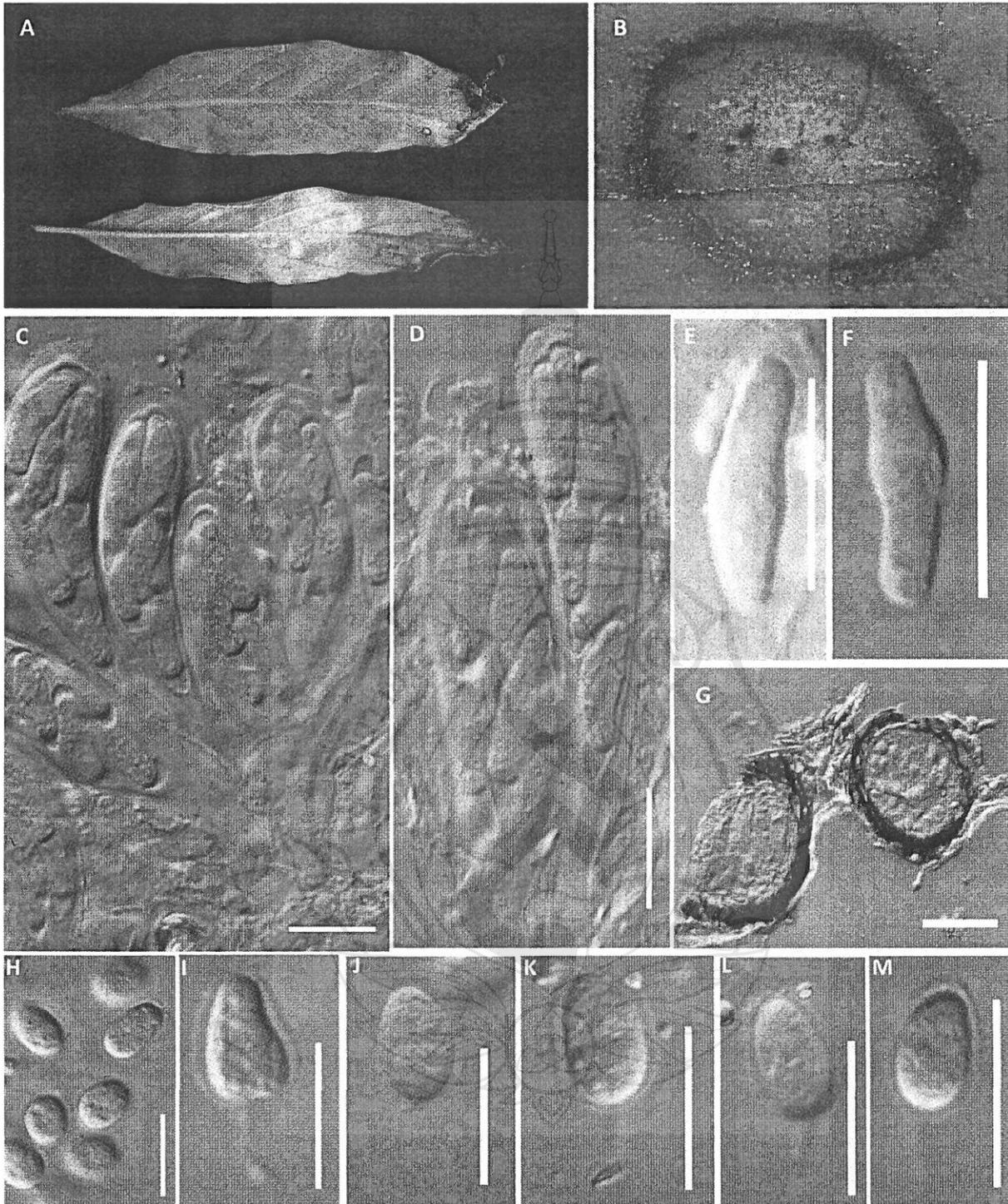


Fig. 4-8 Ascomata growing on infected *Spathiphyllum wallisei* (A-B) Cross section through ascomata (G) asci (C-D) ascospore (E-F) conidia (H-M); scale bar = 10 μ m.

9. *Guignardia* on *Cattleya* John Lindley

Teleomorph stage: *phyllosticta* sp.

Host: *Cattleya* John Lindley

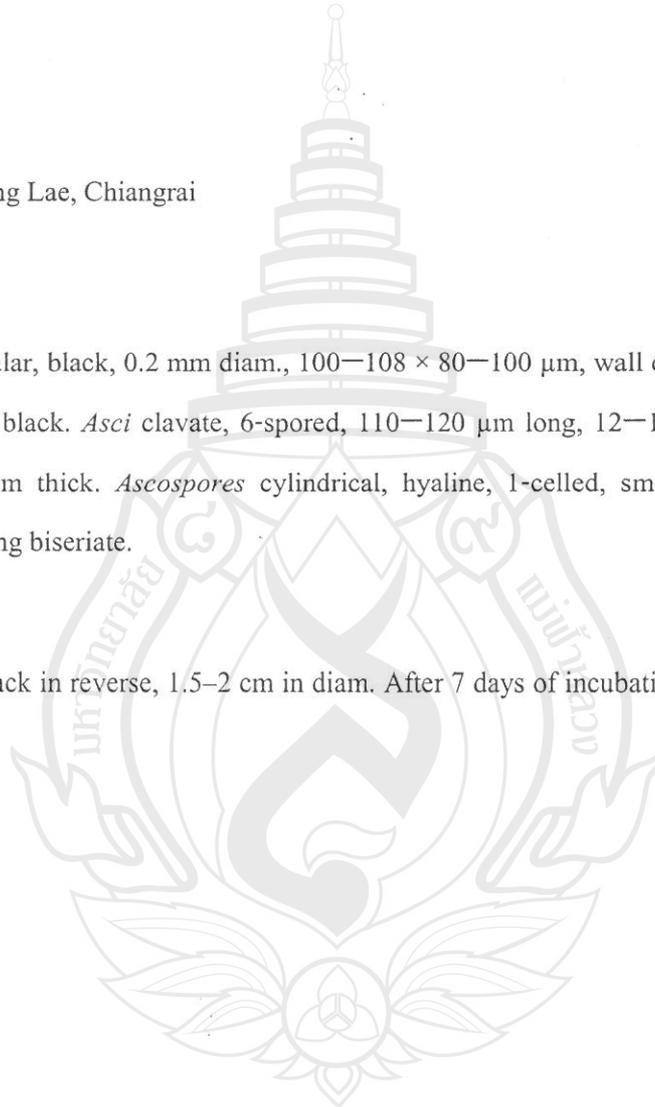
Symptom: Black spot

Habitat: leaf

Collecting Site: Pasang, Nang Lae, Chiangrai

Ascomata epiphyllous, circular, black, 0.2 mm diam., $100-108 \times 80-100 \mu\text{m}$, wall composed of 1-layers, 11–13 μm thick, black. *Asci* clavate, 6-spored, 110–120 μm long, 12–14 μm wide, bitunicate, wall 1.1–1.4 μm thick. *Ascospores* cylindrical, hyaline, 1-celled, smooth-walled, $20-26 \times 6-7 \mu\text{m}$, overlapping biseriate.

Colonies black, fimbriat, black in reverse, 1.5–2 cm in diam. After 7 days of incubation on half – PDA.



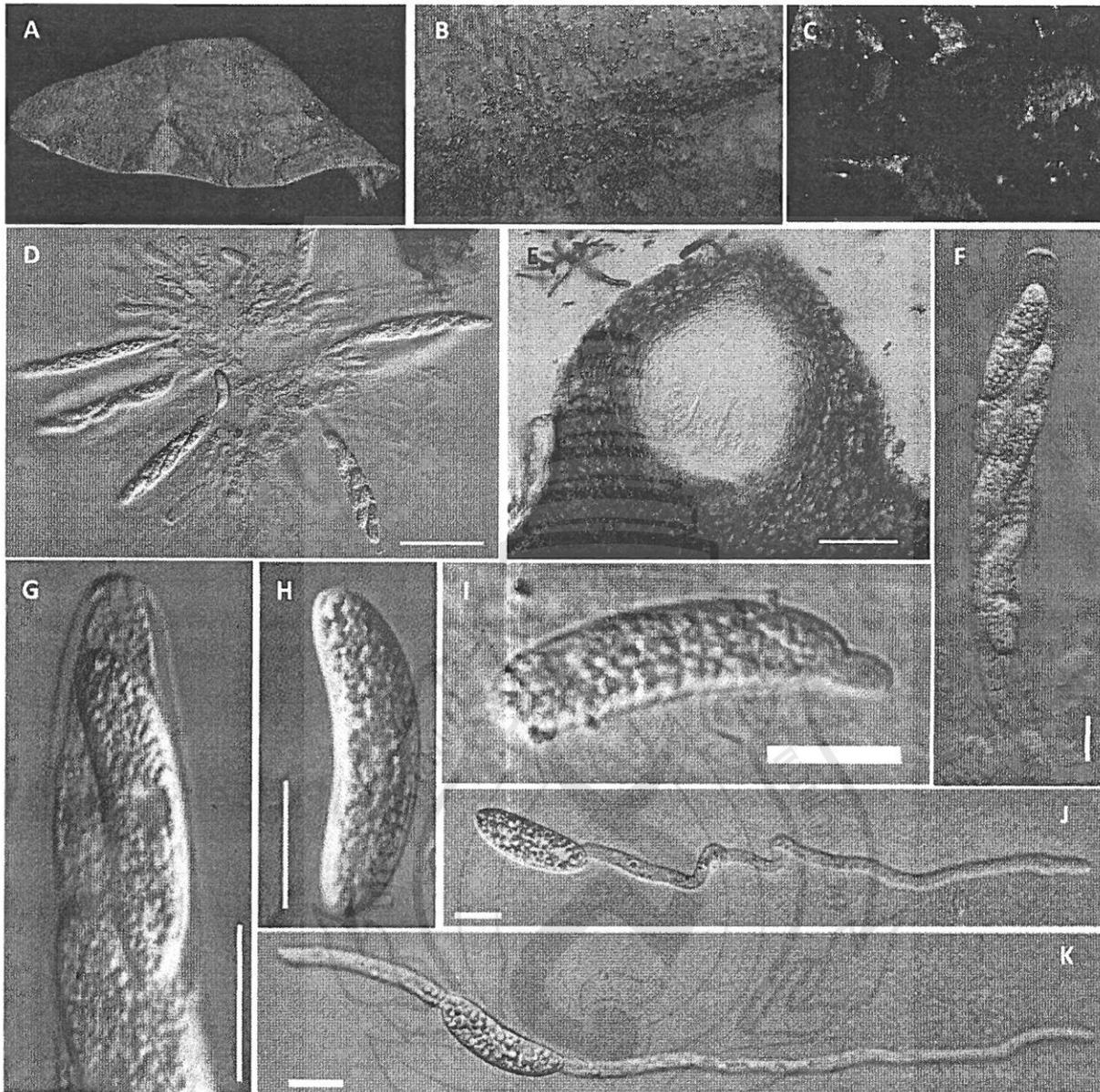


Fig. 4-9 Ascomata growing on infected *Cattleya* John Lindley (A–C) Cross section through Ascomata (D–E); scale bar= 50 μm asci (F–G) ascospores(H) germinated spore (I–K) ; scale bar = 10 μm .

10. *Guignardia on Musa sapientum* Linn.

Teleomorph stage: *Phyllosticta* sp.

Host: *Musa sapientum* Linn.

Symptom: Black spot

Habitat: leaf

Collecting Site: Pasang, Nang Lae, Chiangrai

Ascomata epiphyllous, circular, black, 0.2 mm diam., 90–100 × 70–85 µm, wall composed of 1-layers, 10–12 µm thick, black. *Asci* clavate, 6-spored, 100–104 µm long, 16–20 µm wide, bitunicate, wall 2.3–3 µm thick. *Ascospores* ellipsoidal, hyaline, 1-celled, smooth-walled, surrounded by mucilaginous sheath, 3–4 × 8–10 µm, overlapping biseriate. *Pycnidia* epiphyllous, associated with ascomata, circular, black, 0.1 mm diam., wall composed of 1-layers, black, inside consisting *Conidiogenous cells* lining wall of pycnidium, phialidic, cylindrical, hyaline. *Conidia* ellipsoidal, hyaline, 1-celled, smooth-walled, 20–22 × 9–10 µm surrounded by mucilaginous sheath 2.6–3.0 µm thick bearing single apical appendage, usually 20–23 µm long.

Note: Could not be introduced spore germination on media agar by single spore isolaton.

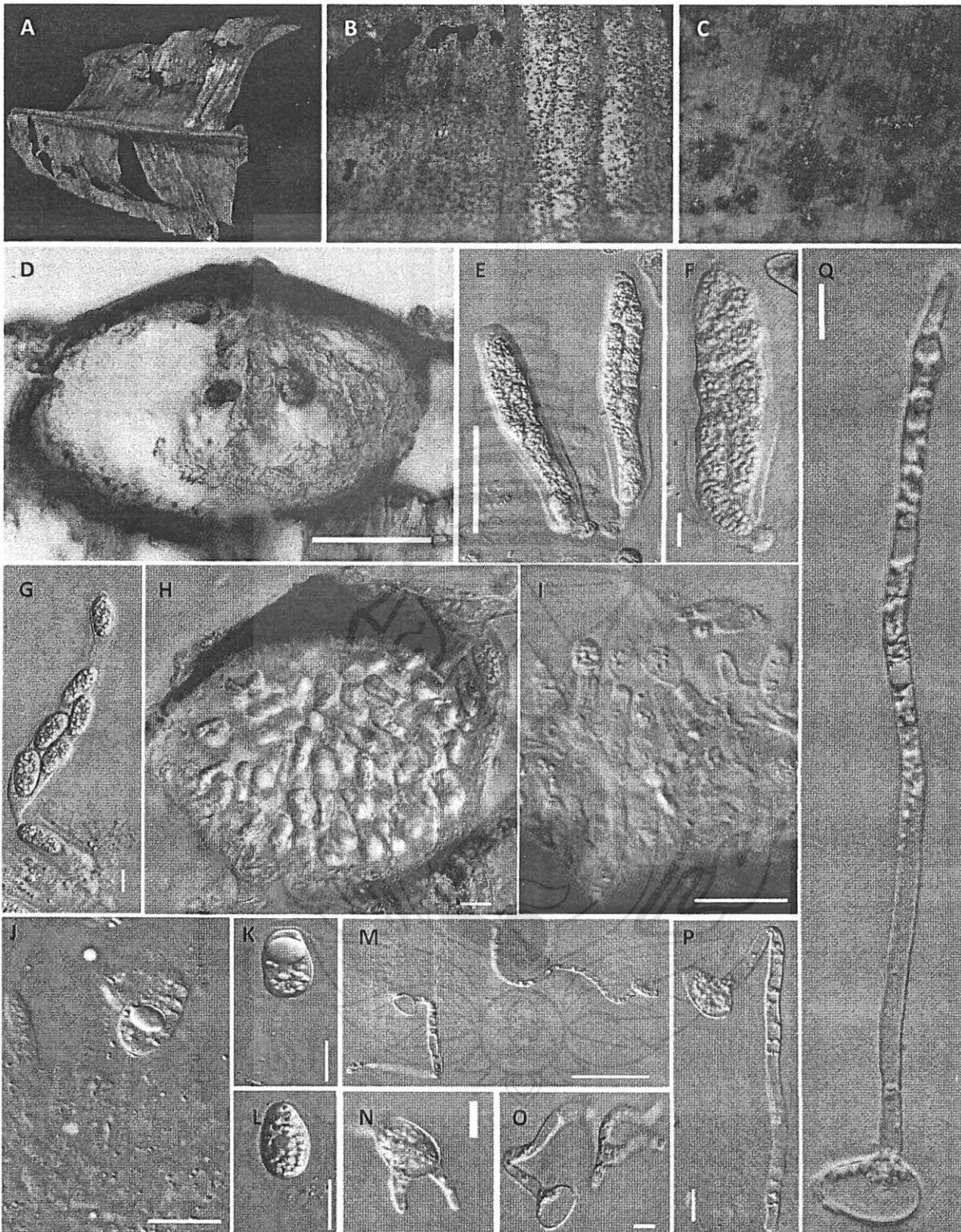


Fig. 4-10 Ascomata growing on infected *Musa sapientum* Linn. (A-C) Cross section through Ascomata (D)asci (E-G) Cross section through pycnidia showing part of the pycnidia wall with developing conidia (D-F); scale bar= 50 μ m conidia (J-L)Germinated spore (M-Q)

11. *Phyllosticta* on unidentified host

Teleomorph stage: *Guignardia* sp.

Host: unknown

Symptom: Black spot

Habitat: leaf

Collecting Site: Doi Inthanon, Chiangmai

Pycnidia epiphyllous, circular, black, 0.2 mm diam., wall composed of 2-layers, inner 90–100 μm wide, 120–130 μm long, 15–17 μm thick, black. Outer 121–130 μm wide 150–160 μm long, 23–35 μm thick, brown, *Conidiogenous cells* lining wall of pycnidium, phialidic, cylindrical, hyaline. *Conidia* ellipsoidal, hyaline, 1-celled, smooth-walled, 11–14 \times 9–10 μm surrounded by mucilaginous sheath 1.1–1.4 μm thick bearing single apical appendage, usually 8–9 μm long.

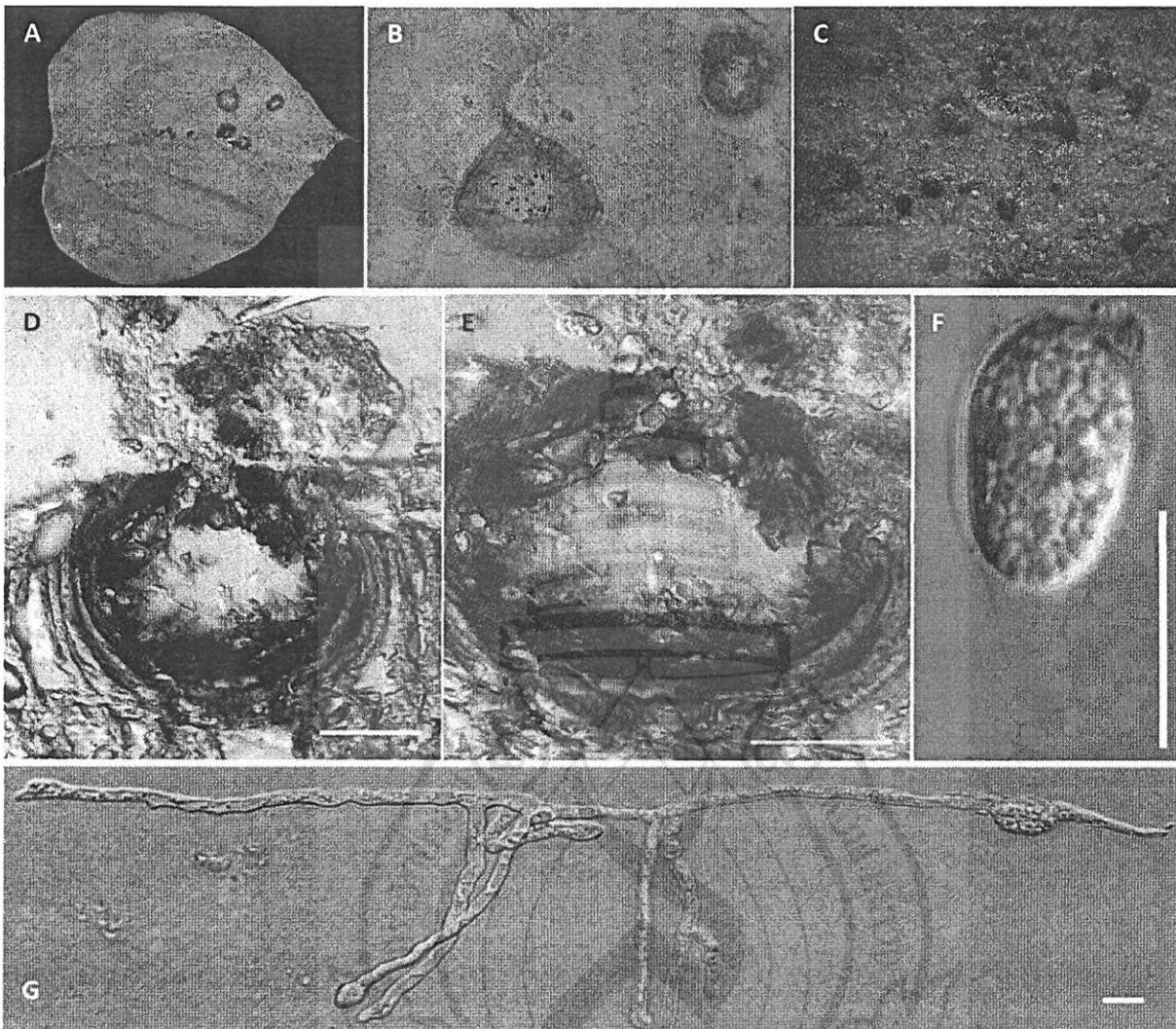


Fig. 4-11 Pycnidia growing on infected leaf of Unknown (A–C) Cross section through pycnidia showing part of the pycnidia wall with developing conidia (D–E); scale bar = 50 μm conidia (F) germinated spore(G); scale bar = 10 μm

12. *Phyllosticta* on *Cordyline*

Teleomorph stage: *Guignardia* sp.

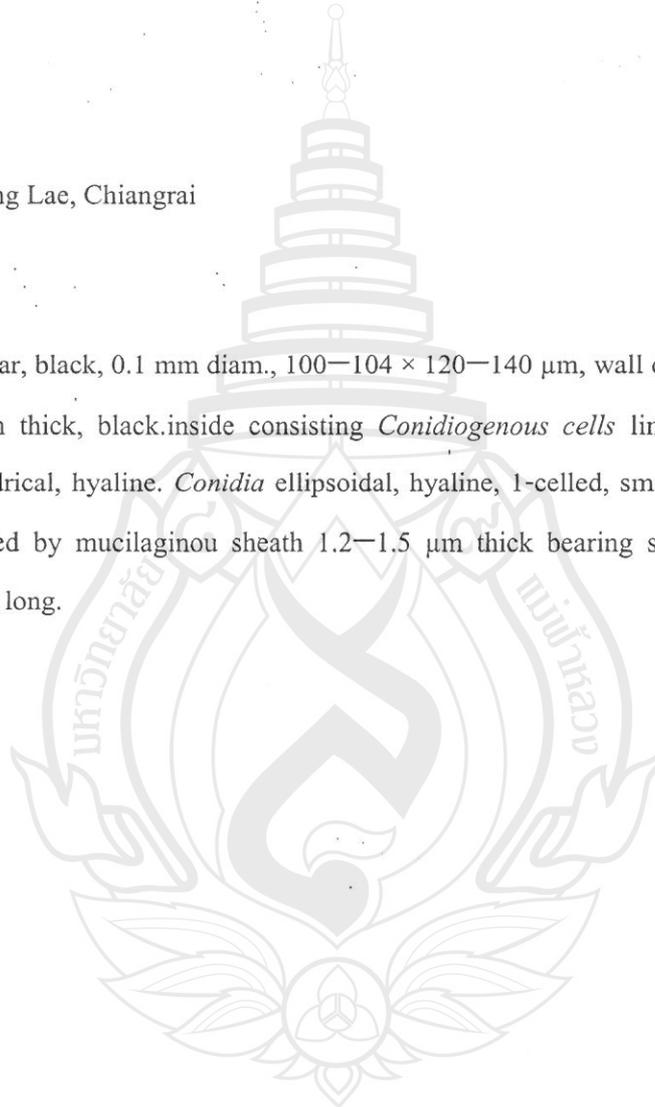
Host: cordyline

Symptom: Black spot

Habitat: leaf

Collecting Site: Pasang, Nang Lae, Chiangrai

Pycnidia epiphyllous, circular, black, 0.1 mm diam., 100–104 × 120–140 μm, wall composed of 1-layers, black, 10–12 μm thick, black inside consisting *Conidiogenous cells* lining wall of pycnidium, phialidic, cylindrical, hyaline. *Conidia* ellipsoidal, hyaline, 1-celled, smooth-walled, 10–11 × 6–7 μm surrounded by mucilaginous sheath 1.2–1.5 μm thick bearing single apical appendage, usually 4–5 μm long.



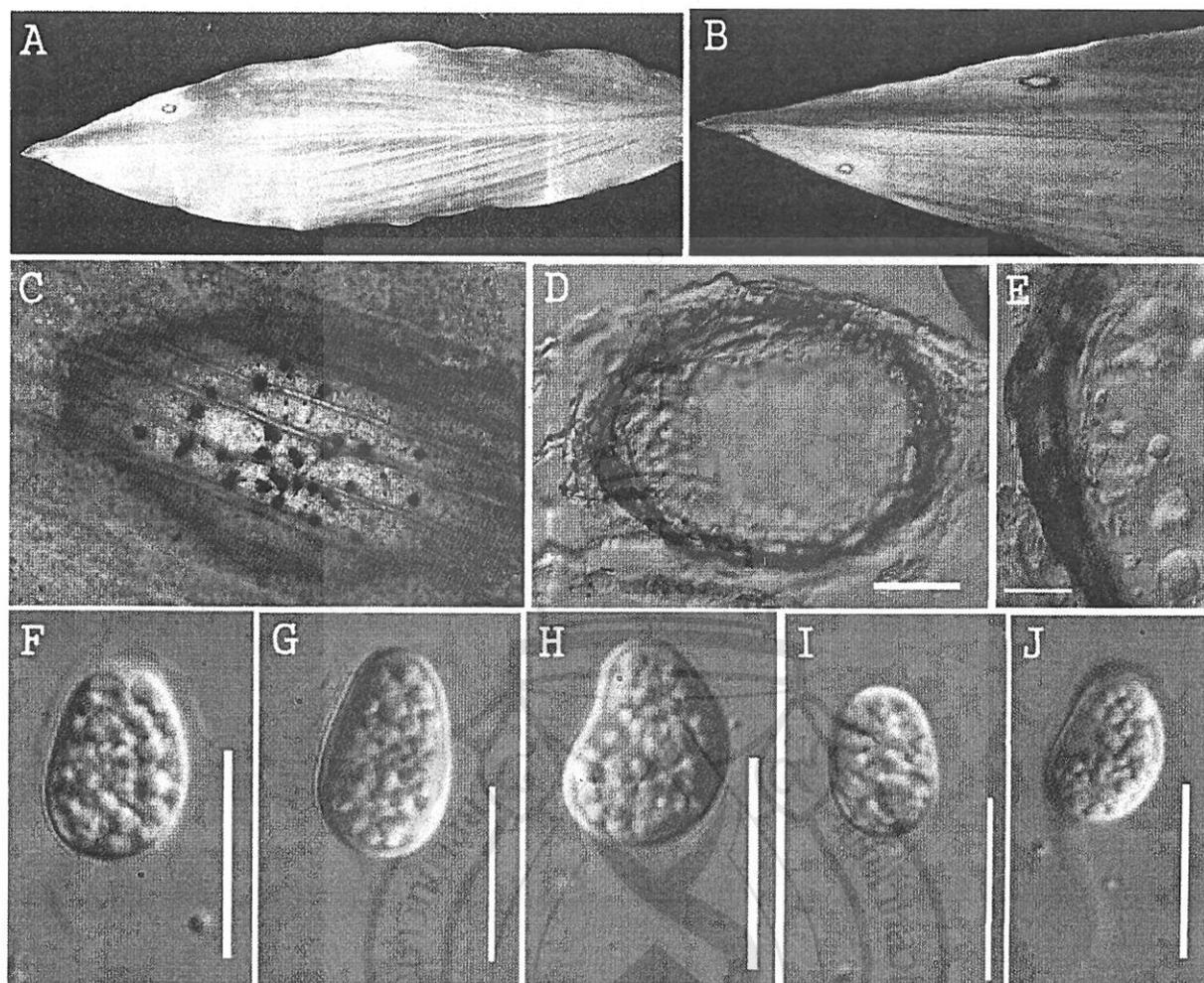


Fig. 4-12 Pycnidia growing on infected living leaf of *Cordyline* (A–C) Cross section through pycnidia showing part of the pycnidia wall with developing conidia (D); scale bar= 50 μ m. Conidiogenous cell (E) conidia (D–J) scale bar= 10 μ m

13. *Phyllosticta* on Liliaceae

Teleomorph stage: *Guignardia* sp.

