The need for re-inventory of Thai phytopathogens

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ABSTRACT

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

Keywords: plant pathogenic fungi, quarantine, systematics.

1. INTRODUCTION

The Index of Plant Diseases in Thailand [1] lists fungi and other pathogens of plants in Thailand recorded up to 1994 and is based on many years of sound morphological identification [2], and was the best methodology that was applicable at that time.
Recent molecular studies, however, have revealed that many of the pathogenic species listed are species complexes, containing several or numerous species [3-8]. In other cases, what was thought to be a good species is no longer tenable. The apparent confusion in species determinations has important implications for plant breeding, quarantine, and disease management and therefore needs to be addressed.

The objective of this paper is to highlight examples of fungal genera that have undergone revision or are being revised, following the application of molecular data and to illustrate the need for modern taxonomic treatments of plant pathogens in Thailand. Recommendations are made for future research that should be carried out by mycologists and plant pathologists in Thailand with a view to improving the disease data available to plant breeders, plant pathologists, agricultural extensionists and quarantine officials. The selected causative genera/species that probably should occur in Thailand are highlighted in summary Table, and their current status and need for revision is discussed below.

2. SELECTED GENERA OF IMPORTANT PLANT PATHOGENS

2.1 Alternaria

*Alternaria* is a cosmopolitan genus that includes saprobic, endophytic and pathogenic species. Plant pathogenic *Alternaria* species may produce host-specific toxins [9, 10] and cause leaf spots, blights, and blights of many agricultural and economical important crops, and produce blemishes or damage to stored products [11, 12]. The genus is also a common and important plant pathogen in Thailand and 19 species are listed as associated with various hosts [1, 13].

Species definition in *Alternaria* previously relied on the morphological characters and their ability to produce host-specific toxins [9,14]. However, the taxonomic system of the genus remains unclear and thus phylogenetic studies have been carried out to define the species of *Alternaria*. ITS sequences demonstrated that the conidial *Alternaria* species form a monophyletic group that is discrete from other members of the genus [15]. Peever *et al*. [16] sequenced citrus-associated isolates of *Alternaria* and showed that a single phylogenetic clade contained more than one morphospecies, and *Alternaria citrimalcarius* was polyphyletic.

Based on ITS and SSU sequence identities, Pryor and Gilbertson [17] revealed that *A. longissima* was only distantly related to other *Alternaria* species, as well as to *Ulocladium* and *Stemphylium* spp. Currently, the name *Prathoda longissima* (Deighton & MacGarvie) E.G. Simmons is used for *A. longissima* [18]; the record of *A. longissima* from Thailand needs to be verified. Furthermore, at least 25% of the *Alternaria* species listed in the checklist of Thailand [1] used old names (for example, *A. gomphrenae*, *A. cucurbitae*). *Alternaria tenuis* is currently accepted as a synonym of *A. alternata*, and both are listed in the checklist.

2.2 Botryosphaeria and Its Anamorphs

The species-rich, cosmopolitan genus *Botryosphaeria* is responsible for die-back and canker diseases of numerous woody host plants [19]. Several members of this group are associated with a wide range of monocotyledonous, dicotyledonous and gymnosperm hosts [20], and are saprobic, parasitic and endophytic on branches, leaves and stems [21-23]. Researchers [5, 24-27] have shown that *Botryosphaeria* is polyphyletic comprising several teleomorphic and anamorphic taxa. Identification is unclear as the teleomorphic characters vary on different hosts and often cannot be differentiated at the species level [4]. The wide host ranges and
the large number of synonyms of certain species suggests that these are species complexes [5]. Because of their unclear identity, many species of Botryosphaeria and their anamorphs are generally accepted in species complexes, for example Botryosphaeria dothidea and Lasiodiplodia theobromae [4, 5, 26]. Slippers et al. [4] epitypified Botryosphaeria dothidea and studied the phylogenetic relationships with its related species, namely B. ribis and B. parva. The combined data of morphological, cultural and multi-allelic DNA sequence from ITS, β-tubulin and EF-1α genes proved that B. dothidea comprises several distinct species [4]. Some ITS sequences named B. dothidea in GenBank do not align with the epitype and are likely to have wrongly applied names [28]. Based on a study of morphological characters combined with nucleotide sequence data of ITS and EF-1α gene, Alves et al. [5] defined Lasiodiplodia theobromae as a complex of three different cryptic species.

In Sontirat et al. [1], Botryosphaeria ribis and 13 species belonging to anamorphic Botryosphaeria genera such as Diplodia, Dothiorilla, Fusicoccum and Lasiodiplodia are listed from various hosts in Thailand. The complex species Lasiodiplodia theobromae [5] was previously known as Diplodia theobromae and both names are listed separately in the Thai pathogen index [1].

2.3 Colletotrichum

Colletotrichum species are important tropical pathogens responsible for anthracnose disease of agricultural, horticultural, fruit, forestry and native hosts [7,29]. Thirty-one putative Colletotrichum species are listed from Thailand by Sontirat et al. [1]. Of these, 16 are old names [8], while C. dematium var. truncatum was accepted as C. truncatum [30] and already appears in the checklist. This means that only 15 names in the Thai pathogen checklist are currently used species and even these names are unreliable and may be species complexes [6].

The genus Colletotrichum has recently undergone molecular revision [6] and what were previously easily named species complexes (e.g. C. acutatum, C. gloeosporioides, C. graminicola) are now considered to comprise numerous cryptic species [31, 32]. Work on the species complex C. graminicola has shown this to comprise at least 12 distinct species [33, 34].

There has been some recent work on Thai Colletotrichum species. Than et al. [35] established that C. acutatum, C. capsici and C. gloeosporioides were the causal agents of chili anthracnose. This work predated molecular revision of the genus and now the “C. acutatum” from this paper has been renamed C. simmondsii [32], the “C. gloeosporioides” from this paper does not align with the epitype and the “C. capsici” from this paper has recently been shown to be a probable synonym of C. truncatum [31].

Coffee berry anthracnose was previously thought to be caused by C. gloeosporioides and C. kahawae, the latter being responsible for a serious coffee berry disease in Africa [36]. A recent molecular characterization of Colletotrichum species causing anthracnose of coffee in northern Thailand showed three species to be responsible; C. asianum, C. fructicola, C. siamense [37]. Colletotrichum gloeosporioides did not occur on the diseased coffee berries, nor was it found as an endophyte or epiphyte. Similarly, C. gloeosporioides was previously thought to be the causal agent of most fruit anthracnoses in the tropics. Phoulivong et al. [38] surveyed eight tropical fruits with anthracnose symptoms in Laos and Thailand. The associated species were isolated and morphological characters studied. Following molecular analysis it was established that none of the isolated strains...
aligned with the epitype of *C. gloeosporioides* and it was concluded that this species was not responsible for anthracnose of the fruits studied. It should be noted that *Colletotrichum gloeosporioides* is rare and is only known from *Citrus* and *Vanda* sp. [38].

### 2.4 Fusarium

*Fusarium* is an important pathogen of agricultural, horticultural and forest crops [39] as well as the causal agents of superficial and systemic infections in humans and other animals [40-42]. The genus causes economically important plant diseases in Thailand and 28 species appear in the country [1, 43]. Sixteen of these records are formae speciales of *F. oxysporum* and two are formae speciales of *F. solani*. Both of these are actually species complexes [3, 44].

The species concept for the genus *Fusarium* was previously based on morphological characters [45]. It is presently accepted that biological [46] and phylogenetic [3, 47, 48] species concepts are important. Currently, biological, morphological and phylogenetic species concepts are being combined to understand the species definition of *Fusarium* [39]. The information from DNA sequence data has shown that taxa previously thought to be a single species (e.g. *F. graminearum*) are actually species complexes comprising numerous distinct taxa [47, 49]. Members of the *F. graminearum* species complex are responsible for *Fusarium* head blight (FHB) of wheat and barley [3, 49, 50]. O’Donnell *et al.* [3] recognized that *F. graminearum* species complex comprises at least 13 phylogenetically distinct species. *Fusarium graminearum* is listed as a causal agent of wheat FHB disease in the checklist of Thailand pathogens [1].

Kvas *et al.* [51] reviewed the molecular revision of *Fusarium* in the *Gibberella fujikuroi* complex, which comprises an assemblage of similar and overlapping morphological species. The information based on combined DNA sequence for various gene regions showed that the *G. fujikuroi* complex comprises at least 50 distinct phylogenetic species. Of these, 34 species are defined using a morphological species concept and ten are defined using a bio-species concept (sexual fertility).