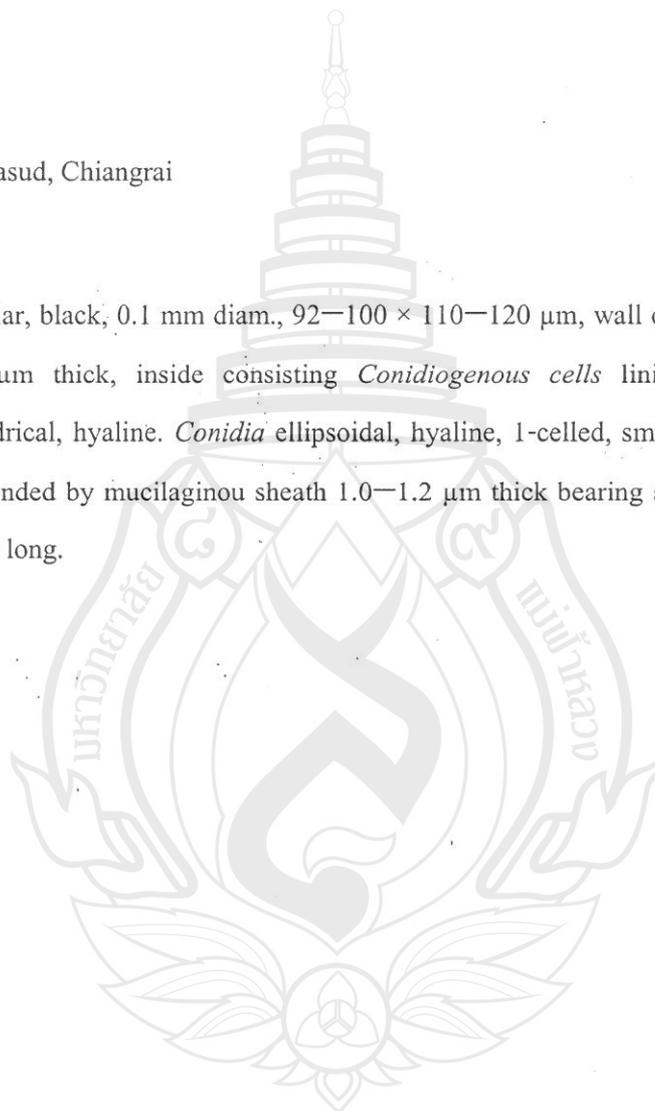
Host: LILIACEAE

Symptom: Black spot

Habitat: leaf

Collecting Site: Doi Op, Thasud, Chiangrai

Pycnidia epiphyllous, circular, black, 0.1 mm diam., 92–100 × 110–120 µm, wall composed of 1-layers, brown, 10–15 µm thick, inside consisting *Conidiogenous cells* lining wall of pycnidium, phialidic, cylindrical, hyaline. *Conidia* ellipsoidal, hyaline, 1-celled, smooth-walled, 11–12×6.2–6.8 µm surrounded by mucilaginous sheath 1.0–1.2 µm thick bearing single apical appendage, usually 5–7 µm long.



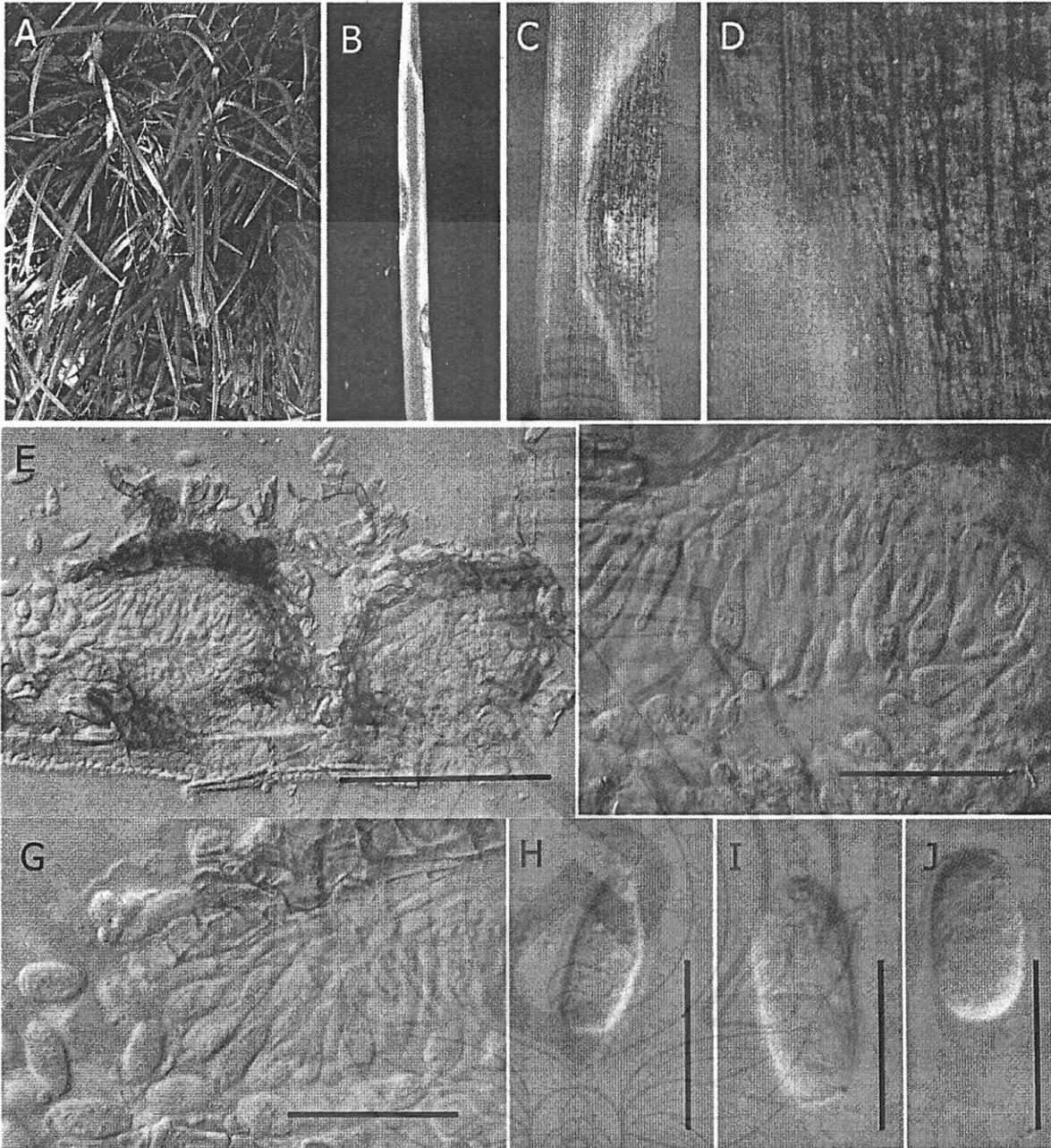


Fig. 4-13 Pycnidia growing on infected leaf of Liliaceae (A–D) Cross section through pycnidia showing part of the pycnidial wall with developing conidia (E) ; scale bar = 100 μm Conidiogenous cell (F–G) conidia (H–J) ; scale bar= 10 μm .

14. *Guignardia on elaeocarpus hygrophilus* Kurz

Anamorph stage: *Phyllosticta* sp.

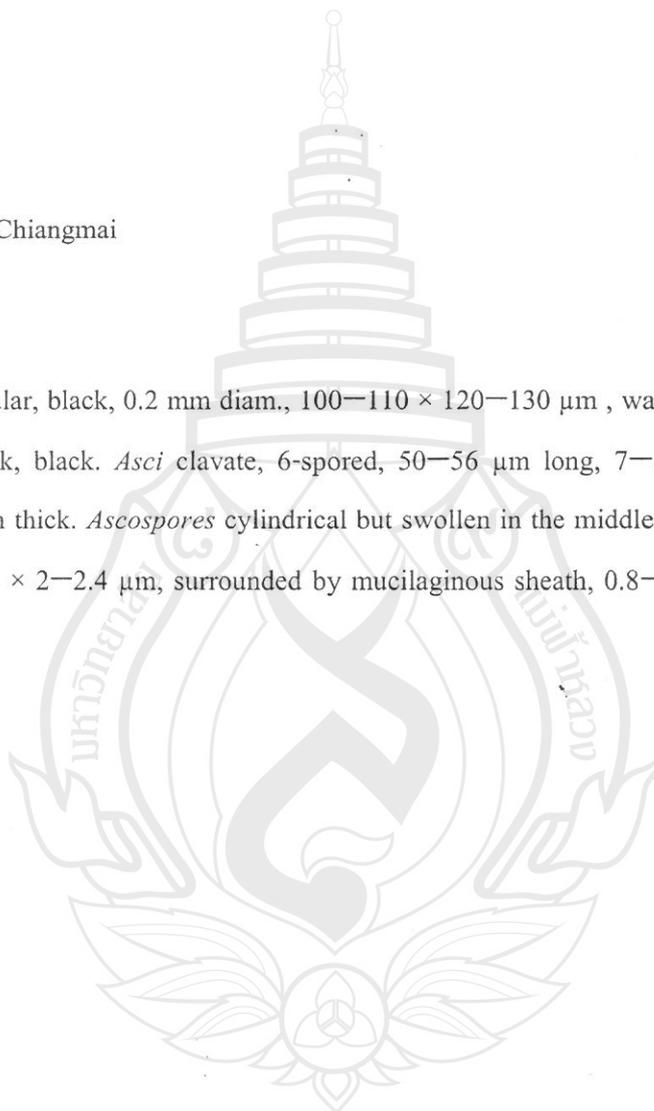
Host: *elaecarpus hygrophilus* Kurz

Symptom: Black spot

Habitat: Living leaf

Collecting Site: Mae Chan, Chiangmai

Ascomata epiphyllous, circular, black, 0.2 mm diam., 100–110 × 120–130 μm, wall composed of 1-layers, 9–12 μm thick, black. *Asci* clavate, 6-spored, 50–56 μm long, 7–8 μm wide, bitunicate, wall 0.8–1.2 μm thick. *Ascospores* cylindrical but swollen in the middle, hyaline, 1-celled, smooth-walled, 5–6 × 2–2.4 μm, surrounded by mucilaginous sheath, 0.8–1 μm thick overlapping biseriate.



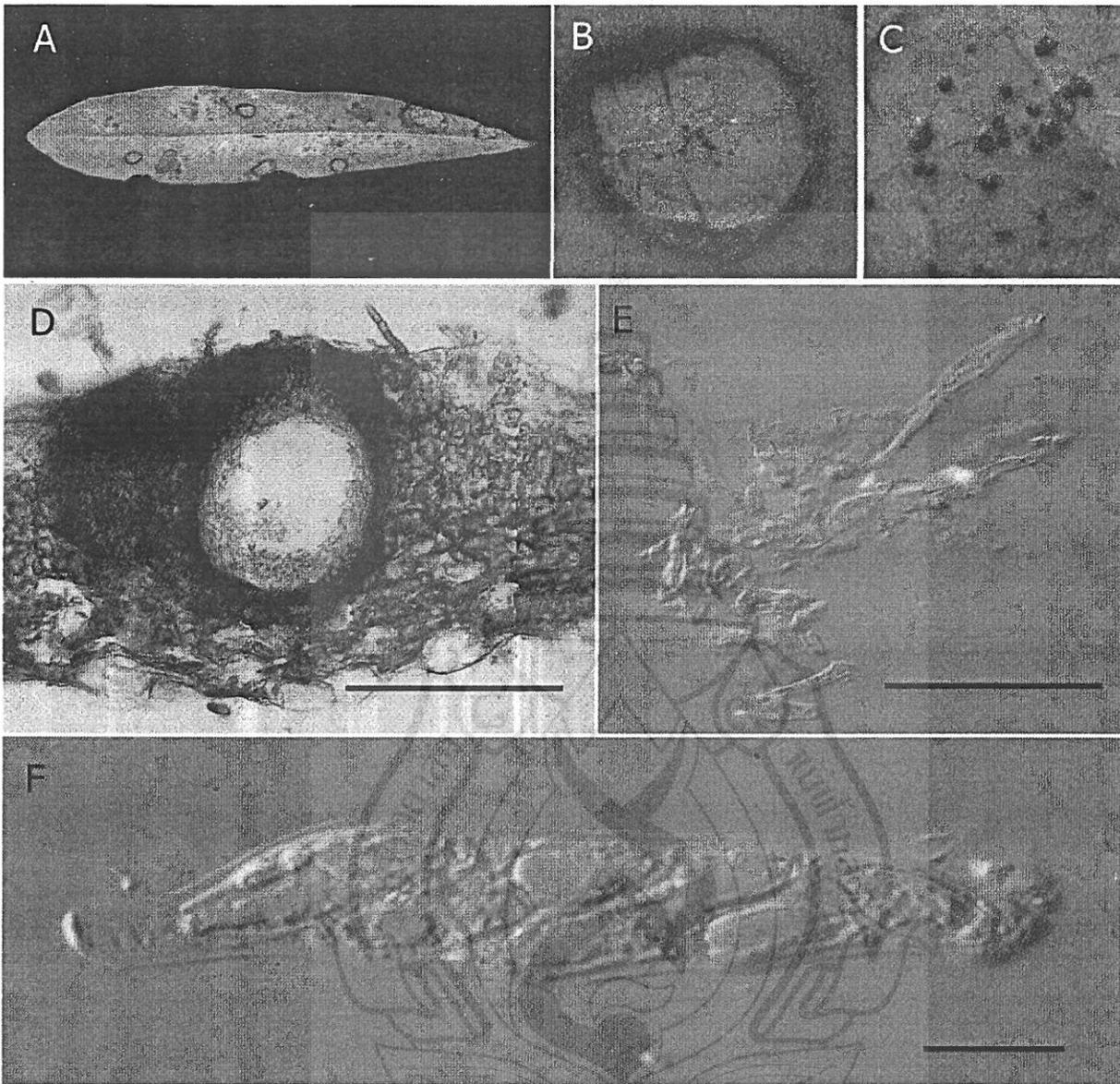


Fig. 4-14 Ascomata growing on infected *elaecarpus hygrophilus* Kurz (A-C) Cross section through perithecium showing ascospore with mucilaginous sheath (E) ; scale bar = 100 μm . Ascus (F) ; scale bar= 10 μm

15. *Guignardia* on Palm

Anamorph stage: *Phyllosticta* sp.

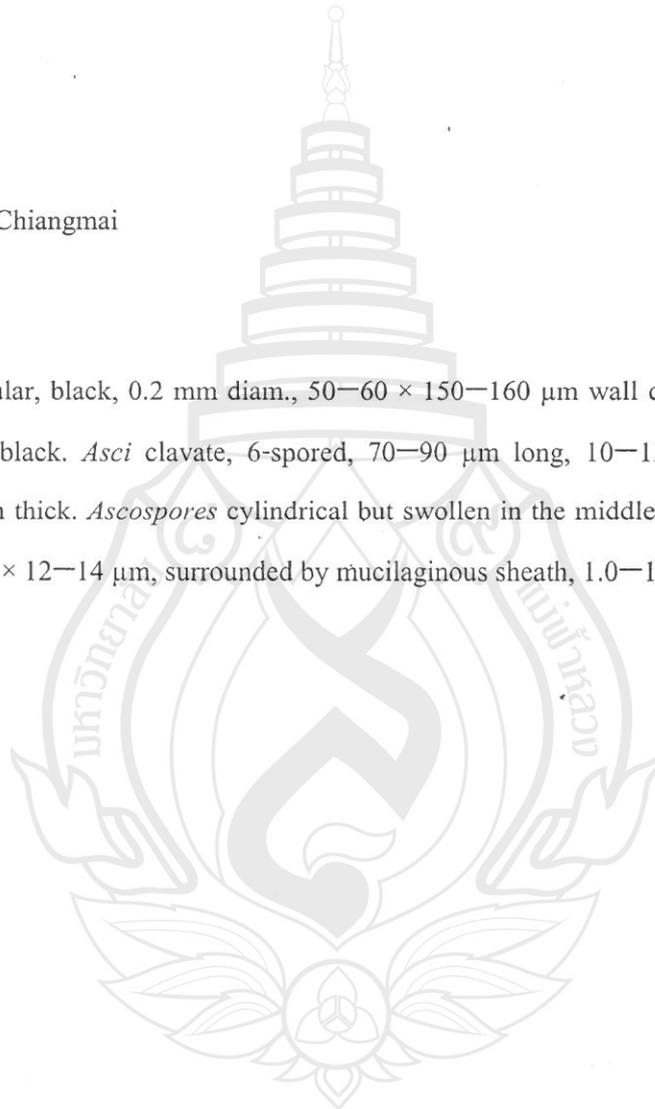
Host: Palm

Symptom: Black spot

Habitat: Living leaf

Collecting Site: Mae Chan, Chiangmai

Ascomata epiphyllous, circular, black, 0.2 mm diam., 50–60 × 150–160 µm wall composed of 1-layers, 9–11 µm thick, black. *Asci* clavate, 6-spored, 70–90 µm long, 10–12 µm wide, bitunicate, wall 1.2–1.5 µm thick. *Ascospores* cylindrical but swollen in the middle, hyaline, 1-celled, smooth-walled, 3–4 × 12–14 µm, surrounded by mucilaginous sheath, 1.0–1.3 µm thick, overlapping biseriate.



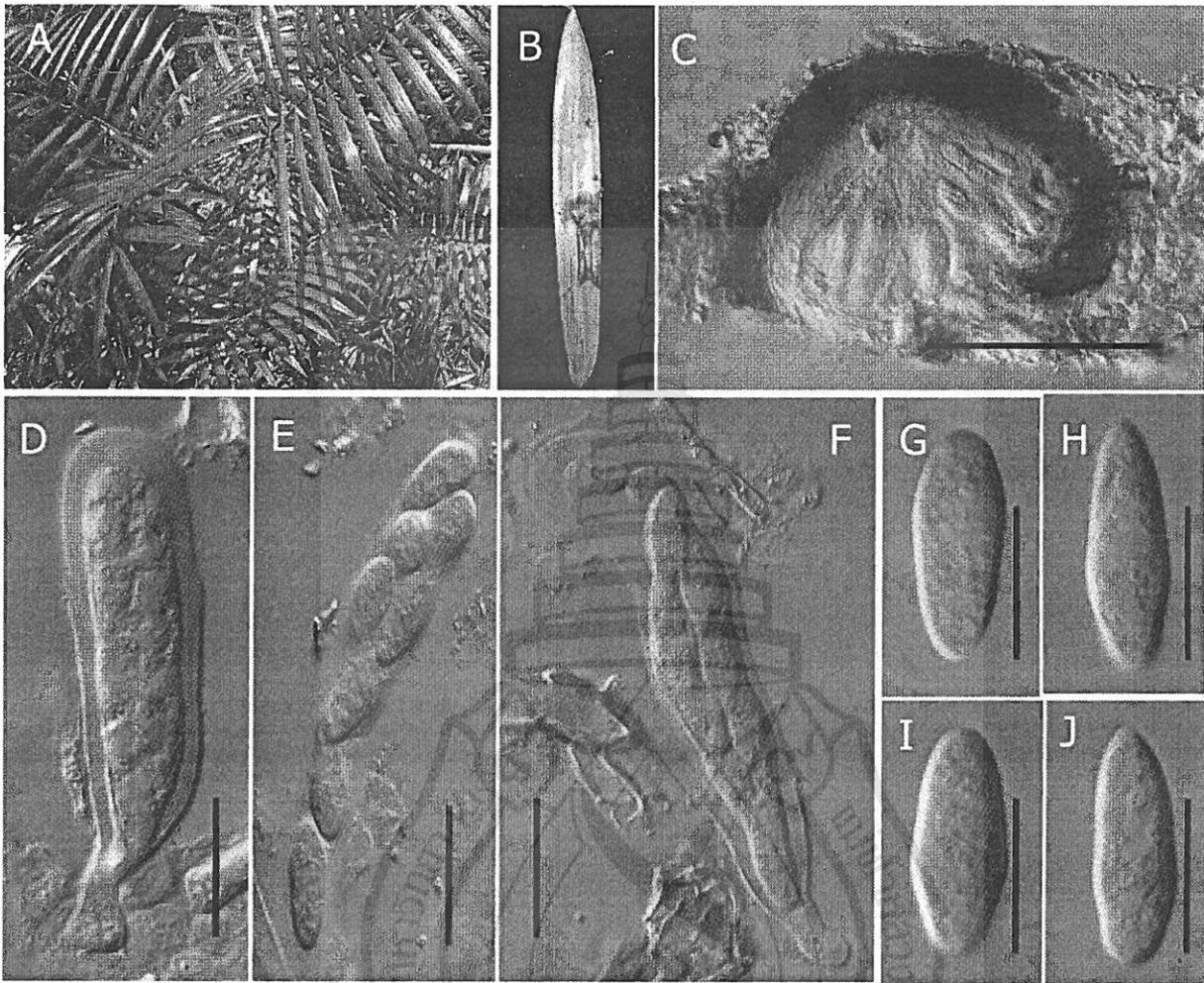


Fig. 4-15 Ascomata growing on infected palm (A–B) Cross section through perithecium showing ascospore with mucilaginous sheath (C) ; scale bar = 100 μm . Ascus (D–F) Ascospore (G–H) ; scale bar= 10 μm

Isolation of endophytic stage of *Phyllosticta*

After piece of explants put on agar plate, fungi in side the plant would grow out of explants to colonize on agar plate. Then fungi were moved to new PDA plate by cut on the tip of mycelium. *Phyllosticta* from many species of plants could be isolated by endophytic technique follow this direction;

1. Plant parts were sterile twice by 70% alcohol for 10-15 min for removed another fungi on surface.
2. Suspended in distilled water for 15 min.
3. Plant parts were cultured on PDA+antibiotic.
4. Incubated in room temperature for 1 week and observed ever other day.

In this study, *Phyllosticta* could be isolated from *Mangifera indica* L (Fig.4-16A) and *Croton Codiaeum* variegatum (Fig. 4-16B). Also from *Michelia champaca* L (Fig. 4-17F).

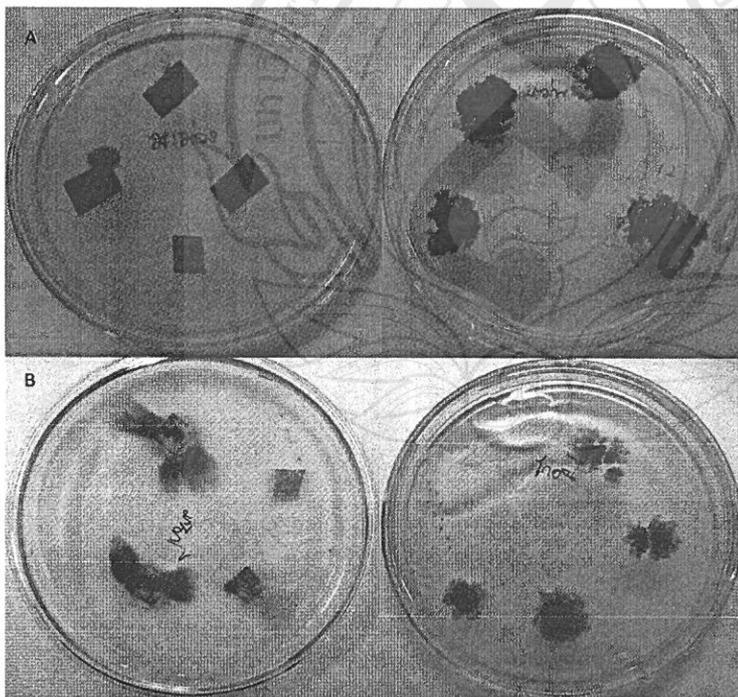


Fig. 4-16 *Phyllosticta* on *Mangifera indica* L. (A) *Croton Codiaeum* variegatum (B) isolated by endophytic technique

Moreover, endophyte stage of *Phyllosticta* could be found in Arecaceae (Fig 4-18)

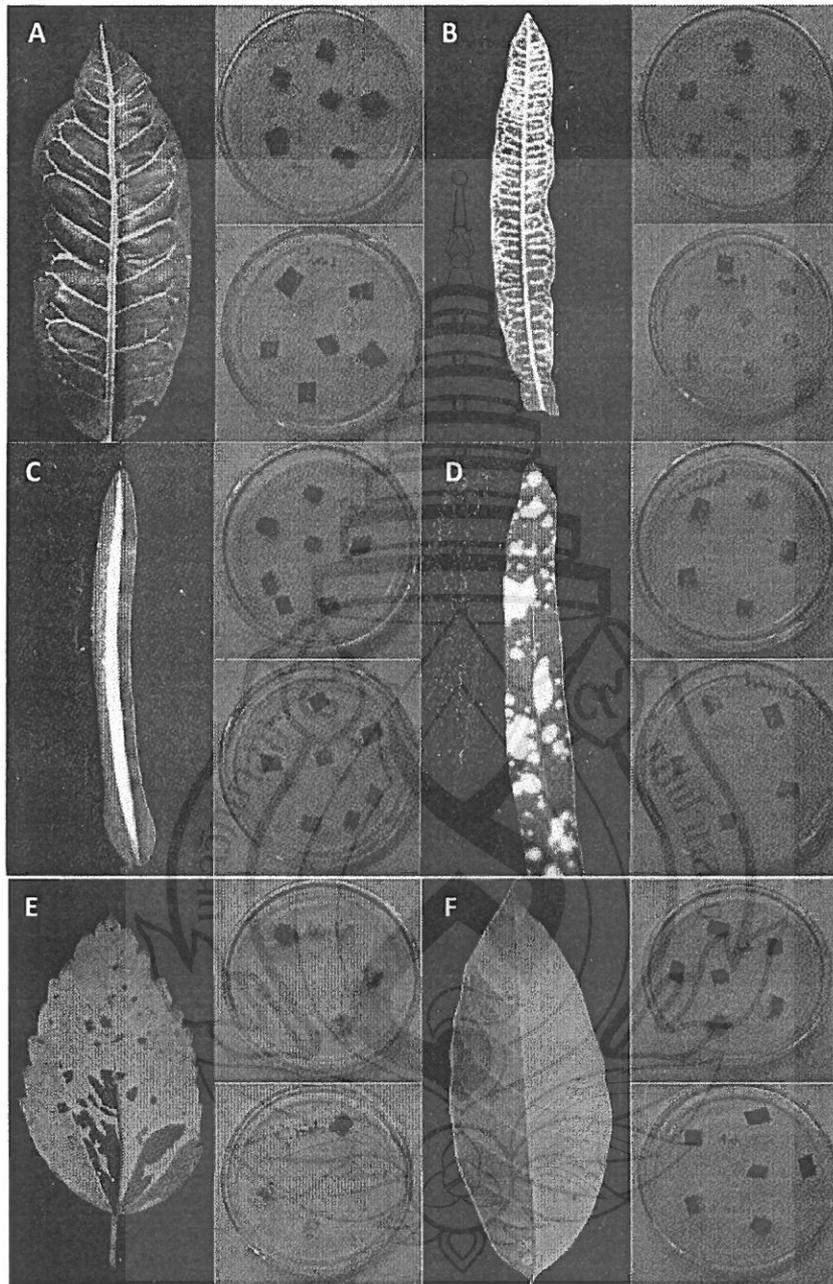


Fig. 4-17 Leaves from many plants were used in this experiment (A–D) Euphorbiaceae

(E) *Hibiscus rosa-sinensis* L. (F) *Michelia champaca* L.

About five species of Euphorbiaceae could observe *Phyllosticta* on media only one species.

Phyllosticta on ARECACEAE (Palm)

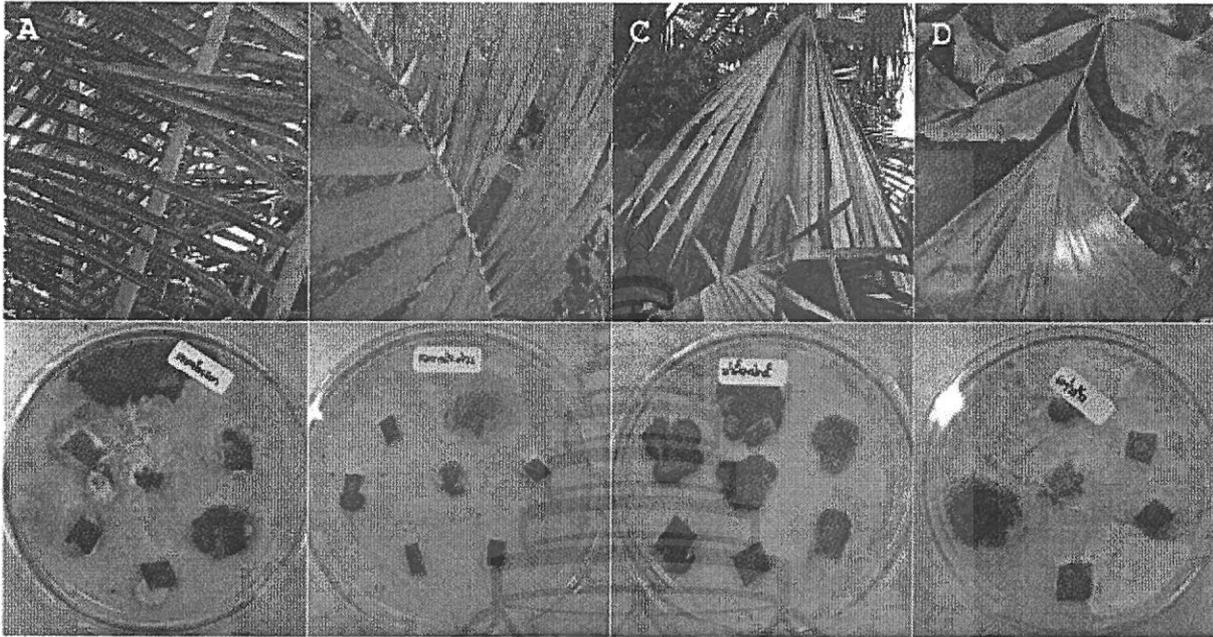


Fig.4-18 Arecaceae hosts were used in this study *Phyllosticta* (A) *Cocos nucifera*(B) *Dypsis lutescens* (C) *Caryota mitis*(D) *Borassus* sp.

From five species of Arecaceae, four species of plants host in this genus present *Phyllosticta* associated with this plant but *Phyllosticta* could not be harmful agent for host.

The germination profile

After *Phyllosticta* sp. was isolated by single spore isolation technique for 24 hrs. The germinated spore was transferred in potato dextrose agar and spores were observed germinating under microscope. Generally, spore would produce germ tube for penetrating through host layer. The mostly of fungi were produce mycelium and extend endlessly by apical growth. From this study, apical growths of *phyllosticta*'s conidia are usually found. The growth of germ tube would develop from appendage of conidia. Then genetic material and organelles were moved to tip of mycelium (Fig. 4-19).

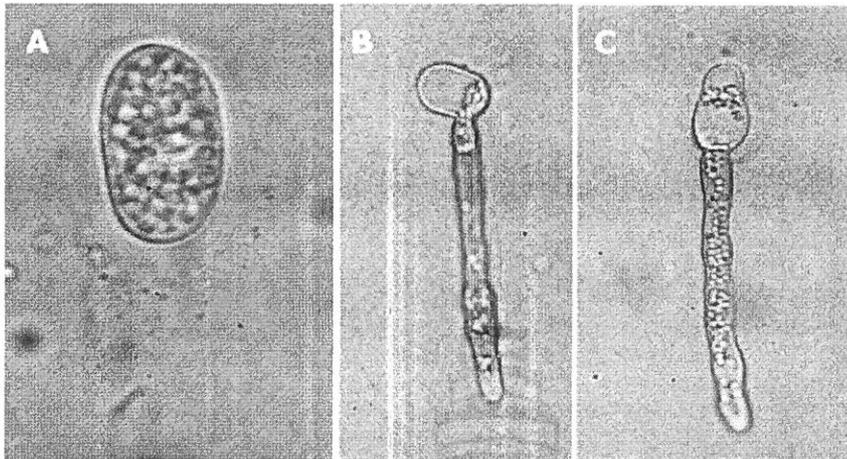


Fig. 4-19 Development of spores of *Phyllosticta musarum*

From observation, there is another characteristic of germination of conidia for *phyllosticta musarum*. In fig4-20 germ tube was developed from appendage of conidia. And all of material and organelles in conidia were moved to tip of germ tube.

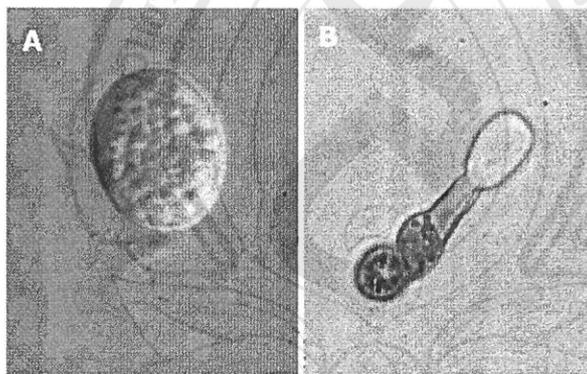


Fig. 4-20 Germinated spores of *Phyllosticta musarum*

After culture were deposited in MFLU Culture Collection and were sent to China for molecular work. Therefore, *Phyllosticta* cultures were analyzed for relation among those species.

CHAPTER 5

Conclusion

Probably the most significant finding of this study is the new species described from *Citrus maxima* (Pomelo) which causes tan spot on fruits. This disease was originally thought to be caused by *Phyllosticta citrocarpa*, an organism that is subject to quarantine control in the European Union. Subsequent molecular work on Thailand and Chinese *Citrus* specimens confirmed that these are distinct species with *P. citrocarpa* occurring on orange and other *Citrus* species and *P. citroasiana* being specific to Pomelo. This finding will mean that Pomelo will no longer be subject to quarantine control in the European Union since Pomelo does not grow in these countries.

Phyllosticta species are common on many hosts in Thailand and some species are host specific. Species of *Phyllosticta* and *Guignardia* sp.(teleomorph) may be pathogenic on a wide range of hosts in northern Thailand for example, *Arthocarpus heterophyllus*, *Dioscorea penthapylla*, *Caryota* sp., *Citrus maxima*, *Dendrobium* sp., *Dioscorea bulbiferae*, *Dracaena sanderiana*, *Jasminum sambac*, *Ficus benjamina* L. var. *variegata*, *Musa acuminata*, *Musa paradisiacal*, *Ophiopogon japonicus*, *Pandanus amaryllifolia*, *Shorea* sp., *Sphatolobus suberectus*, *Vanda* sp. They may also be endophytic in plants such as *Michelia champaca* L., *Hibiscus rosa-sinensis* L., and various genus of host plant such as *Arecaceae*, *Euphorbiaceae*, and *Magnoliaceae*. About 100 collections of *Phyllosticta*/*Guignardia* taxa recorded so far in northern Thailand and all specimens have been placed in Mae Fah Luang University herbarium and 53 cultures have been deposited in Mae Fah Luang University Culture Collection. There have been three new species described resulting from this studied as following; *Guignardia bispora* and *G. ellipsoidea* from palm and *Phyllosticta ophiopogonis* sp. nov. from *Ophiopogon japonicus* (*Liliaceae*) and we have re-examined the cause of banana freckle

The isolation techniques for this genus could be (1) single spore isolation (2) pathogen plant part technique and (3) endophytic fungi isolation, depending on the species and specimen. In some species of *Phyllosticta* on *Musa*, conidia could not be grown in agar media by single

spore isolation technique then pathogen plant part technique and endophytic fungi isolation could be used for isolation of *Phyllosticta musarum*.

Future work will investigate more species at the molecular level and result in a comprehensive understanding of the genus.



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Appendix A Publications



Guignardia morindae frog eye-leaf spotting disease of *Morinda citrifolia* (Rubiaceae)

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Wulandari NF, To-Anun C, Hyde KD. 2010 – *Guignardia morindae* frog eye leaf spotting disease of *Morinda citrifolia* (Rubiaceae). *Mycosphere* 1(4), 325–331.

Frog eye disease of leaves of *Morinda citrifolia* (Rubiaceae) was studied in Indonesia and Thailand. The causative species, *Guignardia morindae*, differs from species of *Guignardia* on other hosts by the distinct shape of its ascospores. The holotype for this taxon is missing, and therefore a neotype from Indonesia is designated. The species is illustrated from the neotype. New collections were also made from Thailand.

Key words – Disease record – Indonesia – New record – *Phyllosticta* – Taxonomy – Thailand

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Introduction

We are studying the important phytopathogen genus *Guignardia* and its anamorph *Phyllosticta* (Arx & Müller 1954, Reusser 1964, Aa 1973, Sivanesan 1984, Crous et al. 1993, Hyde 1995, Okane et al. 2001, Aa & Vanev 2002, Kobayashi 2003, Somritiphol & Hyde 2004, Motohashi et al. 2008a, b, Wulandari et al. 2009, Motohashi et al. 2010). A leaf spot caused by a species of *Guignardia* is very common on the plant *Morinda citrifolia* L. Frog eye leaf spot or shot-hole disease is caused by *G. morindae* (Koord.) Aa. This species was introduced as *Physalospora morindae* Koorders (1907) from Kedu Province, Central Java, Indonesia. This taxon was transferred as *Puiggarrina morindae* (Koord.) Speg. by Spegazzini (1919) and later as *G. morindae* (Aa 1973). Petrak & Sydow (1927) introduced the anamorph species *Phyllostictina morindae* Petr. & Syd from *Morinda citrifolia*, while Aa

(1973) transferred it as *Phyllosticta morindae* (Petr. & Syd.) Aa and linked it with the teleomorph *G. morindae*. Farr & Rossman (2010) list *G. morindae* from Australia, India, Indonesia, Japan and Samoa, but it is very common in Pacific island countries of American Samoa, Cook Islands, Federated States of Micronesia, Fiji, French Polynesia, Kiribati, Niue, Palau, Samoa, Tonga, Tuvalu, Vanuatu, Wallis and Futuna (Dingley et al. 1981, McKenzie 1989, 1990a,b, 1996, Shivas 1996).

Morinda citrifolia (Rubiaceae) is a highly prized medicinal plant. Noni juice extracted from the fermented fruit is marketed worldwide and has an estimated value of US\$2 billion annually. Most of the raw product comes from Polynesia. Crude extracts of *M. citrifolia* and *M. elliptica* L. have been shown to have antiviral activity against foot and mouth disease virus (Chungsamarnyart et al. 2007).

Genotoxic and antigenotoxic effects of noni fruit juice produced in Thailand had genotoxic and antigenotoxic effects on human lymphocytes in the chromosome aberration assay and sister chromatid exchange (SCE) assay *in vitro*. (Ratanavalachai et al. 2008, Thani et al. 2010). Noni appears to restore the normal menstrual cycle problems and alleviate menstrual symptoms in mice (Chearskul et al. 2004, Thani et al. 2010) and inhibits murine tumor growth with a definite curative potential in mice (Furusawa 2002). Mathivanan et al. (2005) reviewed current research on *Morinda citrifolia* while Rethinam & Sivaraman (2007) discussed research developments in India and elsewhere and reviewed the literature. The objective of the research is two fold. This paper provides an updated description of *G. Morindae* and, since the type specimen is lost a neotype is designated in order to stabilize the application of this species name.

Results

Collections of *Guignardia morindae* from three different locations are compared. A description and illustration from the neotype specimen is made and a neotype is designated here.

Taxonomy

Guignardia morindae (Koord.) Aa, *Stud. Mycol.* 5: 69 (1973)
Figs 1–3
MycoBank 314756

≡ *Physalospora morindae* Koord., *Verh. K. Akad. Vet. Amsterdam* 13(4): 190 (1907).

≡ *Puiggarina morindae* (Koord.) Speg., *Boletín de la Academia Nacional de Ciencias de Córdoba* 23: 486 (1919).

Anamorph *Phyllosticta morindae* (Petr. & Syd.) Aa, *Stud. Mycol.* 5: 69 (1973).

≡ *Phyllostictina morindae* Petr. & Syd., *Feddes Repert.*, Beih. 42: 200 (1927).

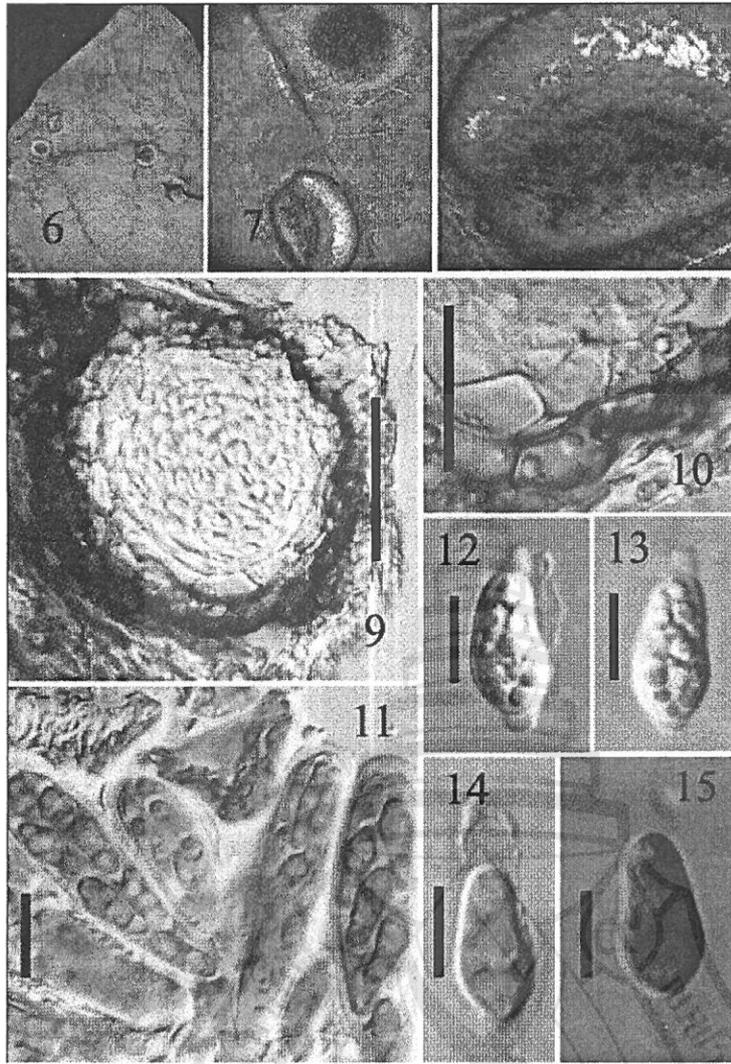
Causing frog eye or shot-hole leaf spots, 0.3–0.8 × 0.5–1.2 cm, which are rounded to irregular with red to dark brown borders; the area where the fungi sporulates is transparent and often falls from the leaf (Figs 1, 2, 3). Ascromata 60–120 µm diameter, 90–100 µm high, on the upper and lower surfaces of the leaves, black, globose to subglobose, immersed in plant tissues, coriaceous, clustered, ostiolate,



Figs 1–5 – *Guignardia* frog eye disease on leaves. 1–3 Symptoms on leaves. 4–5 Appearance of ascoma on the host surface.

ostioles as black dots in the centre (Figs 4, 5, 6, 7, 8). Peridium 10–15 µm wide, composed of two to three layers of cells, of *textura angularis*, pigmented outwardly and around ostiole and paler inside (Figs 9, 10, 16). Pseudoparaphyses hypha-like, 2–3 µm in diam. Asci 39–65 × 11–14 µm (\bar{x} = 50 × 12 µm, n = 20), 8-spored, bitunicate, fissitunicate, broadly cylindro-clavate, rounded at the apex, tapering gradually to a pedicel attached to the basal peridium (Figs 11, 17, 18). Ascospores 7–12 × 4–6 µm (\bar{x} = 10 × 5 µm, n = 20) biseriolate, obovoid, obtusate, clavate, diamond shaped when viewed from above and inequilaterally ellipsoidal or ellipsoidal with one side flattened dorsally when viewed from side, hyaline or greenish, 1-celled, coarse-guttulate, smooth-walled, with rounded elongate ends and bipolar mucilaginous appendages (Figs 12–15, 19).

Pycnidia 85–95 µm diameter, 64–85 µm high, on the upper and lower leaf surface, black, globose to subglobose, immersed in plant tissues, coriaceous, solitary to clustered, ostiolate,

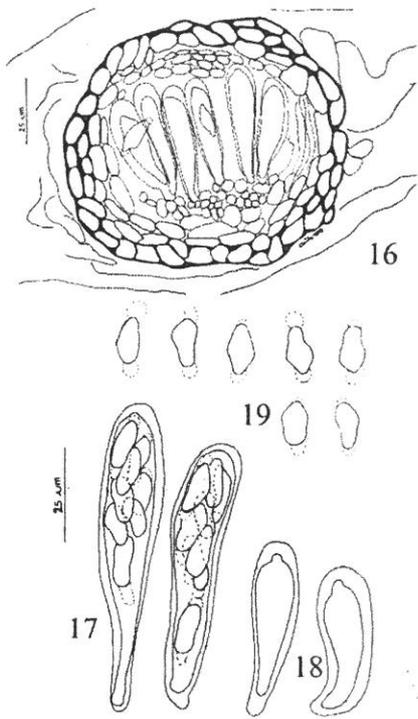


Figs 6–15 – *Guignardia morindae* (neotype). **6** Leaf spots. **7, 8** Appearance of ascomata on the host surface. **9** Section of ascoma. **10** Peridium comprising one strata of *textura angularis* comprising 2–3 layers of cells with an apex of thickened brown walls. **11** Asci. **12, 13, 14, 15** Ascospores with bipolar mucilaginous appendages with rounded elongate ends – Bars 12 = 50 μm , 13 = 20 μm , 14 = 10 μm , 15 = 5 μm .

ostioles as black dots in the centre, often growing together with ascomata. Peridium 11–15 μm wide, composed of two to three layers of cells, *textura angularis* and pigmented outwardly and around ostiole and paler inside (Figs 20, 21, 28). Conidiogenous cells 7–12 \times 2–3 μm (\bar{x} = 10 \times 2 μm , n = 20), holoblastic, determinate, discrete, rarely integrated, hyaline cylindrical to doliiform, forming from cells lining the pycnidial locule (Figs 20, 29). Conidia 8–10 \times 5–7 μm (\bar{x} = 9 \times 6 μm , n = 20), hyaline-greenish, 1-celled, coarse guttulate, smooth-walled, globose, ellipsoidal, clavate or obclavate, with an obtuse apex, sometimes truncate on the base, surrounded by 0.5–1 μm (\bar{x} = 1 μm , n = 20) thick mucilaginous sheath

which persists at maturity with a 2–7 μm (\bar{x} = 4 μm , n = 20) single, hyaline, curved or straight appendage (Figs 23, 30).

Spermogonia 44–45 μm diameter, 42–47 μm high intermixed with pycnidia. Peridium 5–9 μm wide, composed of two to three layers of cells, *textura angularis* and pigmented outwardly and around ostiole and paler inside (Figs 24, 25, 31). Spermatogenous cells 11–22 \times 2–3 μm (\bar{x} = 16 \times 2 μm , n = 20), holoblastic, filamentous to cylindrical, simple or branched and easily discernible apical structure (Figs 26, 32). Spermatia 5–9 \times 1–2 μm (\bar{x} = 6 \times 2 μm , n = 20) holoblastic, cylindrical to dumb-bell shaped, guttulate, straight or slightly curved forming singly in basipetal succession and

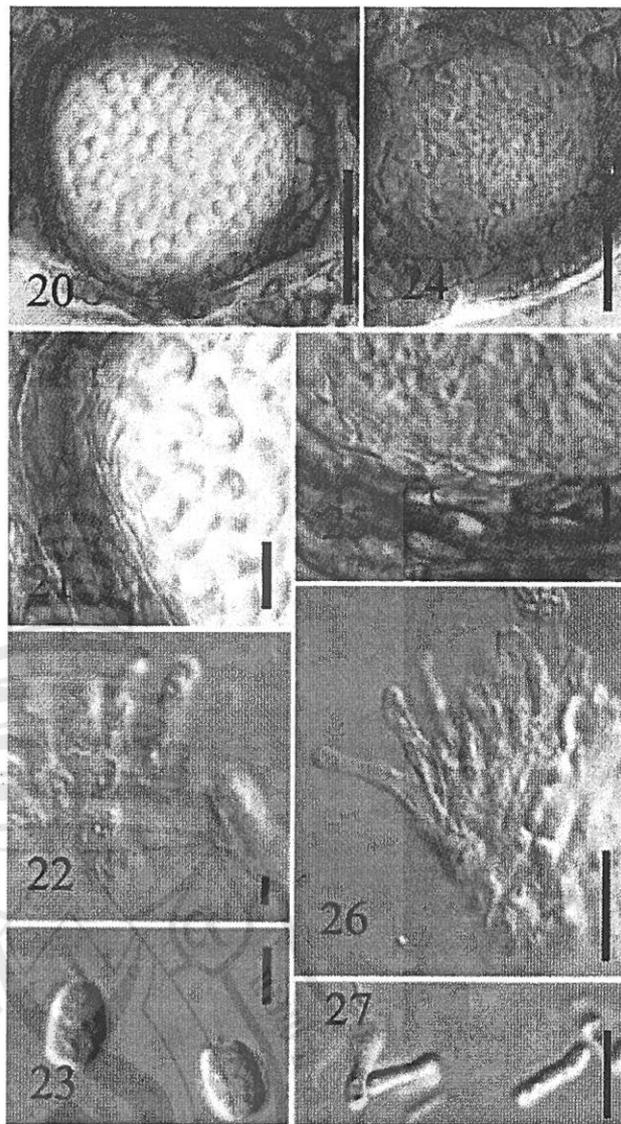


Figs 16–19 – *Guignardia morindae* (neotype) line drawing. **16** Section of ascoma. **17** Mature Asci. **18** Immature Asci. **19** Ascospores.

separating from the spermatogenous cells by a septum (Figs 27, 33).

Material examined – INDONESIA, West Java Province, Bogor, Bogor Botanical Garden, on living leaf of *Morinda citrifolia*, 23 September 2010, N.F. Wulandari, NFW 361 (BO 22648 designated as neotype), spermatial stage, anamorph and teleomorph present; *ibid.*, 23 September 2010, N.F. Wulandari, NFW 363 (BO 22650), teleomorph only present; *ibid.*, 11 May 2006, N.F. Wulandari, NFW 169 (BO 22652), teleomorph and anamorph present; *ibid.*, 27 June 2006, N.F. Wulandari, NFW 168 (BO 22651), anamorph only present; Central Java Province, Ngentak, Ngentak, Kedu, on living leaf of *Morinda citrifolia* 18 September 2010, N.F. Wulandari, NFW 362 (BO 22649), anamorph only present. THAILAND, Chiang Rai, Phahonyothin Road, on leaves of *Morinda citrifolia*, 20 January 2010, N.F. Wulandari, NFW 296 (MFLU 10 0453), teleomorph only present; *ibid.*, 05 March 2010, N.F. Wulandari, NFW 313 (MFLU 10 0466), teleomorph only present.

Known distribution – American Samoa, Australia, Cook Islands, Federated States of Micronesia, Fiji, French Polynesia, Wallis and Futuna, India, Indonesia (Bogor, Kedu), Japan,

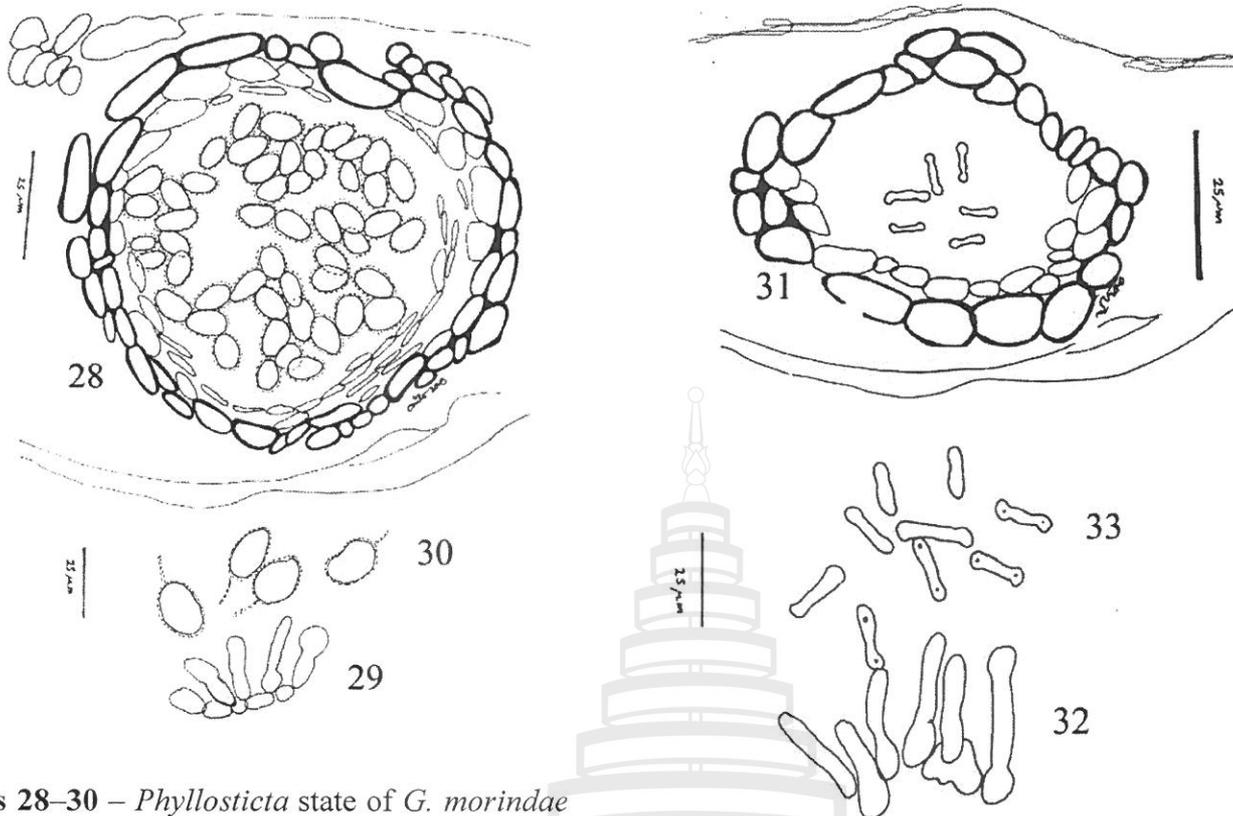


Figs 20–27 – *Phyllostictia* state of *G. morindae* (neotype). **20, 21, 24, 25** Peridium comprising one strata of *textura angularis* comprising 2–3 layers of cells with an apex of thickened brown walls. **22** Conidiogenous cells. **23** Conidia. **24** Spermatogonia. **26** Spermatogenous cells **27** Spermatia – Bars 20 = 45 µm, 21 = 15 µm, 22 = 3 µm, 23 = 7 µm, 24 = 20 µm, 25, 27 = 9 µm, 26 = 22 µm.

Kiribati, Niue, Palau, Samoa, Thailand (Chiang Mai, Chiang Rai), Tonga, Tuvalu, Vanuatu and Wallis.

Discussion

The holotype of *Guignardia morindae* is not in BO; Koorders never deposited his specimens in the herbarium (Mien A. Rifai, pers. comm.) and there is no available ex-type culture. Since there is no type of *G. morindae* the species was recollected from Bogor at the



Figs 28–30 – *Phyllosticta* state of *G. morindae* (neotype) line drawing. **28** Section of pynidium. **29** Conidiogenous cells. **30** Conidia.

original place of collection. A neotype is a specimen or illustration selected to serve as nomenclatural type if no original material is extant, or is missing (Art 9.6) (McNeill et al. 2006). The need of neotypification is important in order to stabilize the application of the species name (McNeill et al. 2006). *Guignardia morindae* is recorded for the first time in Thailand. Molecular work is needed to discern the distinctness of this species. However, despite repeated attempts we could not isolate the fungus.

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Figs 31–33 – *Leptodothiorella* state of *G. morindae* (neotype) line drawing. **31** Section of spermogonium. **32** Spermatogenous cells. **33** Spermata.

Kramadibrata are thanked for specimens certificate permit No. 1184/IPH.1.02/K.S.01.04/2010. Mien A. Rifai and Dewi Susan are thanked for valuable information concerning the holotype of *Physalospora morindae* in BO. Mae Fah Luang University is thanked for the award of a grant no 53101020017 (17/2553) to study *Phyllosticta* in northern Thailand. BRT, Thailand is acknowledged for the award of a grant (BRT No R251181) to study Dothideomycetes in northern Thailand. The Mushroom Research Foundation is thanked for a scholarship to carry our studies towards a PhD. Eric McKenzie is thanked for improving the draft manuscript.

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***Guignardia/Phyllosticta* species on banana**

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Abstract – *Guignardia musae* is the reported causal agent of freckle disease of banana. The epithet has, however, been introduced on three separate occasions and only one name is valid. We therefore investigated this problem. We examined the types of *G. musae* Racib., *G. musae* F. Stevens and *G. musae* Syd. & P. Syd. and also made fresh collections from banana in northern Thailand. *Guignardia musae* Racib. is the earliest name and takes precedence over the other two names which are homonyms. *G. musae* F. Stevens is a different species and therefore a new name *G. stevensii* Wulandari & K.D. Hyde is introduced to accommodate it. The name *G. sydowiana* Trotter has previously been introduced to accommodate *G. musae* Syd. & P. Syd.; type material is, however, depauperate. *Guignardia musicola* Wulandari, L. Cai & K.D. Hyde sp. nov. is introduced as a new species from Thailand. The three species from banana are compared and their differences described.

Banana freckle disease / New species / Taxonomy

INTRODUCTION

Freckle disease occurs on several species and varieties in Musaceae (Jones & Alcorn, 1982; Jones, 1984, 1993, 1994a, b, 1999; Pitakpaivan, 1985; Shivas *et al.*, 1996). The causal agent induces freckling on the leaves and fruits, causing a series of black, raised spots with a sand paper-like texture; this is due to the protruding pycnidia and/or ascomata. Leaves turn yellow with time and eventually senesce. The causal agent of banana freckle is reported to be *Guignardia musae* (Aa, 1973; Aa & Vanev, 2002; CABI, 1990, 2005; Chuang, 1981; Dingley *et al.*, 1981; Hwang *et al.*, 1984; Jones & Alcorn, 1982; Jones, 1984, 1993, 1994a, b, 1999; Meredith, 1968; Pitakpaivan, 1985; Ploetz *et al.*, 2003; Sivanesan, 1984; Shivas *et al.*, 1996; Tsai *et al.*, 1993; Zhou & Xie 1992) and its anamorph is reported to be *Phyllosticta musarum* (Aa, 1973; Aa & Vanev, 2002; Sivanesan, 1984).