O’Donnell *et al.* [52] recently studied the *F. oxysporum* species complex, and suggested that EF-1α gene would be a good genetic marker to define the species of *Fusarium*. There are approximately 80 host specific formae speciales in the *F. oxysporum* complex [53], which are responsible for vascular wilts, rots, and damping-off diseases of economically important crops [44, 54]. More than 50% of *Fusarium* species recorded from Thailand are formae speciales of *F. oxysporum* [1]. The published understanding of *Fusarium* species in Thailand has relied heavily on morphology and host occurrence, and modern approaches (e.g. phylogenetic study) are needed to resurvey the genus.

### 2.5 Mycosphaerella and Cercosporoid Anamorphs

Approximately 1,800 species of *Mycosphaerella* [55] and their anamorphs are known from various ecosystems as saprobes, plant pathogens and/or hyperparasites [56-59]. *Mycosphaerella* is among the most common and destructive plant pathogens, causing considerable economic losses on a wide range of host plants worldwide [60-63]. The most common plant pathogenic anamorphic genera resulted from a reassessment of cercosporoid forms [63-65]. Their plant pathogenic species are mainly foliicolous, although some are associated with stem cankers [60] or fruit speck [61]. The main damage is reduction of photosynthetic capacity of crop which due to defoliation or leaf necrosis [66,67].
Many *Mycosphaerella* and cercosporoid species complex are taxonomically problematic, there have been many changes in their systematic taxonomy and many synonyms proposed [68-69]. The majority of the plant pathogenic of *Mycosphaerella* and cercosporoid species were previously defined as host-specific [57, 70], while some species have been reported to occur on multiple hosts [71]. The identification of *Mycosphaerella* species is extremely difficult because of the overlapping morphological characteristics [72, 73] and the co-occurrence of many different taxa in the same leaf lesion [74, 75].

Incorporating DNA sequence data allowed more accurate species delimitation, separating *Mycosphaerella* complex into *Davidiella* species with *Cladosporium* anamorphs (*Davidiellaceae*), *Schizothyrium* species with *Zygophiala* anamorphs (*Schizothyriaceae*), *Teratosphaeria* species with many anamorphs (*Teratosphaeriaceae*) and *Mycosphaerella* species, also with numerous anamorphic genera (*Mycosphaerellaceae*) [61, 68-69, 76].

Four species of *Mycosphaerella* and 109 cercosporoid taxa comprising 94 species of *Cercospora* and 15 species of *Pseudocercospora* are listed as infecting various hosts in Thailand [1, 13], with species identification being largely based on morphological data. Several additional works have showed that Thai *Mycosphaerella* and cercosporoid taxa are very common [77-80]. Nevertheless, numerous synonyms of *Mycosphaerella* and its anamorphs lack thorough inventory [61, 68-69].

Recently, Cheewangkoon *et al.* [74] studied *Mycosphaerella* and its related anamorphic taxa on *Eucalyptus* in Thailand. They discussed the difficulties of using only morphological identification for species. By using the combined dataset of morphological and molecular characters, they reported several new species associated with the foliar deformation of *Eucalyptus* in Thailand. This work redefined the species of a group of Thai *Mycosphaerella* and cercosporoid taxa, and highlights the need for further taxonomic study in Thailand using a polyphasic approach to integrate the multi-gene regions phylogenetic discussion.

### 2.6 Phyllosticta and Guignardia

*Phyllosticta* and its teleomorph *Guignardia* species are plant pathogenic, endophytic and saprobic taxa associated with a wide range of host plants [81-83]. Several species of *Phyllosticta/ Guignardia* are known to be responsible for leaf spotting diseases of various plants worldwide [84].

Species of *Phyllosticta* were traditionally defined by morphological characters and their host specificity [84]. Due to a small number of morphological characters for defining a species, the identification of *Phyllosticta* is complicated. Speciation based on host association, without consideration of morphological characters, has resulted in unclear taxonomy [84] as some *Phyllosticta* species have a wide host range. Okane *et al.* [82, 83] studied the endophytic *Phyllosticta* from 67 different plant species in Japan, and by morphology and molecular analysis showed that a single species *Phyllosticta capitalensis* is associated with all of these various hosts.