The name *Guignardia musae* has been introduced on three occasions. It was first introduced by Raciborski (1909) for a fungus on *Musa paradisiaca* from Indonesia. This was followed by *G. musae* F. Stevens from *Musa* sp. in Hawaii (Stevens, 1925) and *G. musae* Syd. & P. Syd from *Musa* sp. in the Democratic Republic of Congo (Sydow & Sydow, 1912). The latter two names are homonyms and thus invalid. In the literature and generally on the world-wide web, the cause of freckle is listed as *Guignardia-Phyllosticta* sp. (<http://www.pestnet.org/Summaries/Crops/Plantationcrops/Banana/Fungi/Frecklediseaseofbanana/tabid/1350/Default.aspx>; <http://www2.dpi.qld.gov.au/horticulture/7926.html> and <http://www.indexfung-orum.org>) and the exact name of the species is not often listed.

Banana freckle occurs worldwide (Table 1). It is common in Asia where the causative agent is usually listed as *Guignardia musae* Racib. (anamorph *Phyllosticta musarum* (Cook) Aa). In Thailand freckle has been recorded on various *Musa* species (Sontirat *et al.*, 1994). Photita *et al.* (2002) recorded *G. musae* Racib., *G. musae* Syd. & P. Syd and *G. sydowiana* Trotter from Musaceae, while Photita *et al.* (2001) reported *G. cocoicola* Punith. as a common endophyte from wild banana in northern Thailand. Brown *et al.* (1998) reported *P. musicola* F. Stevens nom. inval. as a common endophyte from *Musa acuminata* in Hong Kong. There is obviously confusion surrounding the species of these genera occurring on banana.

The purpose of this paper is to investigate the *Guignardia/Phyllosticta* spp. associated with freckle disease on leaves. We re-examined the holotype of each epithet and also made fresh collections from banana in Asia.

MATERIAL AND METHODS

Specimens examined

Holotype specimens were loaned from S (Sweden), KRA (Poland) and BISH (Hawaii), while fresh specimens of freckle disease on banana were collected from Thailand. Herbarium acronyms follow Index Herbariorum (Holmgren & Holmgren, 1998).

Guignardia/Phyllosticta species on banana

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Table 1. Countries in which banana freckle disease has been recorded

Region/country	Reference	Species name
Australia – New South Wales, Queensland, Western Australia	CABI (1990), Farr & Rossman (2010), Jones & Alcorn (1982), Jones (1984)	<i>Guignardia musae</i> , <i>Phyllosticta musarum</i>
Fiji	Dingley <i>et al.</i> (1981), CABI (1990)	<i>G. musae</i>
New Caledonia	CABI (1990)	<i>G. musae</i>
Niue	Dingley <i>et al.</i> (1981)	<i>G. musae</i>
Hawaii (USA)	Steven (1925)	<i>G. musae</i>
Papua New Guinea	CABI (1990)	<i>G. musae</i>
Samoa (USA)	CABI (1990)	<i>G. musae</i>
Solomon Island	McKenzie & Jackson (1986), CABI (1990)	<i>G. musae</i>
Tonga	Dingley <i>et al.</i> (1981), CABI (1990)	<i>G. musae</i>
Bangladesh	CABI (1990)	<i>G. musae</i>
Bhutan	CABI (1990)	<i>G. musae</i>
Brunei Darussalam	CABI (1990), Farr & Rossman (2010)	<i>G. musae</i> , <i>P. musarum</i>
China (Fujian, Guangdong, Guangxi, Yunnan)	Zhou & Xie (1992), Farr & Rossman (2010)	<i>G. musae</i> , <i>P. musarum</i>
Hong Kong, Taiwan	CABI (1990)	<i>G. musae</i>
Christmas Island	Shivas & Hilton (1990)	<i>G. musae</i>
India (Karnataka, Uttar, Pradesh)	CABI (1990), Farr & Rossman (2010)	<i>G. musae</i> , <i>P. musarum</i>
Indonesia (Java, Irian Jaya)	Raciborski (1908), Shivas <i>et al.</i> (1996)	<i>G. musae</i>
Malaysia (Peninsular Sabah, Sarawak)	Jones (1993), CABI (1990)	<i>G. musae</i>
Myanmar	CABI (1990), Farr & Rossman (2010)	<i>G. musae</i>
Nepal	CABI (1990)	<i>G. musae</i> , <i>P. musarum</i>
Pakistan	CABI (1990)	<i>G. musae</i>
Philippines	CABI (1990)	<i>G. musae</i>
Sri Lanka	CABI (1991)	<i>G. musae</i>
Thailand	Sontirat & Jones (1994)	<i>G. musae</i>
Vietnam	Anon (1994), CABI (1990)	<i>G. musae</i>
Cook Islands	Dingley <i>et al.</i> (1981)	<i>G. musae</i>
Samoa	Dingley <i>et al.</i> (1981)	<i>G. musae</i>
Solomon Islands	McKenzie & Jackson (1986)	<i>G. musae</i>
Vanuatu	McKenzie (1989)	<i>G. musae</i>
Palau	McKenzie (1990a)	<i>G. musae</i>
Federated States of Micronesia	McKenzie (1990b)	<i>G. musae</i>

Morphology

Specimens were studied using a Nikon eclipse 80i with EOS 450 D Nikon camera ($\times 1000$ magnification) and an Olympus CX-41 research microscope fitted with a drawing tube and Olympus SMZ 168. Hand sections were made for microscopic examination. Preparations and measurements were made in lactoglycerol (lactic acid: water: glycerol = 1:2:1) for semi-permanent slide and lactophenol cotton blue. The 95% confidence intervals were derived from 30 observations of spores formed on water agar plates, with extremes in parentheses.

RESULTS

Taxonomy

Guignardia musae Racib., *Bull. int. Acad. Sci. Lett. Cracovie*, Cl. sci. math. nat. Sér. B, sci. nat. 3: 388 (1909)
Mycobank: MB 271864

(Figs. 2-9, 17-19)

Ascomata 100-125 μm high, 75-150 μm diam, on upper and lower surface of leaves and on banana fruit skin, globose to subglobose, black, semi-immersed in plant tissues, coriaceous, solitary to clustered, ostiolate, ostioles as black central dots (Fig. 2). *Peridium* 12.5-20 μm wide, upper part composed of compressed, brownish, thin-walled cells, 1-4 cells thick, lower part hyaline, composed of flattened, dark brown cells, darkest around the ostiole (Figs. 3-5, 17). *Pseudoparaphyses* not observed. *Asci* 49-105 \times 16-28 μm (\bar{x} = 74 \times 21 μm , n = 20), 8-spored, bitunicate, broadly cylindro-clavate, rounded at the apex, where the diameter is 8-21 μm , tapering gradually to a 5-10 μm diam. \times 5-10 μm long pedicel attached to the basal peridium, ocular chamber 3-8 μm high (Figs. 6-7, 18). *Ascospores* 20-25 \times 8-13 μm (\bar{x} = 22 \times 10 μm , n = 20), uniseriate or occasionally overlapping biseriate, clavate to oblong, not laterally compressed, having the same shape when viewed from above or from the side, hyaline to greenish, 1-celled, guttulate, smooth-walled, lacking a mucilaginous sheath or appendages at the ends (possibly due to nature of old specimens) (Figs. 8-9, 19).

Material examined. INDONESIA, Bogor, on leaves of *Musa acuminata*, no date, Raciborski, (KRA 063561, holotype of *Guignardia musae* Racib.), only teleomorph present.

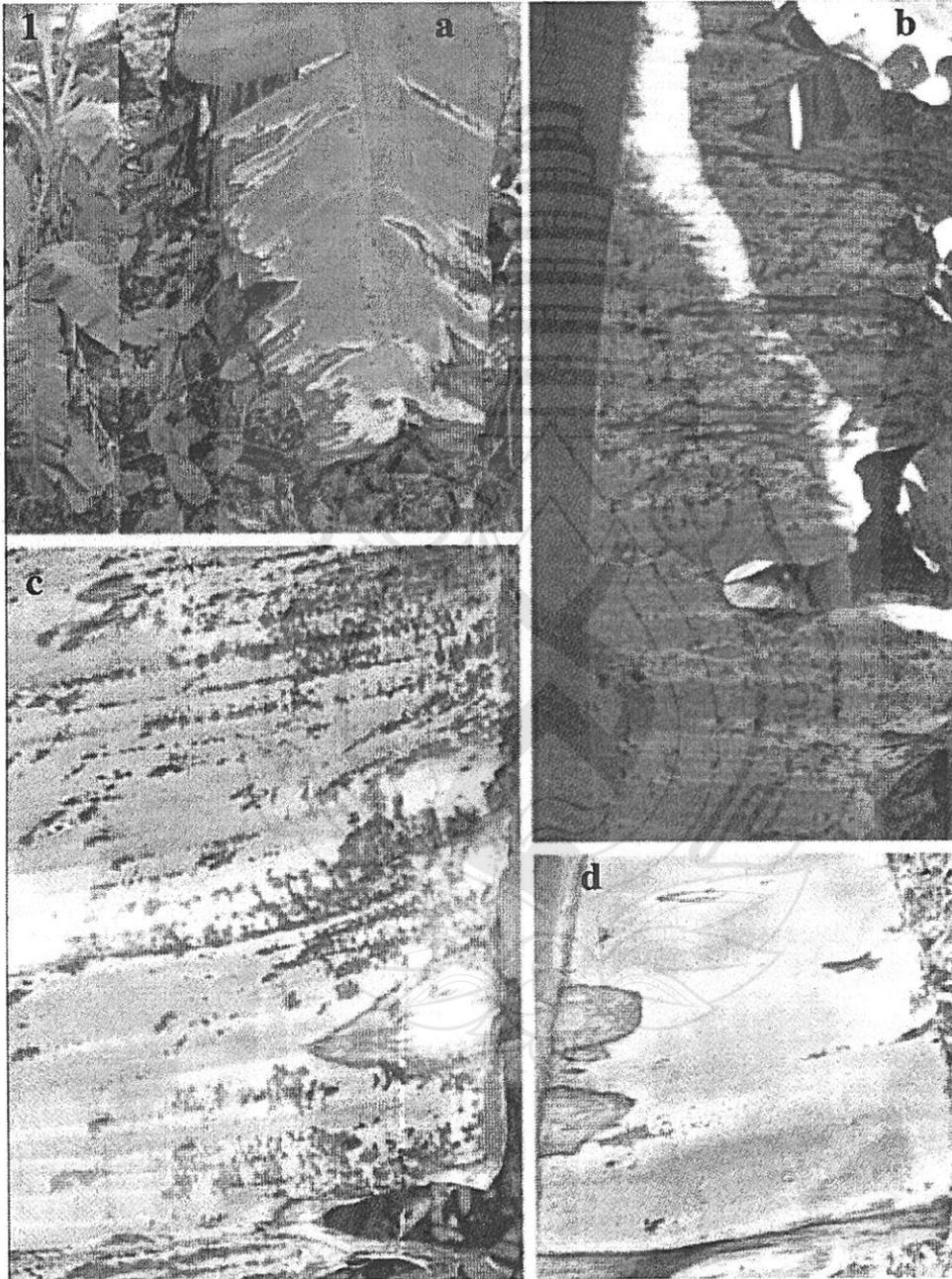
Notes: This is the earliest species of *Guignardia* or *Phyllosticta* described from *Musa* species and therefore takes precedence over *G. musae* F. Stevens and *G. musae* Syd. & P. Syd. The ascospores in this species are distinct because of their size (20-25 \times 8-13 μm) and shape (clavate to oblong, not laterally compressed having the same shape when viewed from above or the side) (Table 2).

Guignardia stevensii Wulandari & K.D. Hyde, **nom. nov.**

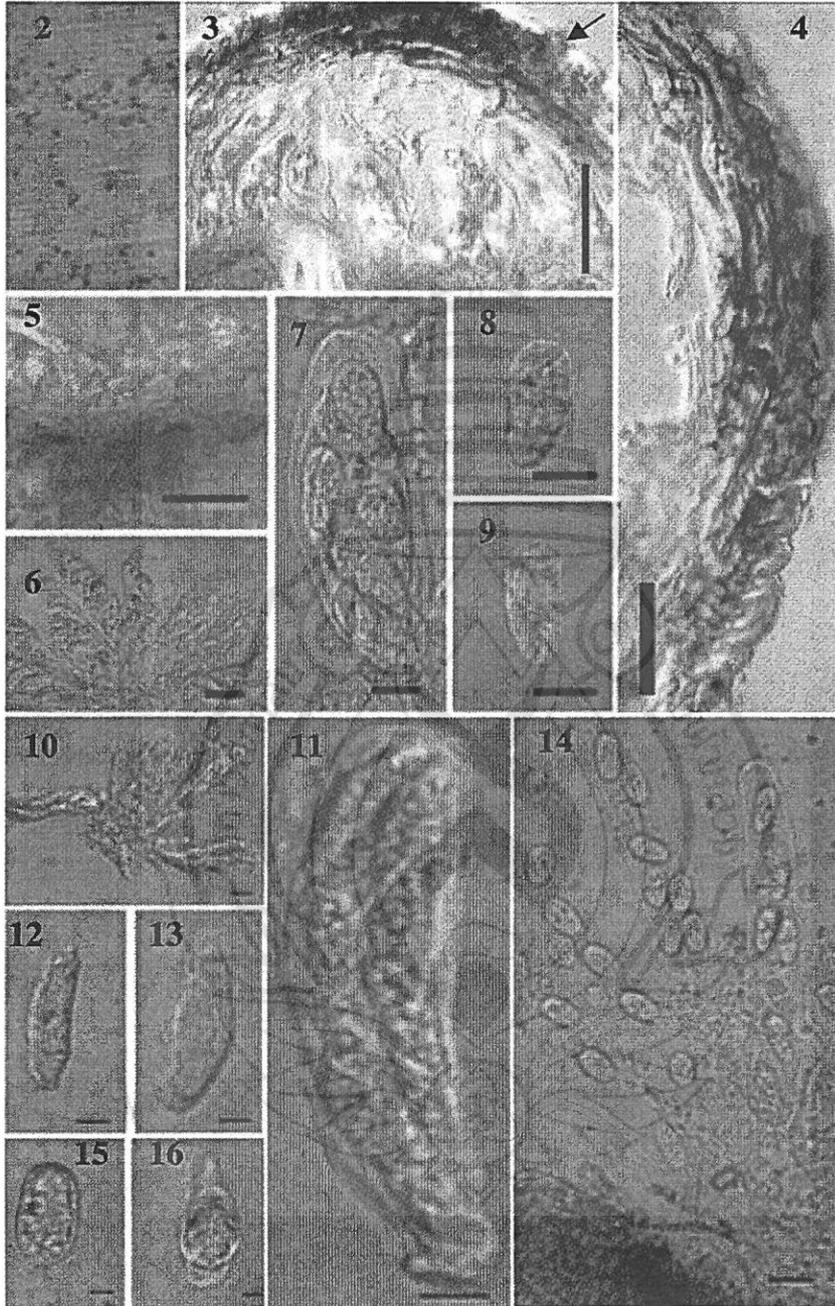
\equiv *Guignardia musae* F. Stevens, Bulletin of the Bernice P. Bishop Museum, Honolulu, Hawaii 19: 101 (1925), nom. illegit., non *G. musae* Racib. 1909.
Mycobank: MB 519089

(Figs. 10-13, 20-22)

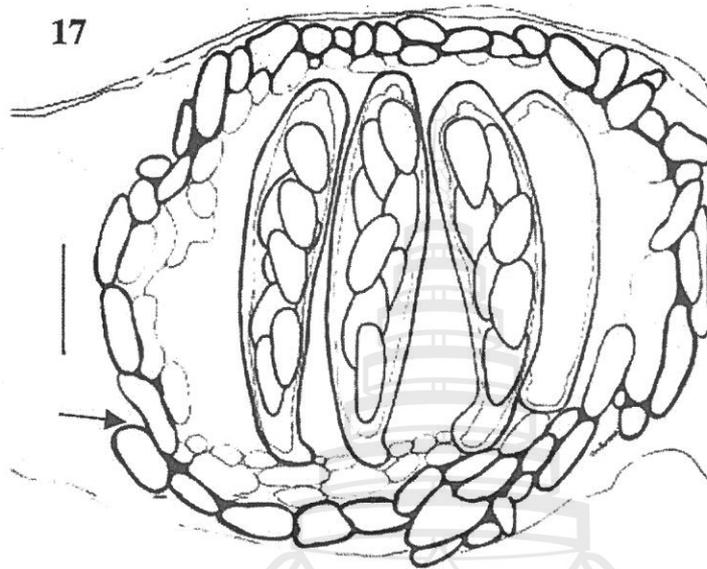
Etymology: Named after its collector, F.L. Stevens.



Figs. 1a-d. Freckle disease on *Musa* spp. in Thailand caused by *Guignardia musicola*.



Figs. 2-16. Micrographs of *Guignardia* spp. on *Musa* sp. 2-9. *G. musae* Racib., 10-13. *G. stevensii*, 15-16. *G. musicola*. 2. Appearance of ascomata on host surface (bar = 100 μ m). 3, 4, 5. Section of ascoma in the leaf (darkened area fungal cells-arrowed) (bar = 20 μ m). 6, 7. Asci (bars 6 = 30 μ m, 7 = 10 μ m). 8, 9. Ascospores (bar = 10 μ m). 10, 11. Asci (bar = 15 μ m). 12, 13. Ascospores (bar = 5 μ m). 14. Asci (bar = 10 μ m). 15, 16. Ascospores (bar = 10 μ m).



Figs. 17-19. Line drawing of *G. musae* Racib. (holotype): **17**. Section of ascoma in the leaf (fungal cells arrowed) (bar = 25 μ m). **18**. Asci (bar = 25 μ m). **19**. Ascospores which are clavate to oblong and symmetrical (bar = 25 μ m).

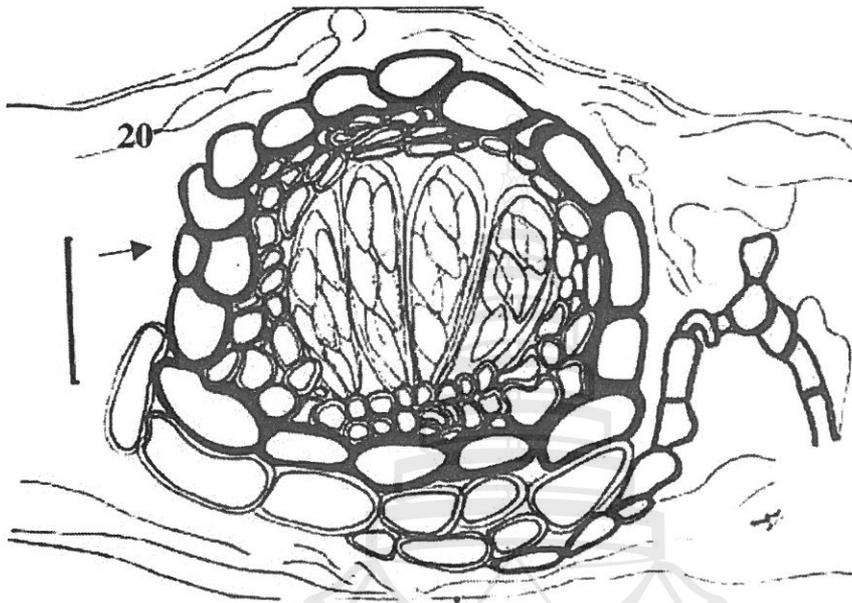
Table 2. Synopsis of ascospores and conidia of *Guignardia* species on *Musa* spp.

	<i>G. musae</i> Racib.	<i>G. stevensii</i>	<i>G. musicola</i>
Ascus (μm)	49-105 \times 16-28, broadly clavate	40-59 \times 11-15, cylindro-clavate	133-150 \times 19-20, cylindrical to cylindro-clavate
Ascospores (μm)	20-25 \times 8-13, clavate to oblong symmetrical, without appendages	14-17 \times 5-6, widest 2/5 near the apex (obtrullate), inequilateral, or ellipsoidal with one side flattened, and with appendages	12-21 \times 7-10, obclavate to oblong, symmetrical, with appendages
Line drawing of ascospores, bars (a = 25 μm ; b-c = 20 μm)			
Phyllosticta state (μm)	Not present	Not present	Conidia 12-17 \times 8-11, with appendage 10-15 long, sheath 2-4 wide

Ascomata 50-125 μm high, 60-95 μm diam, on upper surface of leaves, globose to subglobose, black, semi-immersed in plant tissues, coriaceous, solitary to clustered, ostioles as black central dots. *Peridium* 20-25 μm wide, composed of compressed, brownish, thin-walled cells, in the upper part 1-4 cells thick, composed of flattened, dark brown cells, darkest around the ostiole, hyaline towards the lower region (Fig. 20). *Pseudoparaphyses* not observed. *Asci* 40-59 \times 11-15 μm (\bar{x} = 50 \times 13 μm , n = 20), 8-spored, bitunicate, cylindro-clavate, rounded at the apex, where the diameter is 10-12 μm , tapering gradually to a 2-7 μm diam. \times 3-7 μm long pedicel attached to the basal peridium, ocular chamber 3-8 μm high (Figs. 10-11, 21). *Ascospores* 14-17 \times 5-6 μm (\bar{x} = 15 \times 5 μm , n = 20), uniseriate or occasionally overlapping biseriata, ellipsoidal, widest 2/5th from the apex (obtrullate) in one plane, inequilaterally ellipsoidal, or ellipsoidal with one side flattened when viewed from the side, hyaline to greenish, 1-celled, guttulate, smooth-walled, with a mucilaginous appendage at each end (Figs. 12-13, 22).

Material examined. HAWAII, Oahu, Hakipu, on leaves of *Musa* sp., 12 June 1921, F.L. Stevens, No. 565 (BISH 596860, holotype; BISH 499904 isotype of *Guignardia musae* F. Stevens), teleomorph only present.

Notes: The ascospores of *Guignardia musae* F. Stevens differ markedly from those of *G. musae* Racib. being 14-17 \times 5-6 μm , obtrullate from above, inequilaterally ellipsoidal, or ellipsoidal and flattened on one when viewed from



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Figs. 20-22. Line drawing of *G. stevensii* (holotype): 20. Section of ascoma in the leaf (fungal cells arrowed) (bar = 25 μ m). 21. Asci (bar = 20 μ m). 22. Ascospores which are obtrullate from above, inequilaterally ellipsoidal, or ellipsoidal with one side flattened, and with mucilaginous appendages at the ends (bar = 20 μ m).

the side (Table 2). Since *G. Musae* F. Stevens is a homonym of *G. Musae* Racib. we provide a new name. Fresh living collections from Hawaii are needed to fully circumscribe this taxon from *Musa* sp. with DNA sequence comparison.

Guignardia sydowiana Trotter, in Saccardo, Syll. Fung. (Abellini) 24(2): 788 (1928)

Basionym. ***Guignardia musae*** Syd. & P. Syd., *Annls mycol.* 10: 80 (1912) [name is invalid as homonym of *G. musae* Racib.].

Material examined. Democratic Republic of Congo, on dead leaf of *Musa* sp., Vanderyst, ex Herb. Sydow (S, 10753, holotype of *Guignardia musae* Syd. & P. Syd).

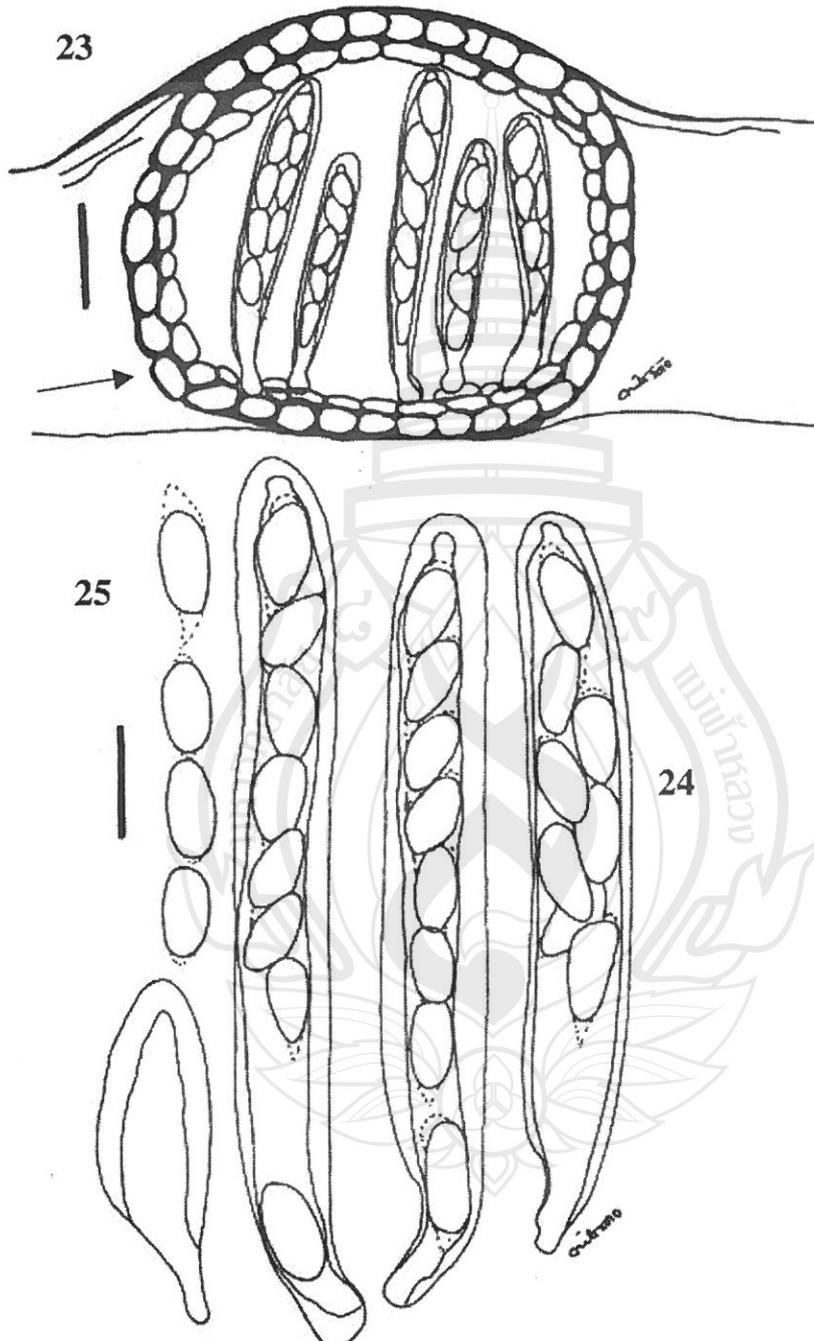
Notes: The name *Guignardia sydowiana* Trotter was introduced to replace *G. musae* Syd. & P. Syd., which is a homonym of *G. musae* Racib., and thus invalid. The type material examined is not in a good condition as ascomata were dry and depauperate.

Guignardia musicola N.F. Wulandari, L. Cai & K.D. Hyde, *sp. nov.*
Mycobank no.: MB 519088

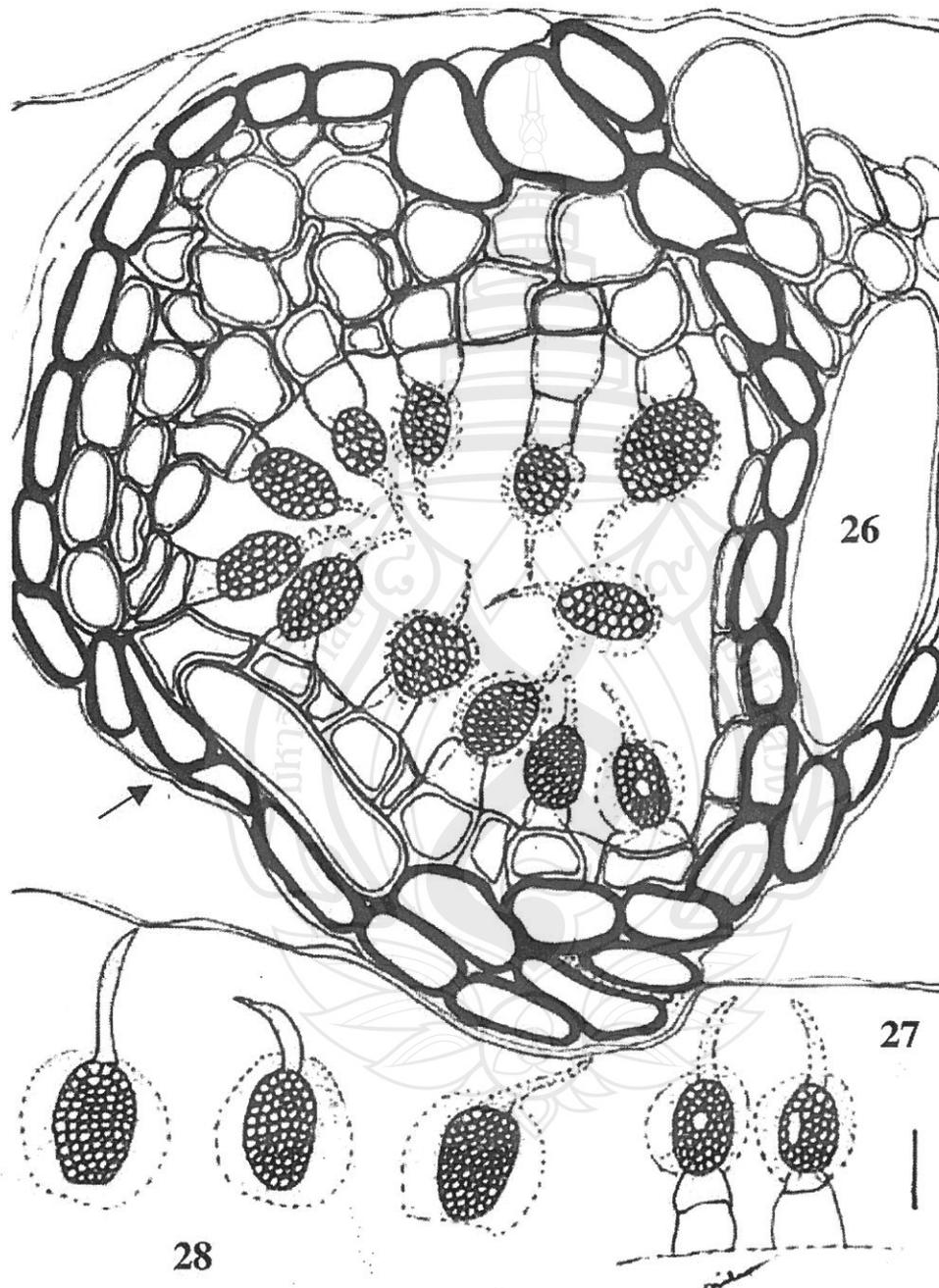
(Figs. 14-16, 23-31)

Etymology: Named after its host plant, *Musa* sp. and *-cola* meaning dwelling on. *Guignardiae musae* Racib. similis, sed ascosporae $12\text{-}21 \times 7\text{-}10 \mu\text{m}$.

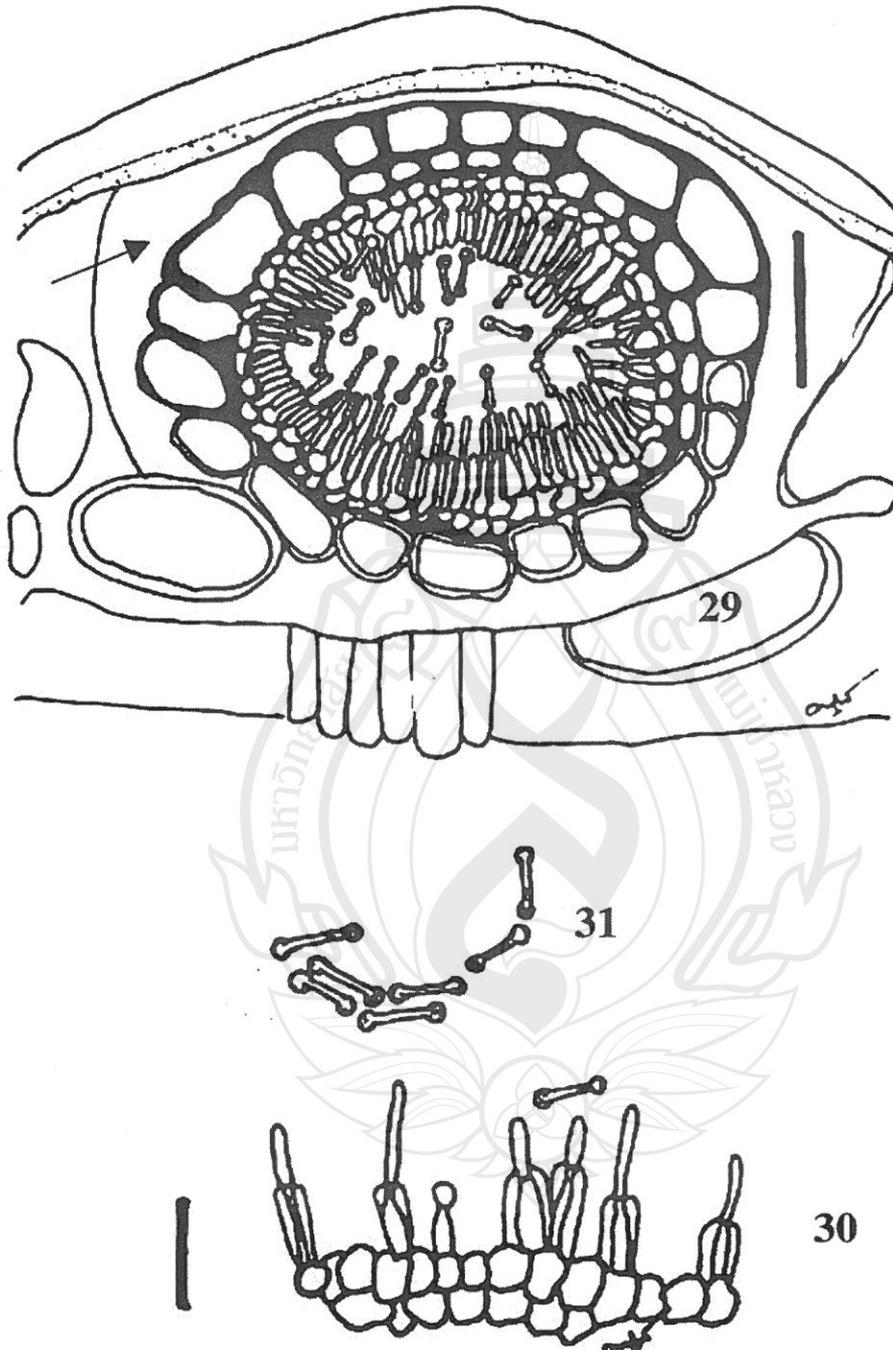
Leaf spot occupying marginal areas of the leaf and pinna, bleached, the leaf breaking at the edge to the middle of lamina, ascomata visible to the unaided eye on surface of the leaves, surface rough indicating protruding ascomata and pycnidia (Fig. 1). *Ascomata* $100\text{-}125 \mu\text{m}$ diam, $100\text{-}125 \mu\text{m}$ high, on upper surface of leaves, globose to subglobose, black, semi-immersed in plant tissues, coriaceous, solitary to clustered, ostiolate, ostioles as black central dots. *Peridium* $22.5\text{-}25 \mu\text{m}$ wide, comprising 2 layers of *textura angularis* cells with thickened brown walls around ostiole (Fig. 23). *Pseudoparaphyses* not observed. *Asci* $133\text{-}150 \times 19\text{-}20 \mu\text{m}$ ($\bar{x} = 137 \times 20 \mu\text{m}$, $n = 20$), 8-spored, bitunicate, fissitunicate, cylindrical to cylindro-clavate, rounded at the apex, where the diameter is $12\text{-}13 \mu\text{m}$, tapering gradually to a $10\text{-}20 \mu\text{m}$ diam. $\times 5\text{-}6 \mu\text{m}$ long pedicel attached to the basal peridium, ocular chamber $2\text{-}5 \mu\text{m}$ high (Figs. 14, 24). *Ascospores* $12\text{-}21 \times 7\text{-}10 \mu\text{m}$ ($\bar{x} = 19 \times 9 \mu\text{m}$, $n = 20$), uniseriate or occasionally overlapping biseriate, ellipsoidal to clavate, not laterally compressed, having the same shape when viewed from above or the side, hyaline to greenish, 1-celled, guttulate, smooth-walled, with a mucilaginous appendage at each end, not (Figs. 15-16, 25). *Pycnidia* $95\text{-}125 \mu\text{m}$ diam, $75\text{-}125 \mu\text{m}$ high, epiphyllous, black, globose to pyriform, immersed in plant tissues, coriaceous, solitary to clustered, ostiolate, ostioles as white dots in the centre. *Peridium* $22\text{-}25 \mu\text{m}$ wide, one stratum of *textura angularis* comprising 2 layers of cells with thickened brown walls around ostiole (Fig. 26). *Conidiogenous cells* $10\text{-}12 \times 8\text{-}9 \mu\text{m}$ ($\bar{x} = 11 \times 8 \mu\text{m}$, $n = 5$), holoblastic, determinate, discrete, sometimes rarely integrated, hyaline, cylindrical to doliiform cells lining the pycnidial locule (Fig. 27). *Conidia* $12\text{-}17 \times 8\text{-}11 \mu\text{m}$ ($\bar{x} = 14 \times 10 \mu\text{m}$, $n = 20$), hyaline to greenish, 1-celled, guttulate, smooth-walled, globose, ellipsoidal, clavate or obclavate, with an obtuse apex, sometimes truncate at the base, surrounded by $2\text{-}4 \mu\text{m}$ thick mucilaginous sheath which persists at maturity and in some specimens with a single, hyaline, curved or straight, $10\text{-}15 \mu\text{m}$ long appendage (Fig. 28). *Spermogonia* $95\text{-}125 \mu\text{m}$ in diameter, $75\text{-}125 \mu\text{m}$ high, epiphyllous, black, globose to subglobose, immersed in plant tissues, coriaceous, solitary to clustered, ostiolate, ostioles as white dots in the centre, similar to pycnidia. *Peridium* $22\text{-}25 \mu\text{m}$ wide, one stratum of *textura*



Figs. 23-25. Line drawing of *G. musicola* (holotype): **23.** Section of ascoma in the leaf (fungal cells arrowed) (bar = 25 μ m). **24.** Asci (bar = 20 μ m). **25.** Ascospores which are obclavate to oblong, symmetrical, with appendages (bar = 20 μ m).



Figs. 26-28. Line drawing of *Phyllosticta* state of *G. musicola* (holotype): 26. Section of pycnidium in the leaf (fungal cells arrowed) (bar = 10 μ m). 27. Conidia and conidiogenous cells (bar = 10 μ m). 28. Conidia (bar = 10 μ m).



Figs. 29-31. Line drawing of *Leptodothiorella* state of *G. musicola* (holotype): 29. Section of spermatogonium in the leaf (fungal cells arrowed) (bar = 25 μ m). 30. Spermatogenous cells (bar = 10 μ m). 31. Spermatia (bar = 10 μ m).

angularis comprising 2 layers of cells with thickened brown walls around ostiole (Fig. 29). *Spermatogenous cells* $7-10 \times 1 \mu\text{m}$ ($\bar{x} = 9.8 \times 1 \mu\text{m}$, $n = 20$), holoblastic, filamentous to cylindrical, simple or branched as distinct phialides with a very characteristic and easily discernible apical structure (Fig. 30). *Spermatia* $5-8 \times 1-2 \mu\text{m}$ ($\bar{x} = 7 \times 1 \mu\text{m}$, $n = 20$), cylindrical to dumb-bell shaped, guttulate, straight or slightly curved forming singly in basipetal succession and separating from the spermatogenous cells by a septum (Fig. 31).

Material examined. THAILAND, Chiang Mai Province, Chiang Mai, Tung Jaow Village, on leaves of *Musa acuminata*, 18 July 2007, N.F. Wulandari, NFW 154 (MFLU 10 0235, **holotype**) teleomorph and anamorph present; exatype cultures CBS 123405; *ibid.*, Srilanna, on leaves of *Musa paradisiaca*, 12 July 2007, N.F. Wulandari, NFW 140 (MFLU 10 0233) teleomorph and anamorph present; Bahn Pa Deng, T. Pa Pae, Mae Taeng, Mushroom Research Centre, on leaves of *M. paradisiaca*, 24 August 2006, N.F. Wulandari, NFW 084 (MFLU 10 0222), teleomorph only present; *ibid.*, 3 June 2007, N.F. Wulandari NFW 128 (MFLU 10 0231), teleomorph only present; *ibid.*, 20 July 2007, N.F. Wulandari, NFW 161 (MFLU 10 0236), teleomorph only present; *ibid.*, 13 August 2007, N.F. Wulandari, NFW 176 (MFLU 10 0237), teleomorph and anamorph present; *ibid.*, 21 August 2007, N.F. Wulandari, NFW 182 (MFLU 10 0238), teleomorph and anamorph present; *ibid.*, 12 September 2007, N.F. Wulandari, NFW 219 (MFLU 10 0244), teleomorph only present. Tumbon, Chiangdoaw, on leaves of *M. paradisiaca*, 5 September 2007, N.F. Wulandari, NFW 184 (MFLU 10 0239), teleomorph, anamorph and spermatial stage present; *ibid.*, 5 September 2007, N.F. Wulandari, NFW 185 (MFLU 10 0240), teleomorph only present; *ibid.*, 5 September 2007, N.F. Wulandari, NFW 188 (MFLU 10 0242), teleomorph only present. Bahn Pha Deng, Mae Lod, Royal Project, on leaves of *M. paradisiaca*, 11 September 2007, N.F. Wulandari, NFW 210 (MFLU 10 0243), teleomorph only present; *ibid.*, Chiang Mai, Chiang Mai University on leaves of *M. paradisiaca*, 16 June 2006, N.F. Wulandari, NFW 114 (MFLU 10 0225), teleomorph only present; *ibid.*, 19 June 2007, N.F. Wulandari, NFW 117 (MFLU 10 0228), teleomorph only present; *ibid.*, 19 June 2006, N.F. Wulandari, NFW 118 (MFLU 10 0229), teleomorph only present; Chiang Mai University Shop garden, on leaves of *M. paradisiaca*, 15 September 2007, W. Tajeena & N.F. Wulandari, NFW 221 (MFLU 10 0245), teleomorph and anamorph present; Medicinal Plant Garden on leaves of *Musa paradisiaca*, 15 September 2007, N.F. Wulandari, NFW 230 (MFLU 10 0246), teleomorph only present. Bahn Pha Deng, Pathummikaram Temple, on leaves of *M. paradisiaca*, 1 July 2007, N.F. Wulandari, NFW 123 (MFLU 10 0230), teleomorph only present; Bahn Pha Deng Mushroom Research Centre, on leaves of *M. paradisiaca*, 24 August 2006, N.F. Wulandari, NFW 079 (MFLU 10 0220), teleomorph only present; *ibid.*, 22 August 2006, N.F. Wulandari NFW 080 (MFLU 10 0221), teleomorph only present; *ibid.*, 18 June 2007, N.F. Wulandari, NFW 115 (MFLU 10 0226), teleomorph only present; *ibid.*, 18 June 2007, N.F. Wulandari, NFW 116 (MFLU 10 0227), teleomorph only present; *ibid.*, N.F. Wulandari, NFW 131 (MFLU 10 0232), teleomorph only present; *ibid.*, 17 July 2007, N.F. Wulandari NFW 151 (MFLU 10 0234), teleomorph only present. Chiang Rai, Nam Tok Huey Mesak Forest Park, on leaves of *M. paradisiaca*, 6 February 2010, N.F. Wulandari & P. Syshophanthong, NFW 306 (MFLU 10 0281), teleomorph only present.

Notes: *Guignardia musicola* is distinct from *G. musae* Racib. as ascospores in *G. musicola* are smaller $12-21 \times 7-10 \mu\text{m}$ ($\bar{x} = 19 \times 9 \mu\text{m}$), compared with those of *Guignardia musae* Racib. ($20-25 \times 8-13 \mu\text{m}$, $\bar{x} = 22 \times 10 \mu\text{m}$) (Table 2).

DISCUSSION

This study redescribes *G. musae* Racib. and shows it to be a morphologically distinct species. Fresh collections are needed from Indonesia, however, to epitypify this species for molecular study. *Guignardia musae*

F. Stevens and *G. musae* Syd. & Syd. are homonyms of *G. musae* Racib. and thus invalid. *Guignardia musae* F. Stevens is, however, a distinct species and a new name *G. stephensii* is introduced for this taxon. One new species of *Guignardia* isolated from leaves of banana with freckle symptoms in Thailand (Fig. 1) is also introduced. The study shows that more than one species is responsible for freckle symptoms of banana and a worldwide study is justified. Several other species, e.g. *Macrophoma musae* (Sacc.) Berl. & Voglino, *Phoma musae* Sacc., *Phoma musae* C.W. Carp., *Phyllosticta musarum* (Cooke) Aa, *Sphaeropsis musarum* Cooke and *Phyllostictina musarum* (Cooke) Petr. have at one time or another been considered to be synonyms of *G. musae* Racib. (Aa, 1973; Carpenter, 1919; Petrak and Ciferri, 1931; Raciborski, 1909; Sivanesan, 1984). The synonymies, however, were based on morphological data and the taxa need recollecting and subjecting to molecular analysis. Further collections and sequence analysis are needed from different continents and various musaceous hosts to establish which species induce freckle disease of banana.

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Guignardia bispora and *G. ellipsoidea* spp. nov. and other *Guignardia* species from palms (Arecaceae)

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Two *Guignardia* species collected in northern Thailand are differ morphologically from previously known *Guignardia* species recorded on palms. *Guignardia bispora* sp. nov. is distinguished by having two ascospore types and *G. ellipsoidea* sp. nov. is distinguished by having reduced mucilaginous appendages compared to the holotype of *G. candeloflamma*, also found on palms. The new species are described and illustrated and compared with similar taxa.

Key words – Ascomycetes – Botryosphaeriaceae – Dothideales – Leaf spot – Pathogen – Taxonomy

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Introduction

Guignardia species on palms have been relatively well studied (Rehm 1914, Arx & Müller 1954, Aa 1973, Punithalingam 1974, Sivanesan 1984, Hanlin 1990, Hyde 1995, Fröhlich & Hyde 2000, Hyde et al. 2000, Yanna et al. 2001, Aa & Vanev 2002, Taylor & Hyde 2003, Pinruan et al. 2007) and much of the data was summarized by Hyde (1995). Hyde (1995) introduced two new species, redescribed a further six species from palms, and provided a key to the palm *Guignardia* species. Taylor & Hyde (2003) found three species of *Guignardia* on palms, each having a *Phyllosticta* anamorph and a *Leptodothiorella* spermatial state. Sontirat et al. (1994) recorded one species of *Phyllosticta* occurring on *Areca* sp. in Thailand.

Phyllosticta species have been recorded on palms as endophytes (Lumyong et al. 2009). *P. cocoicola* is a common species found as an endophyte, saprobe and a pathogen and has *Guignardia cocoicola* as the teleomorph (Punithalingam 1974, Taylor 1999, Hyde & Taylor 2003, Lumyong et al. 2009).

The objective of this research was to investigate *Guignardia* species occurring on palms in northern Thailand. In this paper we describe two new species based on morphological characters.

Methods

Specimens

The holotype specimen of *Guignardia candeloflamma* J. Fröhl. & K.D. Hyde was

borrowed from BRIP and is illustrated here as ascospores are comparable to one of the new species from palms. Fresh collections of the new *Guignardia* species were collected from Chiang Mai and Chiang Rai.

Morphology

Microscopy was carried out using standard techniques. Ascomata were examined with an Olympus SZ40 microscope, and micro-characters examined using a Nikon 80i microscope with Tarosoft program for measuring spores. A camera lucida attached to an Olympus CX 41 microscope was used for preparation of line drawings. Specimens for microscopic observation were prepared by hand sectioning. Lactophenol cotton blue and lactoglycerol solution were used as mounting media. Details of taxonomic novelties are deposited in MycoBank (www.MycoBank.org, Crous et al. 2004).

Results

Two new *Guignardia* species were identified and are described, illustrated and compared with related species from palms. *Guignardia candeloflamma* is also illustrated as it has comparable ascospores to the two new *Guignardia* species described on palms.

Taxonomy

Guignardia bispora N.F. Wulandari & K.D. Hyde, **sp. nov.** Figs 1 2, 7 23, 44 49
MycoBank: MB 519097

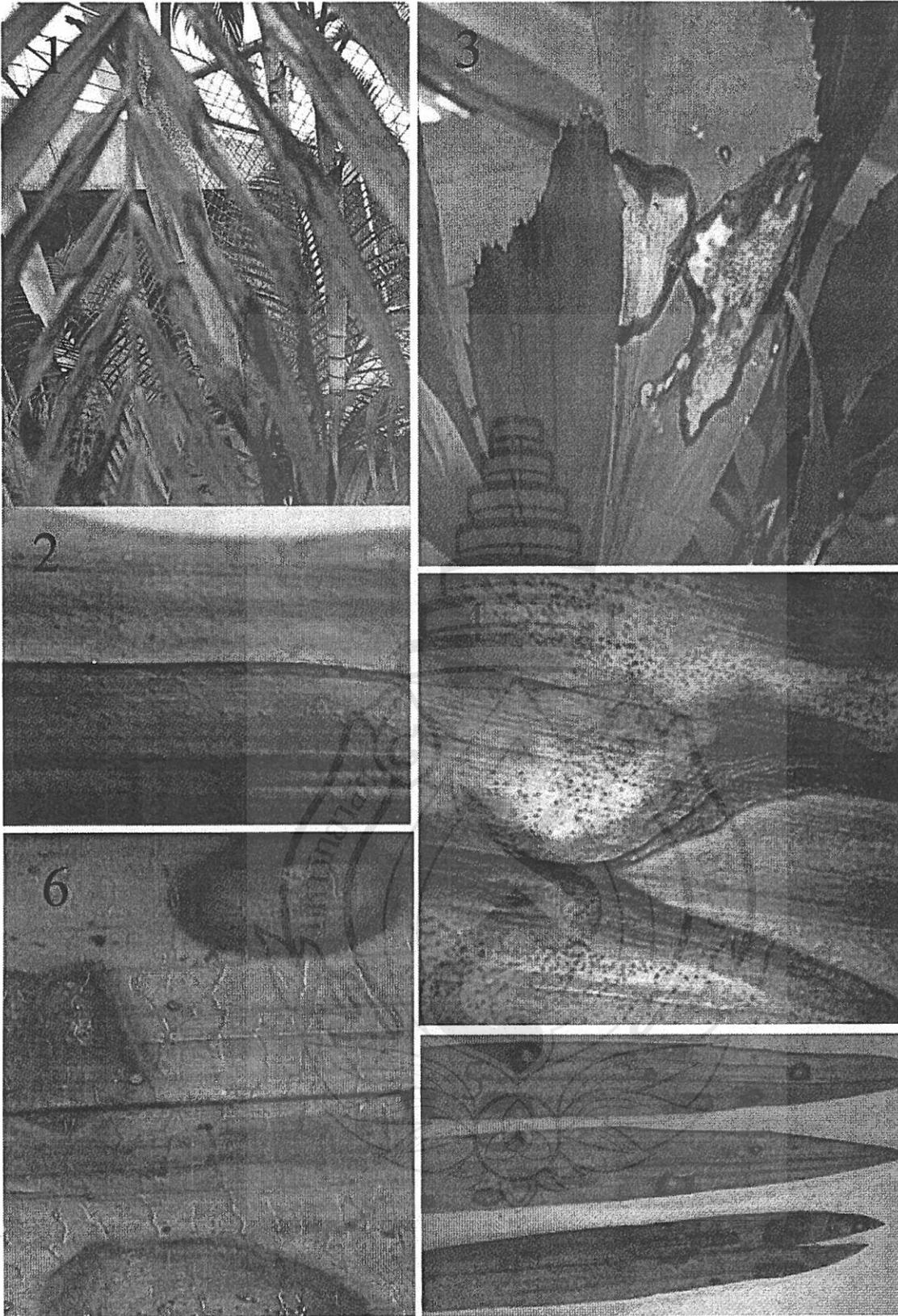
Etymology – named for the two types of ascospores.

Guignardia candeloflamma similis sed ascosporae bisporae, ellipsoideae vel cylindricae, $10\ 16 \times 3\ 5$ vel $13\ 14 \times 3\ 4\ \mu\text{m}$.

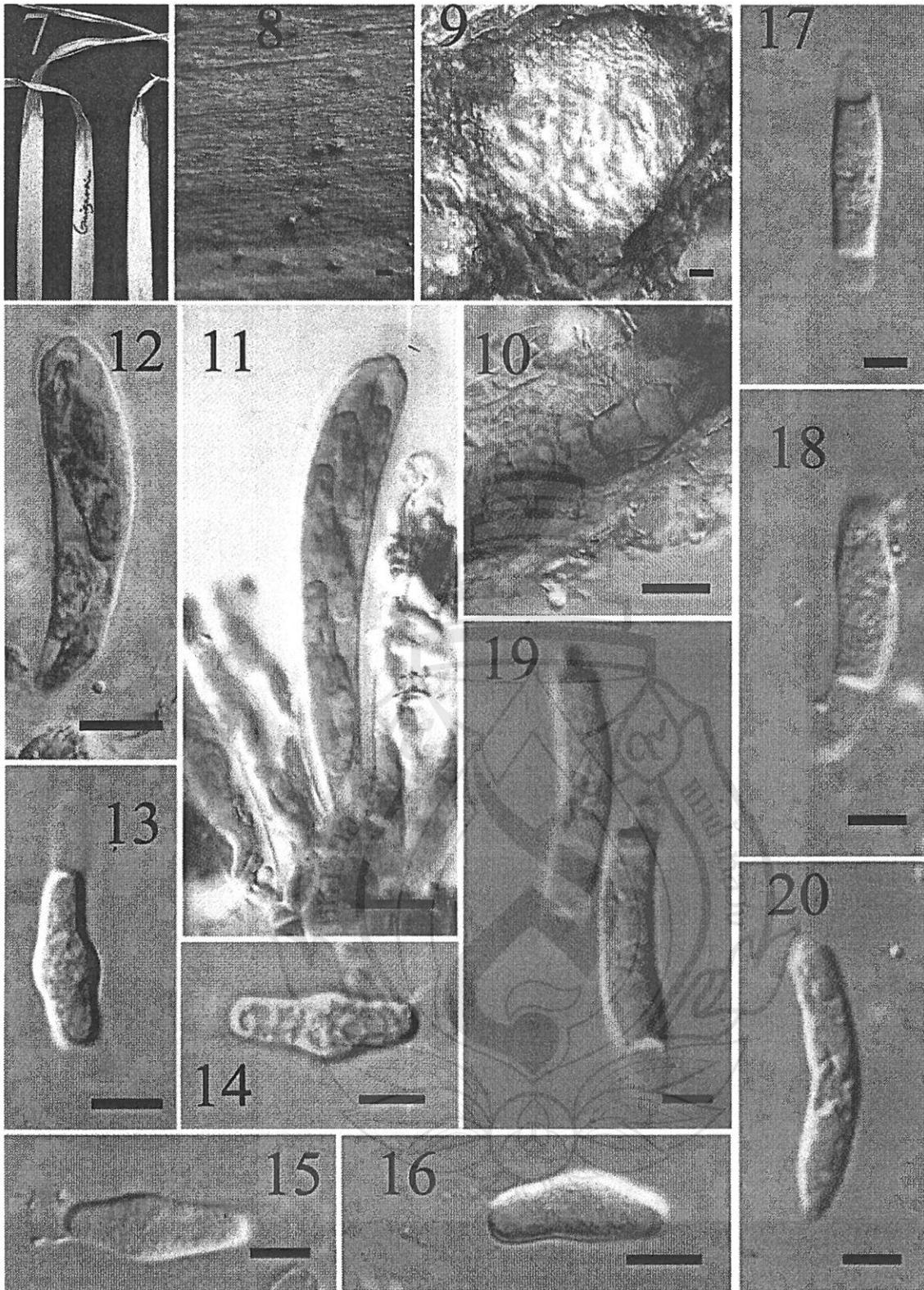
Associated with blight at the apex and on the lamina of the leaves, lesions pale brown, with thin, dark brown border, necrotic tissues containing numerous conspicuous ascomata (Figs 1, 2, 7, 8). Ascomata $95\ 105\ \mu\text{m}$ diameter, $115\ 125\ \mu\text{m}$ high, on the upper surface of the leaves, black, globose to subglobose, immersed in plant tissues, coriaceous, solitary to clustered, ostiolate, ostioles as black dots in the centre. Peridium $10\ 25\ \mu\text{m}$ wide, composed of two to three layers of cells, *textura angularis* and pigmented outwardly and around

ostiole, paler inside (Figs 9, 10, 21). Pseudoparaphyses short chains of fusiform to ovoid cells. Asci $36\ 48 \times 11\ 12\ \mu\text{m}$ ($\bar{x} = 42 \times 12\ \mu\text{m}$, $n = 20$), 8-spored, bitunicate, fissitunicate, clavate to cylindro-clavate, rounded at the apex, where the diameter is $7\ 10\ \mu\text{m}$, tapering gradually to a pedicel attached to the basal peridium, ocular chamber $2\ 4\ \mu\text{m}$ high (Figs 11, 12, 22). Ascospores of two types, $10\ 16 \times 3\ 5\ \mu\text{m}$ ($\bar{x} = 13 \times 5\ \mu\text{m}$, $n = 20$) or $13\ 14 \times 2\ 4\ \mu\text{m}$ ($\bar{x} = 14 \times 2\ \mu\text{m}$, $n = 20$), biseriate, ellipsoidal, swollen in the centre, inequilaterally cylindrical when viewed from above for ascospores of type 1, cylindrical to slightly curved when viewed in any plane for ascospores of type 2, hyaline-greenish, 1-celled, coarse-guttulate, smooth-walled, with polar mucilaginous appendage at each end; appendages of ascospores type 1, $2\ 6\ \mu\text{m}$ long for apical appendage and $2\ 3\ \mu\text{m}$ long for basal appendage (Figs 13 16, 23a) and for ascospores of type 2, $2\ 7\ \mu\text{m}$ long for apical appendage and $1\ 6\ \mu\text{m}$ long for basal appendage (Figs 17 20, 23b).