Seventeen and two species of *Phyllosticta* and *Guignardia* respectively were listed as pathogens from various host plants in Thailand [1] including now outdated names such as *Phyllosticta musarum*. The names *P. saccharicola* and *P. sorghina* that are listed in "Index of Plant Diseases" [1] are now known to be species of *Leptosphaeria* (*L. sacchari*, see http://www.speciesfungorum.org/Names/SynSpecies.aspx?RecordID=230571).

Investigation of *Phyllosticta/ Guignardia* species have been carried out on various hosts such as *Musa* spp. [85] and palms [87] in
Northern Thailand. *Guignardia musicola* and *G. moriniae* associated with freckle disease of leaves of banana and frog eye disease of leaves of *Morinda citrifolia* have also been recorded [85, 86].

Recently *Phyllosticta citriasiana* causing Citrus tan spot in Asia including Thailand was defined by a combination of molecular and morphological techniques [88]. This work showed that molecular analysis, along with morphological tools, is an appropriate approach to study *Phyllosticta* species. Wulandari *et al.* [88] used multigene phylogeny (ITS, TEF1 and actin genes) to differentiate *Phyllosticta* species associated with Citrus disease in Asia. The new species described by Wulandari *et al.* [88] causes tan spot of pomelo. This has important implications for Thai exports of pomelo as tan spot was previously thought to be caused by *P. citricarpa*. *Phyllosticta citriasiana* is not of quarantine concern to Europe whereas *P. citricarpa* is strictly controlled. Therefore, it will facilitate Thai export of pomelo to Europe if it can be shown that *P. citrocarpa* does not occur on pomelo in Thailand. However five genes combined analysis could not resolve the *Guignardia mangiferae* species complex (P. W. Crous pers. comm.) and other gene(s) are needed to resolve the species complex.

### 2.7 Phomopsis and Diaporthe

The important phytopathogenic genus *Phomopsis*, and its teleomorph *Diaporthe* has been reported as pathogens, endophytes or saprobes worldwide [89]. Moreover, some species produce secondary metabolites that cause toxicity to animals such as lupinosis of sheep [90]. The genus is responsible for serious diseases of a wide range of cultivated plants [91, 92] and the species are mainly defined based on host association. However, researchers [93-95] have revealed that many species in this genus have wide host ranges, while more than one species can occur on a single host. Therefore, host association should be of minor importance in classification and species concepts within the genus need modernization with the additional data of biological, biochemical and molecular concepts [89, 96, 97]. There have been some works on molecular phylogenies of *Phomopsis/ Diaporthe* to identify species by using the sequences from ITS region [92, 95, 98, 99]. Rensburg *et al.* [98] studied the *Phomopsis* species associated with die-back of rooibos (*Aspalathus linearis*). By using the sequence derived from ITS regions and EF-1α gene, they re-identified *Diaporthe phaseolorum* var. *meridionalis* as *D. aspalathi*. Recently, Cai *et al.* [100] downloaded *D. helianthi* ITS sequences from GenBank, and their analysis showed that some sequences deviate significantly from the ex-type culture.

Worldwide there are about 1,000 species of *Phomopsis* [101] connected to about 180 species of *Diaporthe* [89]; eight species of *Diaporthe* and four species of *Phomopsis* on various host plants are listed in the checklist of Thai pathogens [1]. *Diaporthe sojae* and *Phomopsis sojae* are currently named as *Diaporthe phaseolorum* var. *sojae* (http://www.speciesfungorum.org/Names/SynSpecies.asp?RecordID=123980), and all three names are separately listed in the list [1].

### 3. Future Studies

Species definitions change with time as knowledge of fungal morphology, biology, and evolutionary relationships increases [102]. Nomenclature of plant pathogenic fungi previously relied solely on morphological characteristics or sometimes their host associations played a major role in species delimitation, with little regard to morphology. During the last decade molecular studies have demonstrated the complexity of morphological and host-based nomenclature
in species definition [34, 51, 71]. On the other hand, use of phylogeny only would become questionable as DNA extraction from the correct identified specimens or culture is essential. Nilsson et al. [103] found many sequences deposited in GenBank with incorrect taxon names and assumed that these were generated from wrongly identified specimens or cultures. While this may be the case, many of the earlier identifications were based on morphology, which could not recognise cryptic species or separate species complexes. Therefore, an integrated approach using morphological characters as well as molecular tools is recommended as an appropriate technique to define plant pathogenic taxa [100, 102].

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

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