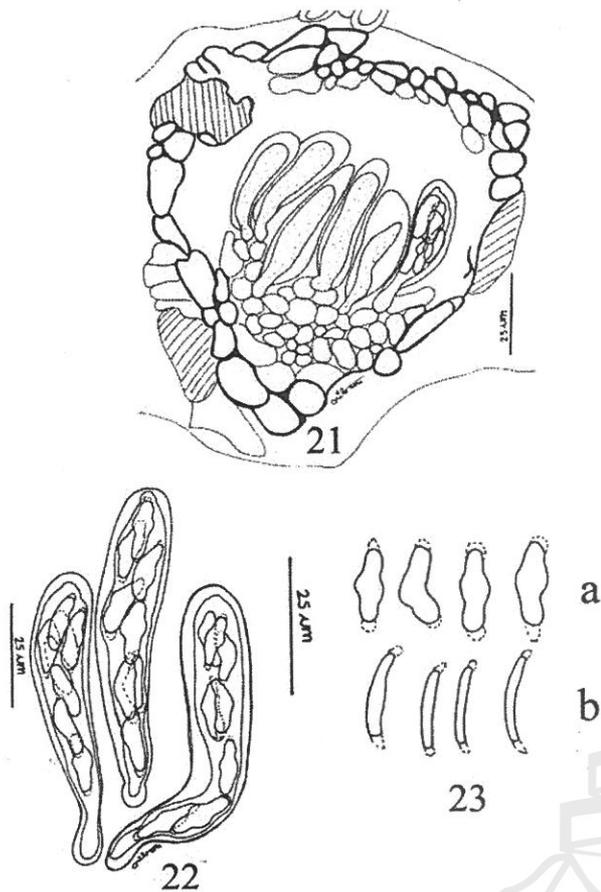
Pycnidia $95\ 115\ \mu\text{m}$ diameter, $105\ 135\ \mu\text{m}$ high, on the surface of the leaf, black, globose to subglobose, immersed in plant tissues, coriaceous, solitary to clustered, ostiolate, ostioles as black dots in the centre, often growing together with ascomata (Fig 8). Peridium $13\ 24\ \mu\text{m}$ wide, composed of two to three layers of cells, *textura angularis* and pigmented outwardly and around ostiole, paler inside (Figs 44, 45). Conidiogenous cells $6\ 14 \times 2\ 3\ \mu\text{m}$ ($\bar{x} = 9 \times 2\ \mu\text{m}$, $n = 20$), holoblastic, determinate, discrete, rarely integrated, hyaline, cylindrical to doliiform, arising from the cells lining the pycnidial locule (Fig 46). Conidia $8\ 13 \times 5\ 7\ \mu\text{m}$ ($\bar{x} = 10 \times 6\ \mu\text{m}$, $n = 20$), hyaline-greenish, 1-celled, coarse-guttulate, smooth-walled, globose, ellipsoidal, clavate or obclavate, with an obtuse apex, sometimes truncate at the base, surrounded by $1\ 5\ \mu\text{m}$ ($\bar{x} = 2\ \mu\text{m}$, $n = 20$) thick mucilaginous sheath which persists at maturity and in some conidia with a single, $2\ 5\ \mu\text{m}$ ($\bar{x} = 3\ \mu\text{m}$, $n = 20$) μm long, hyaline, curved or straight basal appendage (Fig 47). Spermatogenous cells $8\ 13 \times 5\ 7\ \mu\text{m}$ ($\bar{x} = 10 \times 6\ \mu\text{m}$, $n = 20$), holoblastic, filamentous to cylindrical, simple or branched and easily discernible apical structure (Fig 48). Spermatia $4\ 7 \times 1\ 2\ \mu\text{m}$ ($\bar{x} = 5 \times 1\ \mu\text{m}$, $n = 20$)



Figs 1–6 – Leaf blight/spots caused by *Guignardia* spp. on various palms. **1, 2** *G. bispora* leaf blight on *Areca* sp. **3, 4** *G. ellipsoidea* leaf spot on *Caryota* sp. **5, 6** *G. ellipsoidea* leaf spot on *Raphis* sp.



Figs 7–20 –*Guignardia bispora* (MFLU 10 0464, **holotype**). **7** Leaf blight on *Areca* sp. **8** Appearance of ascomata on the host surface. **9** Section of ascoma. **10** Peridium comprising one strata of *textura angularis* comprising 2–3 layers of cells with an apex of thickened brown walls. **11**, **12** Asci. **13–16** Ascospores ellipsoidal, swollen in the centre, inequilaterally ellipsoidal from above with polar mucilaginous appendage (ascospore type 1). **17–20** ascospores cylindrical to slightly curved in any view with polar mucilaginous appendage (ascospore type 2) – Bars **8** = 200 μ m, **9**, **10** = 20 μ m, **11**, **12** = 12 μ m, **13**, **14**, **15**, **16**, **18**, **19** = 5 μ m, **17**, **20** = 4 μ m.



Figs 21–23 – *Guignardia bispora* (MFLU 10 0464, **holotype**) line drawing. **21** Section of ascoma. **22** Asci. **23 a** Ascospores type 1, **b** Ascospores type 2.

holoblastic, cylindrical to dumb-bell shaped, guttulate, straight or slightly curved forming singly in basipetal succession and separating from the spermatogenous cells by a septum (Fig 49).

Material examined – Thailand, Chiang Mai, Medicinal Plant Garden of Faculty of Pharmacy, Chiang Mai University, on living leaf of *Areca* sp., 12 February 2010, N.F. Wulandari, NFW 311 (MFLU 10 0464, **holotype**) anamorph and teleomorph present; on living leaf of *Areca* sp., 12 December 2009, N.F. Wulandari, NFW 317 (MFLU 10 0469) teleo-morph only present. Chiang Rai, Mae Fah Luang University Garden, on living leaf of *Areca* sp., 4 August 2010, N.F. Wulandari, NFW 323 (MFLU 10 0473) anamorph and teleomorph present; Mae Fah Luang Presidential House, on living leaf of *Areca* sp., 13 August 2010, N.F. Wulandari, NFW 334 (MFLU 10 0484) teleomorph only present.

Notes – This species differs from other *Guignardia* species on palms by having two ascospore types. Furthermore, the *Phyllosticta* anamorphic state of *G. bispora* has smaller conidia with shorter appendages when compared to *Phyllosticta cocoicola* (Sivanesan 1984). The *Phyllosticta* anamorph is also different from the anamorph of *G. ellipsoidea* in conidia and appendage size, $8.12.5 \times 5.7 \mu\text{m}$; $1.5.4.5 \mu\text{m}$ in *Phyllosticta* sp. state *G. bispora* vs $6.8.10 \times 4.6.7.7 \mu\text{m}$; $2.7.6.5 \mu\text{m}$ in *P. ellipsoidea*. We therefore consider this species to be new to science.

G. ellipsoidea N.F. Wulandari & K.D. Hyde, **sp. nov.** Figs 3 6, 24 57

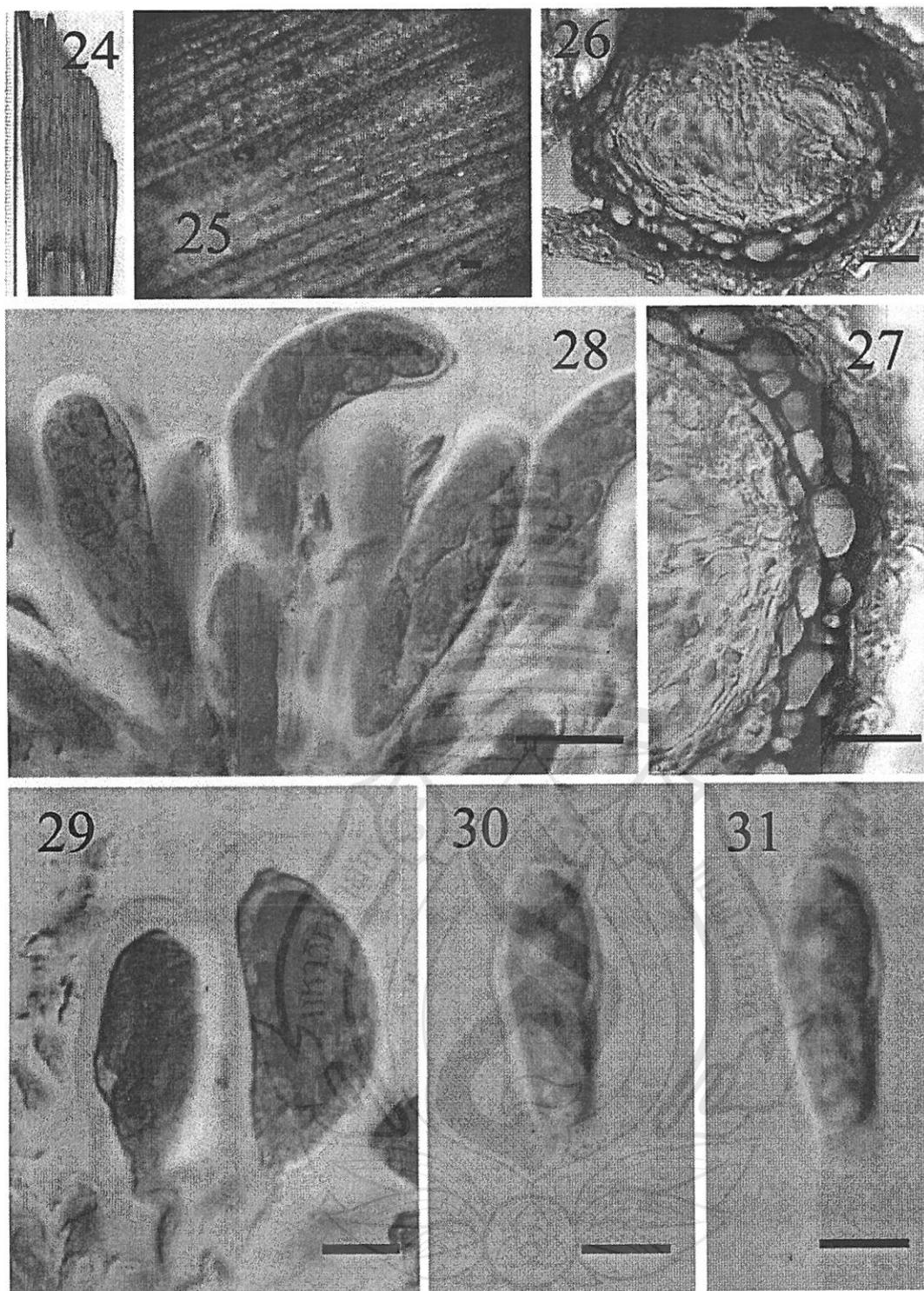
Mycobank MB 519098

Etymology – named for its ellipsoidal ascospores.

Guignardia candeloflamma similis sed ascosporae, ascosporae ellipsoideae, $10.14 \times 4.6 \mu\text{m}$.

Leaf spots irregular, with thick, dark brown border, with numerous ascomata (Figs 3 6, 24, 32, 33, 50, 51). Ascomata $115.130 \mu\text{m}$ diameter, $85.115 \mu\text{m}$ high, immersed, black, globose to subglobose, coriaceous, solitary to clustered, ostiolate, ostioles as black dots in the centre (Figs 25, 34). Peridium $15.22 \mu\text{m}$ wide, composed of two to three layers of cells, *textura angularis* and pigmented outwardly and around ostiole, paler inside (Figs 26, 27, 35, 36, 41). Pseudoparaphyses short chains of filiform to ovoid cells. Asci $33.60 \times 11.14 \mu\text{m}$ ($\bar{x} = 47 \times 13 \mu\text{m}$, $n = 20$), 8-spored, bitunicate, fissitunicate, clavate to cylindro-clavate, rounded at the apex, where the diameter is $5.13 \mu\text{m}$, tapering gradually to a pedicel attached to the basal peridium, ocular chamber $2.3 \mu\text{m}$ high (Figs 28, 29, 37, 38, 42). Ascospores $10.14 \times 4.6 \mu\text{m}$ ($\bar{x} = 12 \times 5 \mu\text{m}$, $n = 20$), biserial, ellipsoidal, clavate to oblong, symmetrical having the same shape when viewed in any plane, hyaline-greenish, 1-celled, coarse-guttulate, smooth-walled, with polar mucilaginous appendage at each end, $2 \mu\text{m}$ long for basal appendage and $1 \mu\text{m}$ long for apical appendage (Figs 30, 31, 39, 40, 43).

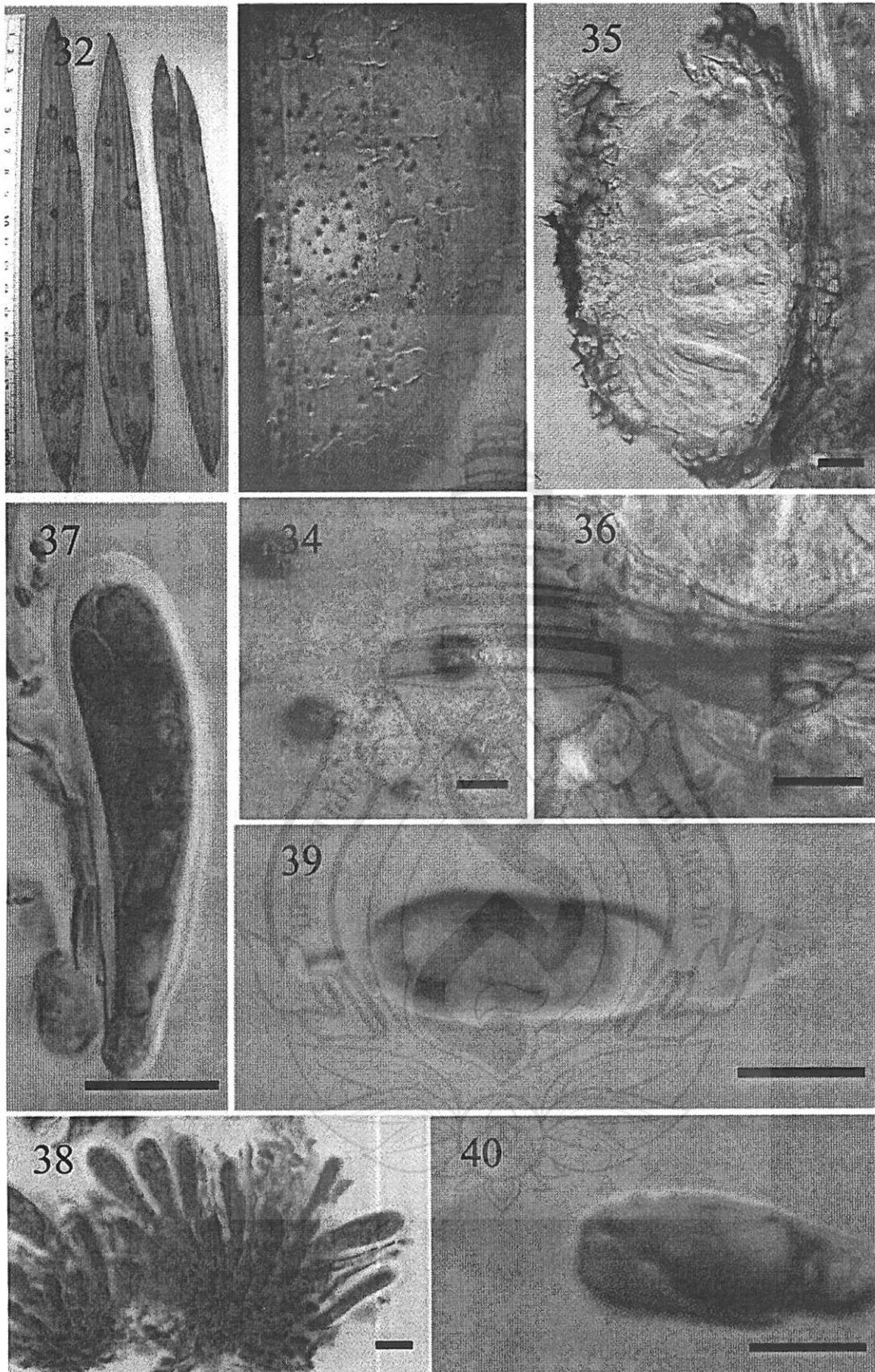
Pycnidia $100.165 \mu\text{m}$ diameter, $100.130 \mu\text{m}$ high, immersed, black, globose to subglobose, coriaceous, solitary to clustered, ostiolate, ostioles as black dots in the centre,



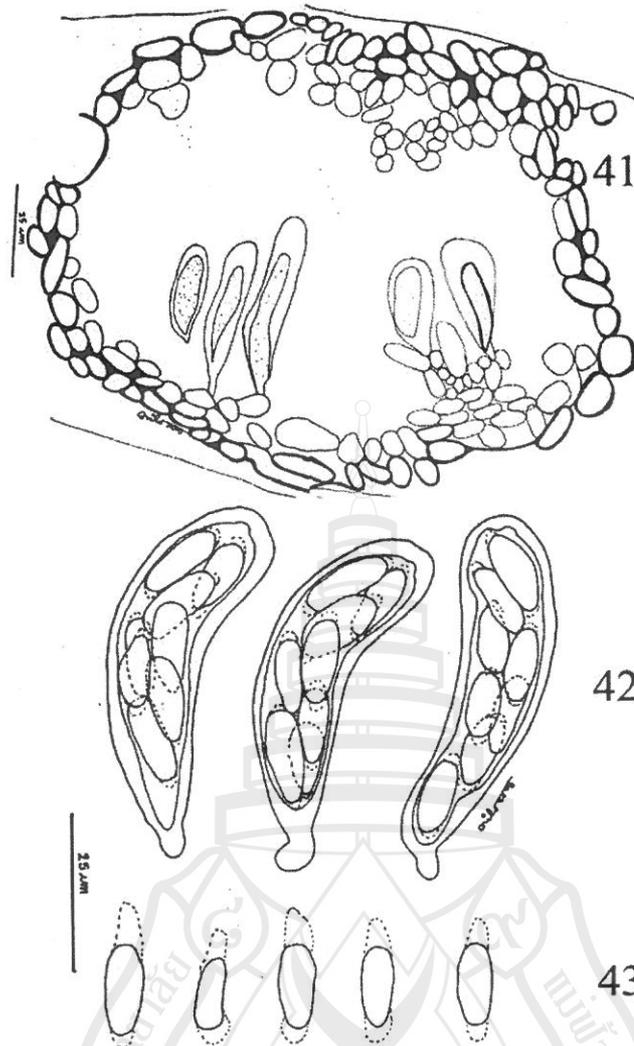
Figs 24–31 – *Guignardia ellipsoidea* (MFLU 10 0431, holotype). **24** Leaf spots on *Caryota* sp. **25** Appearance of ascomata on the host surface. **26** Section of ascoma. **27** Peridium comprising one strata of *textura angularis* comprising 2–3 layers of cells with an apex of thickened brown walls. **28, 29** Asci. **30, 31** Ascospores ellipsoidal to clavate with a polar mucilaginous appendage at each end – Bars 25 = 100 μm , 26, 27 = 20 μm , 28, 29 = 14 μm , 30, 31 = 5 μm .

often growing together with ascomata (Fig 52). Peridium 20 μm wide, composed of two to three layers of cells, *textura angularis* and pigmented outwardly and around ostiole, paler inside (Figs 53, 54). Conidiogenous cells 5–8 \times

2–3 μm (\bar{x} = 6 \times 3 μm , n = 20), holoblastic, determinate, discrete, rarely integrated, hyaline, cylindrical to doliiform forming from cells lining the pycnidial locule (Fig 55). Conidia 7–10 \times 5–8 μm (\bar{x} = 9 \times 6 μm , n = 20), hyaline-



Figs 32–40 – *Guignardia ellipsoidea* (MFLU 10 0475). **32** Leaf spots on *Raphis* sp. **33, 34** Appearance of ascomata on the host surface. **35** Section of ascoma. **36** Peridium comprising one strata of *textura angularis* comprising 2–3 layers of cells with an apex of thickened brown walls. **37, 38** Asci. **39, 40** Ascospores with polar mucilaginous appendage at each end – Bars 34 = 200 μm , 35, 36 = 20 μm , 37, 38 = 12 μm , 39, 40 = 5 μm .

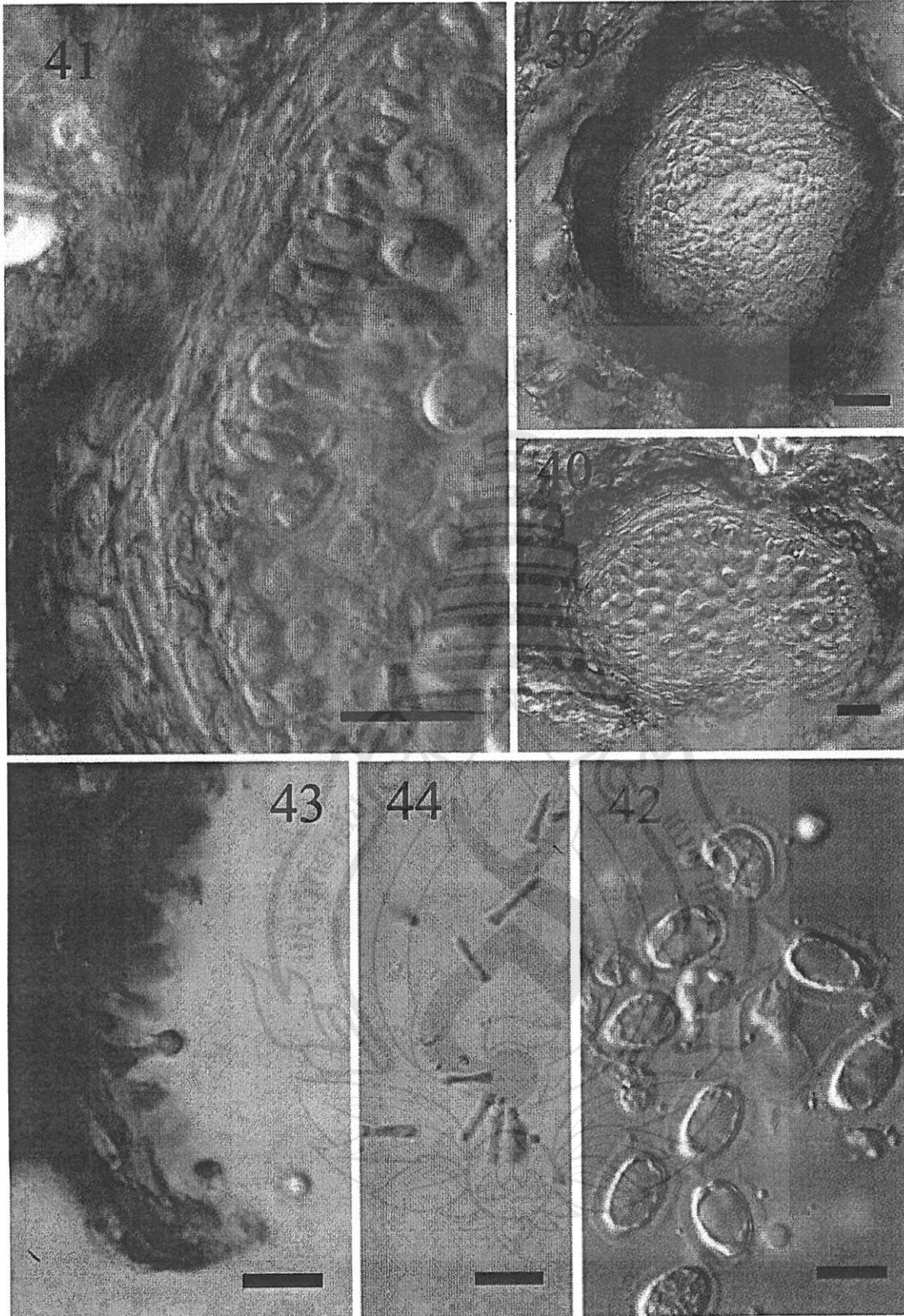


Figs 41–43 – *Guignardia ellipsoidea* (MFLU 10 0475) line drawing. **41** Section of ascoma. **42** Asci. **43** Ascospores with polar mucilaginous appendage at each end.

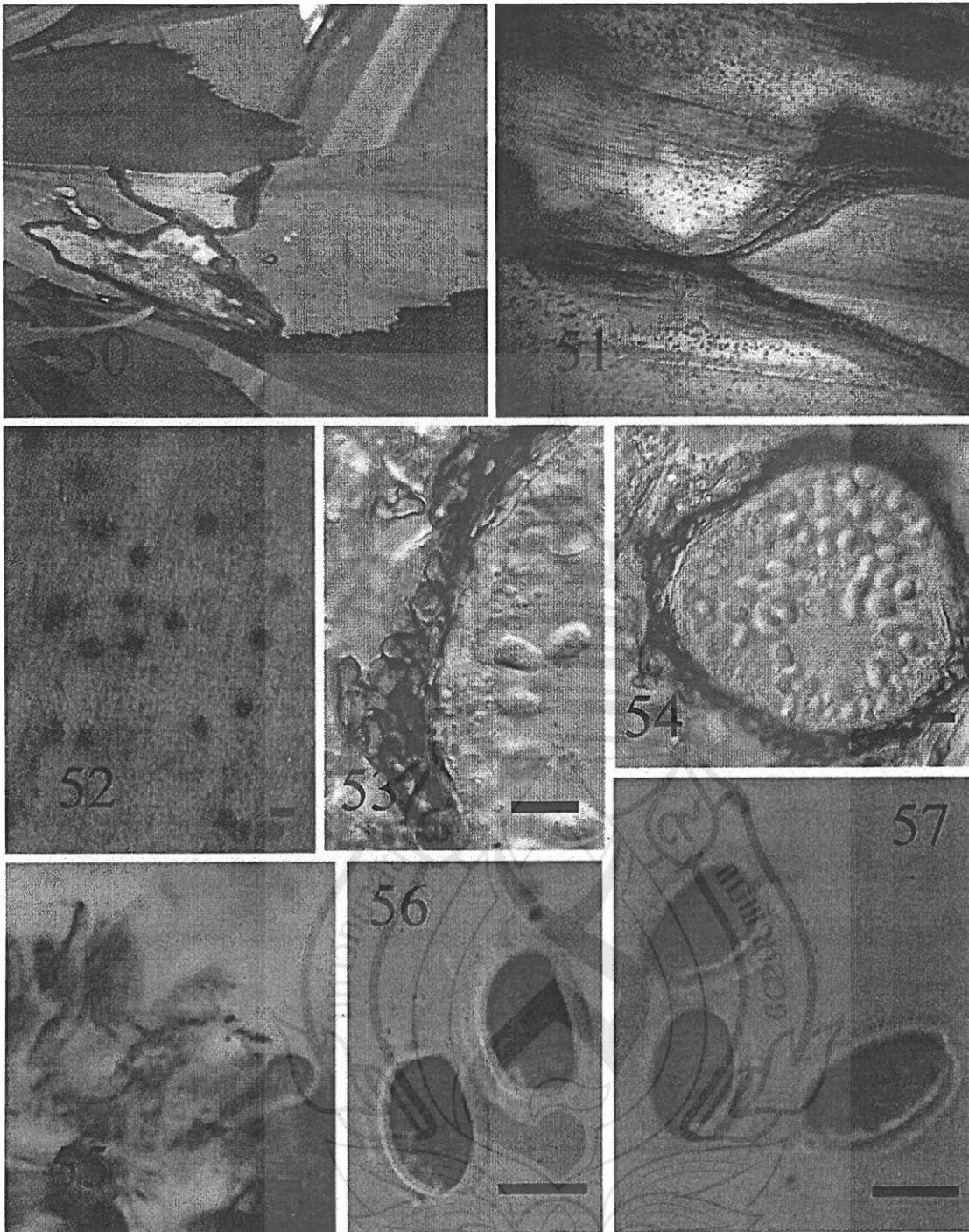
greenish, 1-celled, coarse-guttulate, smooth-walled, globose, ellipsoidal, clavate or obclavate, with an obtuse apex, sometimes truncate at the base, surrounded by 1–2 μm (\bar{x} = 2 μm , n = 20) thick mucilaginous sheath which persists at maturity and in some conidia with a single, 3–7 μm long (\bar{x} = 4 μm , n = 20), hyaline, curved or straight basal appendage (Figs 56, 57). Spermatogenous cells 5.8 \times 1.3 μm (\bar{x} = 6 \times 2 μm , n = 20), holoblastic, filamentous to cylindrical, simple or branched easily discernible apical structure. Spermata 6.8 \times 1.2 μm (\bar{x} = 7 \times 1 μm , n = 20), holoblastic, cylindrical to dumb-bell shaped, guttulate, straight or slightly curved, forming singly in basipetal succession and separating from the spermatogenous cells by a septum.

Material examined – Thailand, Chiang Rai, S3 Building laboratory, School of Science,

Mae Fah Luang University, on living leaf of *Caryota* sp., 28 November 2008, N.F. Wulandari, NFW 239 (MFLU 10 0431, **holotype**) anamorph only present; School of Science, Mae Fah Luang University, on living leaf of *Caryota* sp., 1 January 2010, N.F. Wulandari, NFW 275 (MFLU 10 0441) anamorph only present; School of Science, Mae Fah Luang University, on living leaf of *Caryota* sp., 5 January 2010, N.F. Wulandari, NFW 282 (MFLU 10 0446) anamorph and teleomorph present; School of Science, Mae Fah Luang University, on living leaf of *Caryota* sp., 13 August 2010, N.F. Wulandari, NFW 328 (MFLU 10 0478) anamorph and teleomorph present; School of Science, Mae Fah Luang University, on living leaf of *Caryota* sp., 3 June 2010, N.F. Wulandari, NFW 329 (MFLU 10 0479) teleomorph only



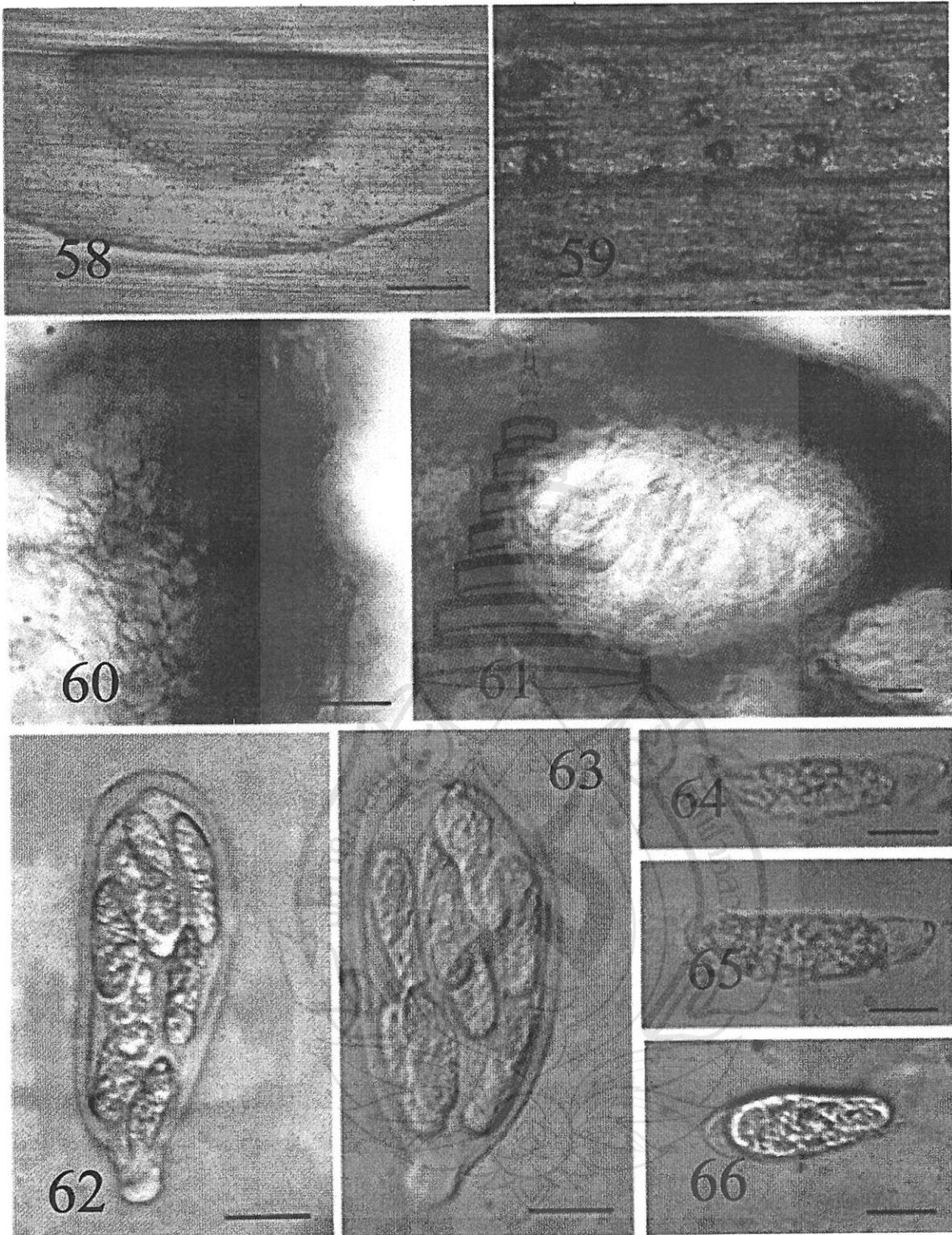
Figs 44–49 – *Phyllosticta* state of *G. bispora* (MFLU 10 0464, **holotype**). **44, 45** Peridium comprising one strata of *textura angularis* comprising 2–3 layers of cells with an apex of thickened brown walls. **46, 48** Conidiogenous cells. **47** Conidia. **49** Spermata – Bars 44, 45, 46 = 20 μm , 47 = 6 μm , 48 = 2 μm , 49 = 7 μm .



Figs 50–57 – *Phyllosticta* state of *G. ellipsoidea* (MFLU 10 0431, **holotype**). **50, 51** Leaf spot on *Caryota* sp. **52** Appearance of pycnidia on the host surface. **53, 54** Peridium comprising one strata of *textura angularis* comprising 2–3 layers of cells with an apex of thickened brown walls. **55** Conidiogenous cells. **56, 57** Conidia. – Bars 52 = 100 μm , 53–54 = 20 μm , 55 = 2 μm , 56–57 = 6 μm .

present; Mae Fah Luang Presidential House, Mae Fah Luang University, on living leaf of *Raphis* sp., 15 March 2010, D. Udayana, D. Manamgoda, R. Phookamsak, NFW 325 (MFLU 10 0475) teleomorph only present.

Notes – This species differs from other *Guignardia* species on palms in having smaller ascospores and the mucilaginous appendage is less well developed as in *G. candeloflamma*. We refer this species as new to science.



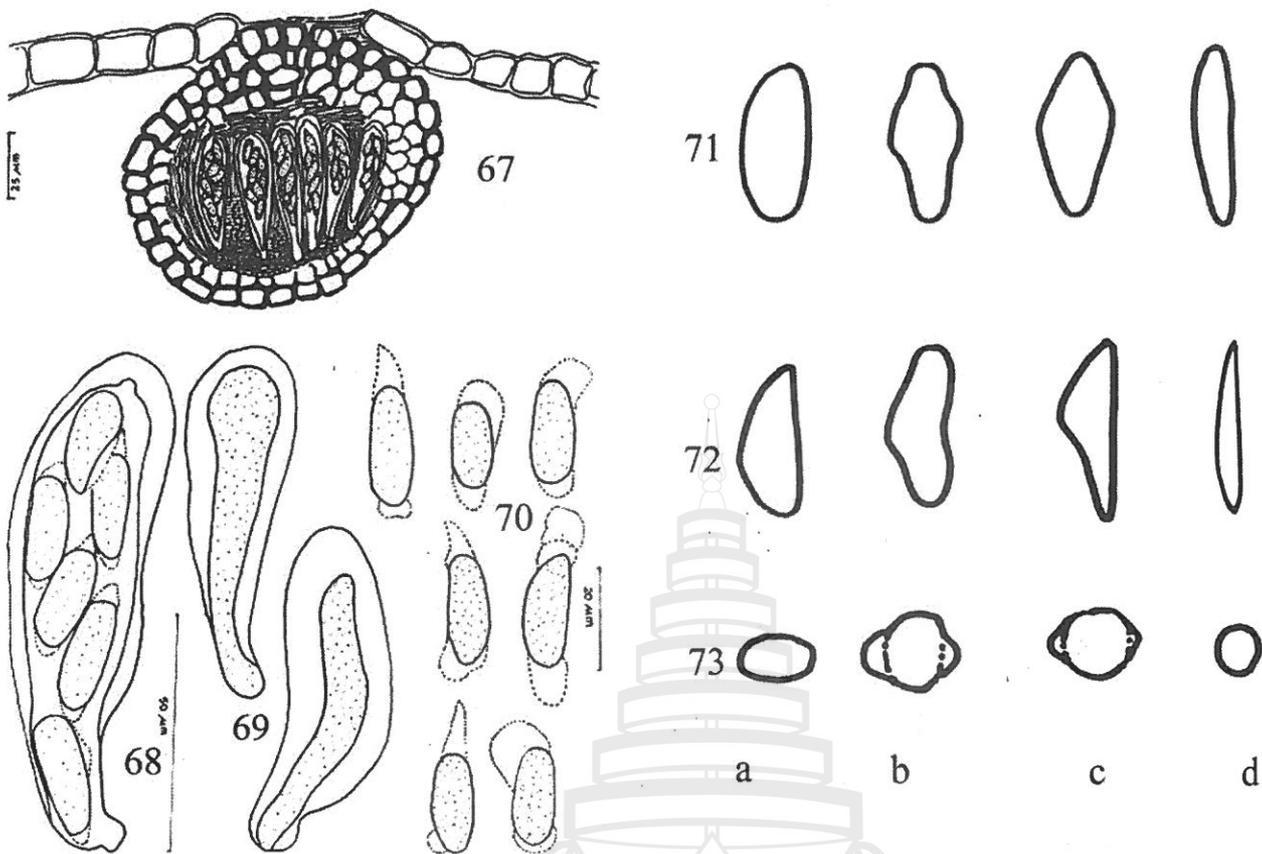
Figs 58–66 – *Guignardia candeloflamma* (BRIP 20472, **holotype**) **58** Leaf spots on *Pinanga* sp. **59** Appearance of ascomata on the host surface. **60** Section of ascoma. **61** Peridium comprising one strata of *textura angularis* comprising 2–3 layers of cells with an apex of thickened brown walls. **62**, **63** Asci. **64**, **65**, **66** Ascospores – Bars 58 = 5 mm, 59 = 100 μ m, 60, 61 = 15 μ m, 62–66 = 10 μ m.

G. candeloflamma J. Fröhl. & K.D. Hyde

Figs 58–70

Literature: Fröhlich & Hyde 1995.

Leaf spots 1.3 × 1.3 mm, ellipsoidal, dark brown in the centre with brown border and becoming paler outside with brown border and contents of numerous ascomata (Fig 58).



Figs 67–70 – *Guignardia candeloflamma* (BRIP 20398, **isotype**) line drawing. **67** Section of ascoma. **68** Ascus. **69** Immature asci. **70** Ascospores.

Ascomata 50–130 μm diameter, 50–80 μm high, on the lower and upper surface of the leaf (amphigenous), black, globose to subglobose, immersed in plant tissues, coriaceous, solitary to clustered, ostiolate, ostioles as black dots in the centre (Fig 59). Peridium 18–31 μm wide, composed of two to three layers of cells, *textura angularis* and pigmented outwardly and around ostiole, paler inside (Figs 60, 61, 67). Pseudoparaphyses not observed. Asci 50–90 × 19–25 μm (\bar{x} = 68 × 22 μm, n = 20), 8-spored, bitunicate, clavate to broadly clavate, rounded at the apex, where the diameter is 14–25 μm, tapering gradually to a 6–7 long × 3–10 μm wide pedicel attached to the basal peridium, ocular chamber 3–7 μm high (Figs 62, 63, 68) with some immature asci (Fig 69). Ascospores 17–22 × 8–11 μm (\bar{x} = 20 × 9 μm, n = 20), biseriate to triseriate and occasionally overlapping triseriate, ellipsoidal, irregular obovoid, ellipsoidal when viewed in any plane, hyaline-greenish, 1-celled, coarse-guttulate, smooth-

Figs 71–73 – Ascospores viewed in any plane or in vertical section (71), when being flattened on one side (72) and in cross section (73). **a.** *Guignardia ellipsoidea* **b, d.** *Guignardia bispora* **c.** *Guignardia cocogena* (Table 1).

walled, with a polar mucilaginous appendage at each end, sheath extended at the base to 3–13 μm long, up to 15 μm long and candle-flame shaped (Figs 64, 65, 66, 70).

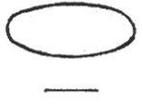
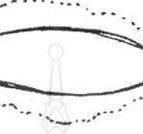
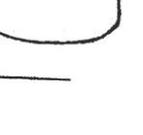
Anamorph – Unknown.

Known distribution – Australia (Queensland), Indonesia (Irian Jaya).

Material examined – Australia, Queensland, Smithfield, on leaves of *Pinanga* sp., 12 February 1992, K.D. Hyde, Queensland Department of Primary Industries Plant Pathology Herbarium (BRIP 20472, **holotype**) teleomorph only present. Indonesia, Irian Jaya, on the leaves of *Pinanga* sp., March 1992, K.D. Hyde, Herbarium of BRIP, National Collection of Fungi (BRIP 20398, **isotype**) teleomorph only present.

Notes – *Guignardia candeloflamma* may form zonate spots on *Pinanga* sp. leaves. This species is distinct from other *Guignardia* species in having a polar mucilaginous appendage at each end of the ascospores, with the

Table 1 Morphological features of asci and ascospores of *Guignardia* species on palms

	<i>G. bispora</i> N.F. Wulandari & K. D. Hyde	<i>G. calami</i> (Syd. & P. Syd.) Arx & E. Müller	<i>G. candelo-flamma</i> J. Fröhl. & K.D. Hyde	<i>G. cocoëns</i> (Petch) K.D. Hyde	<i>G. cocogena</i> (Cooke) Punith.	<i>G. ellipsoidea</i> N.F. Wulandari & K.D. Hyde	<i>G. manokwaria</i> K.D. Hyde	<i>G. migrans</i> (Rehm) K.D. Hyde	<i>G. ryukyensis</i> I. Hino & Katumoto
Asci shape	Ovoid, saccate or clavate	Irregularly ovoid, remnants sheath	Clavate to pyriform	Clavate	Clavate	Clavate, cylindro-clavate	Clavate	Clavate to ovoid	Clavate, cylindro-clavate
Asci size μm	40 100×20 28	42 72×14 18	91 140×17.5 25	75 125×20 25	62 100×10 12	33 60×11 14	70 100×20 24	54 82×22 38	70 85×18 23
Ascospores shape/sheath/appendage	Ovoid, no sheath, roughened wall	Irregularly ellipsoidal with apical button like germ pores and remnants of sheath	Ellipsoidal, pad and candle-flame shaped appendage	Broad and cylindrical to ellipsoidal, obclavate with germ pore	Ellipsoidal, wider in the mid region and rounded with appendage	Fusiform, ellipsoidal to oblong with polar appendage	Fusoid to rhomboid, irregular sheath with germ pore	Ellipsoidal, no sheath	Fusoid, oblong ends rounded or obtuse, no sheath
Ascospores line drawing									
Ascospores size μm	18 26×8 13	15 19×7 8	17.5 22×7.5 11	23 26.5×9 10	13 20×5 6.5	10 14×4 6	22 30×8 12.5	19 24×8.5 12	23 28×6.5 7
Reference	Hyde (1995)	Hyde (1995)	Hyde (1995)	Hyde (1995)	Hyde (1995)	Present study	Hyde (1995)	Hyde (1995)	Hyde (1995)

basal appendage extended in a candle-flame shape.

Discussion

The *Guignardia* species recorded on palms have distinct ascospores and some have mucilaginous appendages. These characters can be useful to identify the taxa to species. A synopsis of *Guignardia* species from palms is provided in Table 1.

Acknowledgements

Nilam Wulandari is grateful to the Graduate School, Chiang Mai University, Thailand for financial support and the School of Science, Mae Fah Luang University for laboratory facilities. BRT, Thailand awarded grant BRT No. R251181 to study Dothideomycetes in northern Thailand, MFLU awarded grant No. 53101020017 to study the genus *Phyllosticta* in northern Thailand and the National Research Council of Thailand awarded grant No. 54201020004 to study the genus *Phyllosticta* in Thailand. The Mushroom Research Foundation is thanked for a scholarship to carry out studies towards a Ph.D. J. Stalpers, CBS, thanked for nomenclatural correctness. P. Crous, CBS, the Netherlands is thanked for partially funding this research.

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Appendix B – CV of Dr Hyde



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Summary

QUALIFICATIONS

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

EXPERIENCE

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

INTERNATIONAL STANDING

Prestigious committees and awards

1. President of the Asian Mycological Committee

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

Fungal Diversity (EC)

Fungal Diversity Research Series (EC)

Agricultural Technology (EC)

Mycology (EC)

Mycological Research (EB)

Biotropica (EB)

Cryptogamie Mycologie (EB)

Journal of Forest Research (EB)

Persoonia (EB)

Fungal Ecology (EB)

Australian Mycologist (EB)

Mycosphere (EB)



PUBLICATIONS

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

- I was Editor-in-Chief and produced 8 issues of the International Journal of Agricultural Biotechnology

TEACHING

Taught and designed courses in:

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



Main CV (Thailand emphasis)

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

Postdoctoral Fellows Supervised

1. **Dr Po Po Than.** January 2008 – July 2009. *Colletotrichum*.
2. **Dr Sutheera Thongkantha.** December 2006 – May 2008. **Basidiomycete expert and Scientific Manager at Mushroom Research Centre.**
3. **Dr Edward Grand.** May 2004 – December 2005. Basidiomycete expert and scientific manager at Mushroom Research Centre.
4. **Dr Yu Jiang.** June 2000 – August 2002. Fungal Biotechnologist (Now Assistant Professor at Baptist University).
5. **Dr Ho Wai Hong.** August 1998 – June 2006. Culture Collection Manager /Teaching Instructor. Samuel is developing Biotechnological Projects and supervising Undergraduate Students.
6. **Dr Sally Fryar.** February 1998 - November 2000. Ecology of Freshwater Fungi. Sally was co-supervised by Dr Hodgkiss.
7. **Dr E.C.Y. Liew.** January 1997 – December 2001. Molecular Phylogeny (Now Principal Scientist in Sydney University).
8. **Dr W.S. Wong.** September 1996 – December 1997. Ultrastructure of the Xylariaceae and Culture Collection Manager. (Now Project Manager, CK Life Science Limited, HK).
9. **Dr T.K. Goh.** July 1996 – June 1999. Molecular taxonomy of Bipolaris-like fungi (Now Assistant Director, Planning and Coordination Office, HKUST)
10. **Dr B. Tread.** August 1995 – April 1997. Culture collection and herbarium development. (Now Pharmacist with a French drug company).

MSC and PhD students completed (Thai Universities)

Thai students

1. **Aom Pinnoi.** October 2004-May 2008. **Palm fungi.** (PhD student, jointly supervised at Princess Songkla University, Thailand).
2. **Rampai Kodsueb.** June 2002 – September 2007. **Biodiversity of Saprobic Fungi on Woody Litter.** (PhD student, jointly supervised at Chiang Mai University, Thailand).
3. **Itthayakorn Promputtha** June 2001 – May 2006. **Fungal succession and diversity.** (PhD student, jointly supervised at Chiang Mai University, Thailand).
4. **Sutheera Thongkantha.** June 2002 – July 2006. **Biodiversity of fungi on Pandanus.**

- (PhD student, jointly supervised at Chiang Mai University, Thailand).
5. **B. Bussaban**, July 1999 – October 2005. **Fungi on *Zingiberaceae* in Thailand**. (PhD student, jointly supervised at Chiang Mai University, Thailand).
 6. **P. Wipornpan**, July 1998 – May 2004. **Fungi on *Musa acuminata* (banana) in northern Thailand** (PhD student, jointly supervised at Chiang Mai University).
 7. **Umpava Pinruan**. October 2004. **Biodiversity and antifungal production by fungi on the palm *Eleiodoxa conferta* in Sirindhorn peat swamp forest, Narathiat, Thailand**. (MSc student, jointly supervised at Chiang Mai University, Thailand).
 8. **Aom Pinnoi**. April 2004. **Biodiversity and antifungal production by fungi on the palm *Eleiodoxa conferta* in Sirindhorn peat swamp forest, Narathiat, Thailand**. (MSc student, jointly supervised at Chiang Mai University, Thailand).
 9. **Warin Techa**. May 2001. **Diversity of saprobic fungi from *Calamus kerrianus* and *Walichia caryotoides* at Suthep-Pui National Park, Thailand**. (MSc student, jointly supervised at Chiang Mai University, Thailand).
 10. **Weraphol Bhilabutra** June 2002 – present. **Grass Fungi**. (Royal Golden Jubilee PhD student, jointly supervised at Chiang Mai University, Thailand) – should submit within 2-3 months.
 11. **Kanchalika Ratanacherdchai**, June 2005-present. **Induced plant immunity to control anthracnose in organic crop production** (Royal Golden Jubilee PhD student, jointly supervised at KMITL, Thailand).
 12. **Mongkol Wongsawas**, June 2007 – present. **Freshwater fungi in Zhejiang Province, China** (PhD student, jointly supervised at Zhejiang University, China).
 13. **Saithong Kaewchay**, June 2006-present. **Biological Control of White Root Disease of Rubber Tree** (Royal Golden Jubilee PhD student, jointly supervised at Chiang Mai University, Thailand).
 14. **Hongli Hu**, September 2005-present. **Brown spored bitunicate ascomycetes** (PhD student, HKU).

Asian students at Thai Universities

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

Thailand (MS student, Mae Fah Luang University).

23. **Haryudian Prihastuti**, November 2007 – present. *Colletotrichum* species on coffee in Thailand. (MS student, Mae Fah Luang University).

MSC and PhD students in progress (Thai Universities)

Thai students

1. **Umpava Pinruan**. October 2004 - present. **Physiology of Palm fungi** (PhD student, jointly supervised at Chiang Mai University, Thailand).
2. **Ratchadawan Cheewangkoon**, June 2006-present **Eucalyptus fungi in Thailand** (PhD student, jointly supervised at Pretoria University, South Africa).
3. **Maythasith Konkarn**, June 2008-present. **Fungi associated with pine-infesting ambrosia beetles in Thailand** (PhD students, jointly supervised at Preoria University, South Africa).
4. **Putarak Chomnunti**, October 2008-present. **Biodiversity and phylogeny of selected Dothideomycetes in Thailand 1** (PhD student, MFLU, Thailand).
5. **Saranyaphat boonmee**, October 2008-present. **Biodiversity and phylogeny of selected Dothideomycetes in Thailand 2** (PhD student, MFLU, Thailand).
6. **Pornthip Ruanpanun**, June 2007-present. **Biodiversity and application of actinomycetes and fungal parasites of invertebrates in organic agricultural system** (Royal Golden Jubille PhD student, jointly supervised at Chiang Mai University, Thailand).
7. **Nathacha Seehan**, June 2008-present. **Biodiversity and utilization of forest litter saprobes** (PhD student, jointly supervised at KMITL, Thailand).
8. **Tanapak Inyod**, June 2008-present. **Biological Control of White Root Disease of Oil Palm** (Royal Golden Jubille PhD student, jointly supervised at KMITL, Thailand).
9. **Parinn Noireung**, June 2009 – present. **Phylogenetic biodiversity of pathogenic *colletotrichum* on plant leaves in northern thailand.** (thailand graduate institute of science and technology (tgist))
10. **Jutamart Monkai**, June 2009 - present. **Utilizing Thailand's biodiversity: ascomycetes taxonomy, phylogeny and screening for insecticides.** (basic research (trf))
11. **Rungtiwa Phookamsak**, June 2009 – present. **Biodiversity,taxonomy and phylogenetic studies on dothideomycetes monocotyledons in thailand.**(royal golden jubille)
12. **Supalak Yacharone**, October 2009 – present. **Utilizing Thailand's biodiversity: ascomycetes taxonomy, phylogeny and screening for insecticides.** (basic research (trf))
13. **Naritsada Thongklang**, October 2009 – present. **Cultivation of agaricus species endemic to thailand and their medicinal properties.**(brn)
14. **Saowanee Wikee**, June 2010 – present. **taxonomy and phylogeny of phyllosticta, diplodia and stemphylium species in northern thailand.**

Asian students at Thai Universities

15. **Sitthisack Phouliyong**, November 2007 – present. **A study of the diversity of *Colletotrichum acutatum* in Thailand.** (PhD student, Mae Fah Luang University).
16. **Samantha Karunarathna**, October 2008-present. **Diversity and taxonomy of *Lentinus* and *Panus* in Northern Thailand and Sri Lanka**(PhD student, Mae Fah Luang University).
17. **Nilam Wulandari**, June 2006-present. **A world monograph of *Guignardia*.** (PhD student, jointly supervised at Chiang Mai University, Thailand).
18. **Phongoun Sysouphanthong**, October 2006-present. **Diversity of *Lepiota* and *Leucoagaricus* (BASIDIOMYCOTA) in Northern Thailand** (MS student, Mae Fah Luang University).
19. **Paul Mungai**, October 2008-present. **Diversity of dung fungi on wild anamils in Kenya** (MS student, Mae Fah Luang University).
20. **Manamgoda Gamage Dimuthu Sandarenu Manamgoda** June 2010 – present **Taxonomy and phylogenetics of *cochliobolus curvularia bipolaris* and *helminthosporium*** (PhD student Mae Fah Luang University).
21. **Dhanushka Udayanga** June. 2010 – present **Taxonomy and phylogeny of the genera *phomopsis*/*diaporthe* and *colletotrichum*** (PhD student Mae Fah Luang University)
22. **Sajeewa Maharachchikumbura** June.2010 – present **Phylogenetic evaluation of genus *pestalotiopsis* using both morphological and molecular character and studies in the natural product chemistry of selected species of *pestalotiopsis*** (PhD student Mae Fah Luang University)
23. **Jiankui Liu** October 2009 – present. **The phylogeny of higher fungi (ascomycota and basidiomycota) from palms.** (PhD student Mae Fah Luang University).
24. **Pheng Phengsintham** October 2009 – present. **Cercospora and allied genera from northern thailand and laos** (PhD student Mae Fah Luang University)

Other student supervision

Research Assistant Professor Supervised

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

Postdoctoral Fellows Supervised

1. **Dr Sutheera Thongkantha.** December 2006 – 2008. Basidiomycete expert and Scientific Manager at Mushroom Research Centre
2. **Dr Rajesh Jeewon.** April 2003-present. **Fungal Molecular Biologist.**
3. **Dr Edward Grand.** May 2004 – December 2005. **Basidiomycete expert and scientific manager at Mushroom Research Centre.**
4. **Dr Yu Jiang.** June 2000 – August 2002. **Fungal Biotechnologist** (Now Assistant Professor at Baptist University).
5. **Dr Ho Wai Hong.** August 1998 – June 2006. **Culture Collection Manager /Teaching Instructor.** Samuel is developing Biotechnological Projects and supervising Undergraduate Students.
6. **Dr Sally Fryar.** February 1998 - November 2000. **Ecology of Freshwater Fungi.** Sally was co-supervised by Dr Hodgkiss.
7. **Dr E.C.Y. Liew.** January 1997 – December 2001. **Molecular Phylogeny** (Now Principal Scientist in Sydney University).
8. **Dr W.S. Wong.** September 1996 – December 1997. **Ultrastructure of the Xylariaceae and Culture Collection Manager.** (Now Project Manager, CK Life Science Limited, HK).
9. **Dr T.K. Goh.** July 1996 – June 1999. **Molecular taxonomy of Bipolaris-like fungi** (Now Assistant Director, Planning and Coordination Office, HKUST).
10. **Dr B. Tread.** August 1995 – April 1997. **Culture collection and herbarium development.** (Now Pharmacist with a French drug company).

PhD students completed

11. **Shenoy Belle Damodar.** September 2003-September 2007. **Grass endophytes.** (PhD student, HKU).
12. **Alvin Tang Ming Chak.** September 2003-October 2006. **Multigene analysis of Sordariomycetes** (PhD student, HKU).
13. **Justin Bahl.** January 2002-June 2006. **Molecular phylogenetics of Hyponectriaceae.** (PhD student, HKU).
14. **Cai Lei.** October 2002- May 2006. **Aquatic fungi.** (PhD student, HKU).
15. **Dhanasekaran Vijaykrishna,** January 2002 - February 2005. **Freshwater Fungi: Biodiversity, Origins and molecular taxonomy.** (PhD student, HKU).
16. **B. Paulus,** June 2000 – October 2004. **Measuring Biodiversity of Fungi in North Queensland, Australia** (PhD student, jointly supervised at James Cooke University, Cairns, NQ, Australia).
17. **S.S.K. Durairajan,** July 2000 – Jan 2004. **Biological screening and isolation of immunomodulatory compounds from endophytic fungi of *Tripterygium wilfordii*** (PhD student, HKU).
18. **V. Bucher,** September 1999 – May 2003. **Enzymes from aquatic fungi** (PhD student, HKU).
19. **G. Smith,** August 1998 – April 2003. **Phylogenetic studies on the Xylariaceae** (PhD student, HKU).
20. **Dr S.R. Ghimire,** Nepal, January 1999 – December 2001. ***Phytophthora infestans* population in Nepal** (Post Doctoral Fellow, USA).
21. **Dr R. Jeewon,** Mauritius, January 1999 – December 2001. ***Pestalotiopsis* Taxonomy: Molecular Phylogenetics, Species nomenclature and Teleomorph Relationships.** (Post Doctoral Fellow, HKU).

22. **Dr O.H.K. Lee.** February 1998 – January 2001. **The Feeding Ecology of *Littoraria* species in Hong Kong Mangroves** (PhD HKU, now Assistant Environmental Protection Officer, Environmental Protection Department, HKSAR).
23. **Dr Yanna.** February 1998 – January 2001. **Biodiversity, Ecology and Taxonomy of Saprobic Fungi on Palm Fronds** (PhD HKU, now Environmental Education Officer, Environmental Protection Department, HKSAR).
24. **Assistant Professor Dr Y.Z. Wang.** December 1997 – November 2000. **Revision of the Ascomycete Genus *Amphisphaeria*** (PhD HKU, now Chief Manager, China General Microbiological Culture Collection, Chinese Academy of Sciences, Beijing).
25. **Dr D. Zhou.** December 1997- November 2000. **Biodiversity of Saprobic Microfungi Associated with Bamboo in Hong Kong and Kunming, China** (PhD student, HKU, now Dean and Professor, Faculty of Conservation Biology, Southwest Forestry University, Kunming, China).
26. **Professor B.H. Lu.** May 1997 - April 2000. **A World Monograph of *Anthostomella*** (PhD HKU, now Professor, Department of Plant Protection, Faculty of Agriculture, Shanxi Agricultural University, Taigu, China).
27. **Dr M.K.M. Wong.** March 1997 – May 2000. **Diversity, Host Preference, and Vertical Distribution of Saprobic Fungi on Grasses and Sedges in Hong Kong** (PhD HKU, now Post Doctoral Fellow in Department of Zoology, HKU).
28. **Dr L.D. Guo.** February 1997 – January 1999. **Identification of Endophytic Fungi in *Livistona chinensis* (Palmae)** (PhD HKU, now a scientist at the Lichen and Mycology Laboratory, Academia Sinica, Beijing).
29. **Dr C. Pearce.** February 1996 – Decemembr 1999. **The *Phyllachoraceae* of Australia: a Taxonomic Treatise** (PhD HKU, now Director of the Australian Tropical Rainforest Research Centre, Cairns).
30. **Dr C.K.M. Tsui.** Septemembr 1996 – August 1999. **Biodiversity and Longitudinal Distribution of Fungi on Submerged Wood, with Reference to Human Disturbance** (PhD HKU, now Post Doctoral Fellow in Botany, HKU).
31. **Dr M. Ranghoo.** December 1995 – November 1998. **Phylogeny of Freshwater Ascomycetes** (PhD HKU, now Molecular Biologist with the Mauritius Sugar Research Institute).
32. **Dr S.R. Whitton.** October 1995 – August 1999. **Microfungi on the *Pandanaceae*** (PhD HKU, Post Doctoral Fellow at Landcare Research, New Zealand).
33. **Dr W.H. Ho.** July 1995 – June 1998. **Biodiversity, Ecology and Ultrastructure Observations of fungi on wood submerged in tropical streams** (PhD HKU, Post Doctoral Fellow and Culture Collection Manager in Ecology & Biodiversity, HKU).
34. **Dr T.K. Yeun.** July 1995 – June 1998. **Wood Decomposition and Competition in Tropical Freshwater Fungi** (PhD HKU, now Consultant, N. Law and Associates Management Consultancy Ltd, Hong Kong).
35. **Dr A. Poonyth.** May 1995 – August 1998. **Biodiversity of Mangrove Fungi in Mauritius.** (PhD University of Mauritius, cosupervised, now Research Officer for Mauritius Wildlife and Parks).
36. **Dr T. Dalisay.** January 1995 – February 1998. **Biodiversity of Microfungi Associated with Species of *Bambusa* and *Dendrocalamus*** (PhD HKU, now Associate Professor in Plant Pathology at The University of the Philippines, Los Banos).
37. **Dr J. Wright.** October 1994 – March 1998. **The Role of Endophytes in Citrus Stem End Rots** (PhD HKU, now Plant Pathologist in Fiji).
38. **Dr J. Taylor.** January 1994 – September 1997. **Biodiversity of distribution of Microfungi on Palms** (PhD HKU, now Lecturer at Botswana University).
39. **Dr Kang Ji Chuan.** December 1993 – March 1997. **Molecular and Morphological**

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

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

MPhil students completed

1. **Hu Dianming.** June 2003-May 2006. **Dung fungi.** (MSc student, jointly supervised at Yunnan University, China).
2. **Li Yan.** June 2003-May 2006. **Nematodes trapping fungi in China.** (MSc student, jointly supervised at Yunnan University, China).
3. **Hong Zhu.** June 2003-May 2006. **Aquatic fungi in Yunnan China.** (MSc student, jointly supervised at Yunnan University, China).
4. **Hongli Hu.** June 2003 – June 2005. **Chinese pine fungi.** (MSc student, jointly supervised at Yunnan University, China).
5. **QuinYeung Sze Yuen.** September 2002-January 2005. **The fungal diversity of Pinaceae in Hong Kong** (MPhil, HKU).
6. **P. Alva.** January 2000 – February 2005. **Internal fungi from seagrasses and their ability to produce enzymes** (MSc student, jointly supervised at Ateneo de Manila University of the Philippines).
7. **Sin Kai Wai.** July 2004. **Molecular biology, physiology and metal resistance of the ligninolytic enzyme system in a newly isolated basidiomycete from a Hong Kong forest.** (Mphil, HKU)
8. **Luo Jing.** May 2004. **Taxonomic and ecological studies on freshwater fungi associated with identified substrates.** (MSc student, jointly supervised at Yunnan University, Kunming, China).
9. **Nguyễn văn Diễm.** December 2003. **Saprobe ascomycetes on *Nypa fruticans* in Can Gio Mangrove Biosphere Reserve, Vietnam** (Student, jointly supervised at Hanoi University of Education).
10. **A. Besitulo.** April 2002. **Occurrence and distribution of fungi in a mangrove forest at Siargao Island, Philippines** (MSc student, jointly supervised at St Carlos University, Cebu).
11. **D. Lacap.** March 2001. **Biodiversity of fungal endophytes on *Taxus baccata* (Taxaceae) and *Polygonum multiflorum*.** (MSc student, jointly supervised at Ateneo de Manila University of the Philippines).
12. **A.M.C. Tang,** January 2001 – present. **Secondary metabolites of wild fruits in Hong Kong: Implications for antimicrobial defense and seed dispersal** (MPhil student, HKU).
13. **C. Lei,** July 2000 – present. **Freshwater Fungi in Yunnan, China** (MSc student, jointly supervised at Yunnan University, Kunming, China).
14. **Y.W. Choi,** January 1999 – present. **Endophytes on *Brucia javanica*** (M.Phil student,

- HKU).
 15. **S.W. Lee**, January 2000 – present. **Post Harvest Diseases of Citrus** (M.Phil student, HKU).

Graduate students (MS and PhD students in Progress)

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

Community Service

- I was **Coordinator of EASIANET** from 2004 until 2007. This was an elected position for the body designated with the role to remove the taxonomic impediment to CBD from the East Asia region.
 - I am **President** of the Asian Mycological Committee. This committee aims to promote the study of mycology throughout the Asian region.
 - In 1997 the **Mycological Association of Hong Kong** was inaugurated. I was **Chairman** of this organization between 2002-2007.
 - I have also been **external examiner** for students at many Universities.
 - I have given more than 20 **keynote and guest lectures** and numerous invited lectures.
- I have **organised** (as Chair or on committee) more than 10 international conferences and numerous workshops

Publication list

Year	International Publications	SCi Papers	Thailand collaboration
1985	2	2	

1986	8	5	
1987	2	2	
1988	8	4	
1989	14	9	1
1990	8	4	1
1991	10	6	
1992	21	9	
1993	22	9	
1994	18	7	1
1995	27	19	
1996	36	26	
1997	37	16	
1998	50	33	
1999	60	30	
2000	51	27	1
2001	56	48	5
2002	39	31	9
2003	43	33	9
2004	48	26	16
2005	24	21	4
2006	31	27	11
2007	23	19	7
2008	24	24	11
2009	23	23	8
2010			

Books (17)

1. **Hyde, K.D.** (ed.) (1997). *Biodiversity of Tropical Microfungi*. Hong Kong University Press, Hong Kong, 421p.
2. **Hyde, K.D.** and Cannon, P.F. (1999). *Fungi Causing Tar Spots on Palms*. IMI, UK, 114p.
3. **Hyde, K.D.** and Pointing, S.B. (eds.) (2000). *Marine Mycology - A Practical Approach*. [Fungal Diversity Research Series 1], Fungal Diversity Press, Hong Kong, 377p.
4. **Hyde, K.D.**, Taylor, J.E. and Fröhlich, J. (2000). *Genera of Ascomycetes from Palms*. [Fungal Diversity Research Series 2], Fungal Diversity Press, Hong Kong, 247p.
5. Fröhlich, J. and **Hyde, K.D.** (2000). *Palm Microfungi*. [Fungal Diversity Research Series 3], Fungal Diversity Press, Hong Kong, 393p.
6. Lu, B.H. and **Hyde, K.D.** (2000). *A World Monograph of Anthostomella*. [Fungal Diversity Research Series 4], Fungal Diversity Press, Hong Kong, 376p.
7. Lu, B.H., **Hyde, K.D.**, Ho, W.H., Tsui, K.M., Taylor, J.E., Wong, K.M., Yanna and Zhou, D.Q. (2000). *Checklist of Hong Kong Fungi*. [Fungal Diversity Research Series 5], Fungal Diversity Press, Hong Kong, 207p.
8. **Hyde, K.D.**, Ho, W.H. and Pointing, S.B. (2000). *Aquatic Mycology across the Millennium*. [Fungal Diversity 5], Fungal Diversity Press, Hong Kong, 207p.
9. Pointing, S.B. and **Hyde, K.D.** (eds.) (2001). *Bio-Exploitation of Filamentous Fungi*. [Fungal Diversity Research Series 6], Fungal Diversity Press, Hong Kong, 467p.
10. **Hyde, K.D.** (ed.) (2002). *Fungi in Marine Environments*. [Fungal Diversity Research Series 7], Fungal Diversity Press, Hong Kong, 397p.
11. Tsui, C.K.M. and **Hyde, K.D.** (2003). *Freshwater Mycology*. *Fungal Diversity Research Series 10*: 1-350.
12. Taylor, J.E. and **Hyde, K.D.** (2003). *Microfungi on Tropical and Temperate Palms*. *Fungal Diversity Research Series 1*-459.
13. Wang, Y.Z., Aptroot, A. and **Hyde, K.D.** (2004). *Review of the Genus Amphisphaeria*. *Fungal Diversity 13*: 1-180.
14. E.B.G. Jones, M. Tantichareon and **K.D. Hyde** (eds.) (2004). *Thai Fungal Diversity*. BIOTEC, Thailand: 281p.
15. Pearce, C. and **Hyde, K.D.** (2006). *Phyllachoraceae of Australia*. *Fungal Diversity Research Series 17*: 1-308.
16. Cai, L. **Hyde, K.D.** and Tsui, C.K.M.T. (2006). *Genera of Freshwater Fungi*. *Fungal Diversity Research Series 18*: 1-261.
17. Sridhar, K.R., Barlocher, F. and **Hyde, K.D.** (eds.) (2008). *Novel Techniques and Ideas in Mycology*. *Fungal Diversity Research Series 20*: 1-373.
18. See SIM ISSUE in December

Journal Articles, Book Chapters and Other Published Papers

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

• 48 Publications (30 SCI)

1. **Hyde, K.D.** (2004). Fungal Conservation: Issues and Solutions. *The Quarterly Review of Biology* **79**: 80-81.
2. Bucher, V.V.C., **Hyde, K.D.**, Pointing, S.B. and Reddy, C.A. (2004). Production of wood decay enzymes, mass loss and lignin solubilization in wood by diverse freshwater fungi. *Microbial Ecology* **48**: 331-337.
3. Fryar, S.C., Davies, J., Booth, W., Hodgkiss, I.J. and **Hyde, K.D.** (2004). Succession of fungi on dead and live wood in brackish water. *Mycologia* **96**: 219-225.
4. Ho, W.H. **Hyde, K.D.**, Hodgkiss, I.J. and Yanna (2004). *Cataractispora receptaculorum*, a new freshwater ascomycete from Hong Kong. *Mycologia* **96**: 411-417.
5. Kodsueb, R., Lumyong, S., Lumyong, P., McKenzie, E.H.C., Ho, W.H. and Hyde, K.D. (2004). *Acanthostigma* and *Tubeufia* species, including *T. claspisphaeria* sp. nov. from submerged wood in Hong Kong sp. nov. *Mycologia* **96**: 667-674.
6. Paulus, B., Gadek, P. and **Hyde, K.D.** (2004). Phylogenetic and morphological assessment of five new species of *Thozetella* from an Australian rainforest. *Mycologia* **96**: 1074-1087.
7. Pinruan, U., Sakayaroj, J., Jones, E.B.G. and **Hyde, K.D.** (2004). Aquatic fungi from peat swamp palms: *Phruensis brunneispora* gen. et sp. nov. and its hyphomycete anamorph. *Mycologia* **96**: 1163- 1170.
8. 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.
9. Tsui, C.K.M. and **Hyde, K.D.** (2004). Biodiversity of fungi on submerged wood in a stream and estuaries in the Tai Ho Bay, Hong Kong. *Fungal Diversity* **15**: 205-220.
10. Ho, W.H., Yanna, and **Hyde, K.D.** (2004). A new type of conidial septal pore in fungi. *Fungal Diversity* **15**: 171-186.
11. 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.
12. 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.
13. Guo, L.D., Xu, L., Zheng, W.H. and **Hyde, K.D.** (2004). Genetic variation of *Alternaria alternata*, an endophytic fungus isolated from *Pinus tabulaeformis* as determined by random amplified microsatellites (RAMS). *Fungal Diversity* **16**: 53-65.
14. 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.
15. 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.
16. Kumar, D.S.S. and **Hyde, K.D.** (2004). Biodiversity and tissue-recurrence of

- endophytic fungi from *Tripterygium wilfordii*. *Fungal Diversity* **17**: 69-90.
17. Pinruan, U., McKenzie, E.H.C., Jones, E.B.G. and **Hyde, K.D.** (2004). Two new species of *Stachybotrys*, and a key to the genus. *Fungal Diversity* **17**: 145-157.
 18. Jeewon, R., Liew, E.C.Y. and **Hyde, K.D.** (2004). Phylogenetic evaluation of species nomenclature of *Pestalotiopsis* in relation to host association. *Fungal Diversity* **17**: 39-55.
 19. Kumar, D.S.S., Cheung, H.Y., Lau, C.S., Chen, F. and **Hyde K.D.** (2004). In vitro studies of endophytic fungi from *Tripterygium wilfordii* with anti-proliferative activity on human peripheral blood mononuclear cells. *Journal of Ethnopharmacology* **94**: 295-300.
 20. Prompttha, I., **Hyde, K.D.**, Lumyong, P., McKenzie, E.H.C. and Lumyong, P. (2004). Fungi on *Magnolia lillifera*: *Cheiromyces magnoliae* sp. nov. from dead branches. *Nova Hedwigia* **78**: 527-532.
 21. Cai, L., Zhang, K.Q., McKenzie, E.H.C. and **Hyde, K.D.** (2004). *Linocarpon bambusicola* sp. nov. and *Dictyoachaeta curvispora* from bamboo submerged in freshwater. *Nova Hedwigia* **78**: 439-445.
 22. Pinnoi, A., Pinruan, U., **Hyde, K.D.** and Lumyong, S. (2004). *Submersisphaeria palmae* sp. nov. and key to genus and notes on Helicoubsia. *Sydowia* **56**: 72-78.
 23. Cai, L., McKenzie, E.H.C. and **Hyde, K.D.** (2004). New species of *Cordana* and *Spadicoides* from decaying bamboo culms in China. *Sydowia* **56**: 222-228.
 24. Prompttha, I., Lumyong, S., Lumyong, P., McKenzie, E.H.C. and **Hyde, K.D.** (2004). A new species of *Pseudohalonestria* from Thailand. *Cryptogamie Mycologie* **25**: 43-47.
 25. Yanna, Ho, W.H., McKenzie, E.H.C. and **Hyde, K.D.** (2004). New saprobic fungi on palm fronds, including *Brachysporopsis* gen. nov. *Cryptogamie Mycologie* **25**: 161-167.
 26. Fryar, S.C. and **Hyde, K.D.** (2004). New species and genera of ascomycetes from fresh and brackish water in Brunei: *Ayria appendiculata* and *Sungaiicola bactrodesmiella* gen. et spp. nov., *Fluviatispora boothii*, *Torrentispora crassiparietis* and *T. fusiformis* spp. nov. *Cryptogamie Mycologie* **25**: 245-260.
 27. Prompttha, I., Lumyong, S., Lumyong, P., McKenzie, E.H.C. and **Hyde, K.D.** (2004). Fungal saprobes on dead leaves of *Magnolia lillifera* (Magnoliaceae) in Thailand. *Cryptogamie Mycologie* **25**: 43-47.
 28. Vijaykrishna, D., Mostert, L., Jeewon, R., Gams, W., **Hyde, K.D.** and Crous, P.W. (2004). *Pleurostomophora*, an anamorph of *Pleurostoma* (Calosphaeriales), a new anamorph genus morphologically similar to *Phialophora*. *Studies in Mycology* **50**: 387-395.
 29. Pinruan, U., Sakayaroj, J., Jones, E.B.G. and **Hyde, K.D.** (2004). *Flammispora* gen. nov., a new freshwater ascomycetes from decaying palm leaves. *Studies in Mycology* **50**: 381-386.
 30. 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.
 31. Pinruan, U., Lumyong, S., McKenzie, E.H.C., Jones, E.B.G., and **Hyde, K.D.** (2004). Three new species of *Craspedodidymum* from palm in Thailand. *Mycoscience* **45**: 177-180.
 32. Kumar, D.S.S., Cheung, H.Y., Zhu, G.Y., Yang, D., Fong, W.F. and **Hyde K.D.** (2004). Isolation and identification of Triptonide and its analogous compounds from a fungal culture of *Pestalotiopsis leucothes*. *Hong Kong Pharmacology Journal* **12**: 158-164.

33. Kumar, D.S.S., Lau, C.S., Chan, W.K., Yang, D., Cheung, H.Y., Chen, F. and **Hyde K.D.** (2004). Immunomodulatory activity of an endophytic fungus isolated from *Tripterygium wilfordii*. In: *Proceedings of the 2nd International Conference on Medicinal Mushroom and the International Conference on Biodiversity and Bioactive Compounds*. BIOTEC, Thailand: 367-373.
34. Ghimire, S.R. and **Hyde, K.D.** (2004). Fungal endophytes. In: *Plant Surface Microbiology* (eds. A. Varma, L. Abbott, D. Werner and R. Hampp). Springer Verlag: 281-288.
35. Phengsinthaam, P. and **Hyde, K.D.** (2004). Checklist of Lao fungi. In: *Building Capacity in Biodiversity Information Sharing 2003* (ed. J. Shimura). NIES, Japan: 184-190.
36. Phengsinthaam, P. and **Hyde, K.D.** (2004). Fungi of Laos I. Ascomycetes from palms. In: *Building Capacity in Biodiversity Information Sharing 2003* (ed. J. Shimura). NIES, Japan: 174-183.
37. **Hyde, K.D.** (2004). Striving to improve mycological expertise in the Asian region. In: *Building Capacity in Biodiversity Information Sharing 2003* (ed. J. Shimura). NIES, Japan: 39-43.
38. Jones, E.B.G. and **Hyde, K.D.** (2004). Introduction to Thai fungal diversity. In: *Thai Fungal Diversity* (eds. E.B.G. Jones, M. Tanticharoen and K.D. Hyde). BIOTEC, Thailand: 7-35.
39. Kodsueb, R., Lumyong, S. and **Hyde, K.D.** (2004). Terrestrial lignicolous fungi. In: *Thai Fungal Diversity* (eds. E.B.G. Jones, M. Tanticharoen and K.D. Hyde). BIOTEC, Thailand: 155-161.
40. Lam, D.M., Lumyong, S. and **Hyde, K.D.** (2004). Fungi on leaf litter. In: *Thai Fungal Diversity* (eds. E.B.G. Jones, M. Tanticharoen and K.D. Hyde). BIOTEC, Thailand: 163-171.
41. Photita, W., McKenzie, E.H.C., **Hyde, K.D.** and Lumyong, S. (2004). Fungi on Musa (Banana). In: *Thai Fungal Diversity* (eds. E.B.G. Jones, M. Tanticharoen and K.D. Hyde). BIOTEC, Thailand: 173-180.
42. Pinnoi, A., Pinuran, U., **Hyde, K.D.**, Lumyong, S. and Jones, E.B.G. (2004). Palm fungi. In: *Thai Fungal Diversity* (eds. E.B.G. Jones, M. Tanticharoen and K.D. Hyde). BIOTEC, Thailand: 181-187.
43. Bussaban, B., Lumyong, P., McKenzie, E.H.C., **Hyde, K.D.** and Lumyong, S. (2001). Fungi on Zingiberaceae (Ginger). In: *Thai Fungal Diversity* (eds. E.B.G. Jones, M. Tanticharoen and K.D. Hyde). BIOTEC, Thailand: 189-195.
44. Lumyong, S., Lumyong, P. and **Hyde, K.D.** (2001). Endophytes. In: *Thai Fungal Diversity* (eds. E.B.G. Jones, M. Tanticharoen and K.D. Hyde). BIOTEC, Thailand: 197-205.
45. Somrithipol, S. and **Hyde, K.D.** (2004). Plant pathogens. In: *Thai Fungal Diversity* (eds. E.B.G. Jones, M. Tanticharoen and K.D. Hyde). BIOTEC, Thailand: 207-212.
46. Jones, E.B.G. and **Hyde, K.D.** (2004). Epilogue. In: *Thai Fungal Diversity* (eds. E.B.G. Jones, M. Tanticharoen and K.D. Hyde). BIOTEC, Thailand: 259-262.
47. **Hyde, K.D.** (2004). Bio-Exploitation of Filamentous Fungi – Opportunities for Research. In: *Proceedings of The 1st KMITL International Conference on Intregation of Science and Technology for Sustainable Development* (eds. K.D. Hyde and K. Soyong). KMITL, Bangkok, Thailand: 3-8.
48. Zhao, R.L., Zhou, T.X., Wang, Y.Y., Yang, J., Soyong, K and **Hyde, K.D.** (2004). Application of RAPD analysis in species classification of *Cyathus*. In: *Proceedings of The 1st KMITL International Conference on Intregation of Science and*

2005

• 23 Publications (20 SCI)

1. Tang, A., Corlett, R.T.C. and **Hyde, K.D.** (2005). The persistence of ripe fleshy fruits in the presence and absence of frugivores. *Oecologia* **142**: 232-237.
2. Kumar, D.S.S., Lau, C.S., Wan, J.M.F., Yang, D. and **Hyde K.D.** (2005). Immunomodulatory compounds from *Pestalotiopsis leucothès* (HKUCC 10197), an endophytic fungus of *Tripterygium wilfordii*. *Life Sciences* **78**: 147-156.
3. Cai, L., Zhang, K.Q. and **Hyde, K.D.** (2005). *Ascoyunnania chameleonia* gen. et sp. nov. a freshwater fungus collected from China and its microcyclic conidiation. *Fungal Diversity* **18**: 1-8.
4. Photita, W., Taylor, P.W.J., Ford, R., Lumyong, P., McKenzie, E.H.C. **Hyde, K.D.** and Lumyong, S. (2005). Morphological and molecular characterization of *Colletotrichum* species from herbaceous plants in Thailand. *Fungal Diversity* **18**: 117-133.
5. 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.
6. 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.
7. 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.
8. Wang, Y., Guo, L.D. and **Hyde, K.D.** (2005). Taxonomic placement of sterile morphotypes of endophytic fungi from *Pinus tabulaeformis* (Pinaceae) in northeast China based on rDNA sequences. *Fungal Diversity* **20**: 235-260.
9. Ho, W.H., Yanna and **Hyde, K.D.** (2005). *Endosporoideus* gen. nov., a mitosporic fungus on *Phoenix hanceana*. *Mycologia* **97**: 238-245.
10. 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.
11. Li, Y., **Hyde, K.D.**, Jeewon, R., Cai, L., Vijaykrishna D. and Zhang, K.Q. (2005). Phylogenetics and evolution of nematode trapping fungi (Orbiliiales) estimated from nuclear and protein coding genes. *Mycologia* **97**: 1034-1046.
12. Bhal, J., Jeewon, R. and **Hyde, K.D.** (2005). Phylogeny of *Rosellinia capetribulensis* sp. nov. and its allies (Xylariaceae). *Mycologia* **97**: 1102-1110.
13. Pointing, S.B., Pelling, A.L., Smith, G.J.D., **Hyde, K.D.** and Reddy, C.A. (2005). Screening of basidiomycetes and xylariaceous fungi for lignin peroxidase and laccase gene-specific sequences. *Mycological Research* **109**: 115-124.
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2006

• 32 Publications (31 SCI)

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2007

- 23 Publications (20 SCI)

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13. Cai, L. and **Hyde, K.D.** (2007). Anamorphic fungi from China freshwater habitats: *Dictyosporium tetrasporum* sp. nov., *Exserticlava yunnanensis* sp. nov., and new records *Pseudofuscophialis lignicola* and *Pseudobotrytis terrestris*. *Cryptogamie Mycologie* (In press).
14. Kodsueb, R., McKenzie, E.H.C., Ho, W.H., **Hyde, K.D.**, Lumyong, P. and Lumyong, S. (2007). New anamorphic fungi from decaying woody litter of *Michelia baillonii* (Magnoliaceae) in northern Thailand. *Cryptogamie Mycologie* **28**: 237-245.
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16. Cai L, **Hyde KD** (2007) *Ascorhombispora aquatica* gen. et sp nov from a freshwater habitat in China, and its phylogenetic placement based on molecular data. *Cryptogamie Mycologie* **28**, 291-300.
17. Hu DM, Zhu H, Cai L, **Hyde KD**, Zhang KQ (2007) *Sirothecium triseriale*, a new chirosporous anamorphic species from China. *Cryptogamie Mycologie* **28**, 311-314.
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2008

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1. Aung OM, Soyong K, **Hyde KD**, 2008. Diversity of entomopathogenic fungi in rainforests of Chiang Mai Province, Thailand. *Fungal Diversity* **30**, 15-22.
2. Cai L, Guo XY, **Hyde KD**, 2008. Morphological and molecular characterisation of a new anamorphic genus *Cheirosporium*, from freshwater in China. *Persoonia* **20**, 53-58.

3. Duong LM, McKenzie EHC, Lumyong S, **Hyde KD**, 2008. Fungal succession on senescent leaves of *Castanopsis diversifolia* in Doi Suthep-Pui National Park, Thailand. *Fungal Diversity* 30, 23-36.
4. **Hyde KD**, Zhang Y, 2008. Epitypification: should we epitypify? *Journal of Zhejiang University-Science B* 9, 842-846.
5. Kodsueb R, McKenzie EHC, Lumyong S, **Hyde KD**, 2008a. Diversity of saprobic fungi on Magnoliaceae. *Fungal Diversity* 30, 37-53.
6. Kodsueb R, McKenzie EHC, Lumyong S, **Hyde KD**, 2008b. Fungal succession on woody litter of *Magnolia liliifera* (Magnoliaceae). *Fungal Diversity* 30, 55-72.
7. Pinruan U, Sakayaroj J, **Hyde KD**, Jones EBG, 2008. *Thailandiomyces bisetulosus* gen. et sp. nov. (Diaporthales, Sordariomycetidae, Sordariomycetes) and its anamorph *Craspedodidymum*, is described based on nuclear SSU and LSU rDNA sequences. *Fungal Diversity* 29, 89-98.
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12. Thongkantha S, Lumyong S, McKenzie EHC, **Hyde KD**, 2008. Fungal saprobes and pathogens occurring on tissues of *Dracaena lourieri* and *Pandanus* spp. in Thailand. *Fungal Diversity* 30, 149-169.
13. Tran HTM, Stephenson SL, **Hyde KD**, Mongkolporn O, 2008. Distribution and occurrence of myxomycetes on agricultural ground litter and forest floor litter in Thailand. *Mycologia* 100, 181-190.
14. Wang HK, **Hyde KD**, Soyong K, Lin FC, 2008. Fungal diversity on fallen leaves of *Ficus* in northern Thailand. *Journal of Zhejiang University-Science B* 9, 835-841.
15. Wongsawas M, Wang HK, **Hyde KD**, Lin FC, 2008. New and rare lignicolous hyphomycetes from Zhejiang Province, China. *Journal of Zhejiang University-Science B* 9, 797-801.

16. Zhang Y, Fournier J, Jeewon R, **Hyde KD**, 2008a. *Quintaria microsporium* sp nov., from a stream in France. *Cryptogamie Mycologie* 29, 179-182.
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20. Hu DM, Dai XH, Guo DY, **Hyde KD**, Zhang KQ, 2008. The diversity of coprophilous fungi from Dahuadian and Zhongdian grasslands, Yunnan, China. *Cryptogamie Mycologie* 29, 355-364.
21. Huang WY, Cai YZ, **Hyde KD**, Corke H, Sun M, 2008. Biodiversity of endophytic fungi associated with 29 traditional Chinese medicinal plants. *Fungal Diversity* 33, 61-75.
22. **Hyde KD**, Soyong K, 2008. The fungal endophyte dilemma. *Fungal Diversity* 33, 163-173
23. Swe A, Jeewon R, **Hyde KD**, 2008a. Nematode-Trapping fungi from mangrove habitats. *Cryptogamie Mycologie* 29, 333-354.
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2009

- 23 Publications (23 SCI)

1. Cai L, Wu WP, and **Hyde KD**, (2009). Phylogenetic relationships of *Chalara* and allied species inferred from ribosomal DNA sequences. *Mycological Progress* 8, 133-143. (IF = 1.79)
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11. Kaewchai, S., Soyong, K. and **Hyde, K.D.** (2009). Mycofungicides and fungal biofertilizers. *Fungal Diversity* 38: 25-50. (IF = 2.28)
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