Phytotaxa 233 (1): 027–048
www.mapress.com/phytotaxa/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

http://dx.doi.org/10.11646/phytotaxa.233.1.2

Taxonomy and phylogeny of *Cercospora* spp. from Northern Thailand

JEERAPA NGUANHOM[1], RATCHADAWAN CHEEWANGKOON[1], JOHANNES Z. GROENEWALD[2], UWE BRAUN[3], CHAIWAT TO-ANUN[1]* & PEDRO W. CROUS[2,4]

[1]*Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, 50200, Thailand*
**email: chaiwat.toanun@gmail.com*
[2]*CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands*
[3]*Martin-Luther-Universität, Institut für Biologie, Bereich Geobotanik und Botanischer Garten, Herbarium, Neuwerk 21, 06099 Halle (Saale), Germany*
[4]*Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa*

Abstract

The genus *Cercospora* represents a group of important plant pathogenic fungi with a wide geographic distribution, being commonly associated with leaf spots on a broad range of plant hosts. The goal of the present study was to conduct a morphological and molecular phylogenetic analysis of the *Cercospora* spp. occurring on various plants growing in Northern Thailand, an area with a tropical savannah climate, and a rich diversity of vascular plants. Sixty *Cercospora* isolates were collected from 29 host species (representing 16 plant families). Partial nucleotide sequence data for two gene loci (ITS and *cmdA*), were generated for all isolates. Results from this study indicate that members of the genus *Cercospora* vary regarding host specificity, with some taxa having wide host ranges, and others being host-specific. Based on cultural, morphological and phylogenetic data, four new species of *Cercospora* could be identified: *C. glycinicola* (from *Glycine max*), *C. cyperacearum* and *C. cyperina* (from *Cyperus alternifolius*), and *C. musigena* (from *Musa* sp.). The most common *Cercospora* sp. found in Northern Thailand was *C.* cf. *malloti*, which occurred on a wide host range. Several collections could not be resolved to species level due to the lack of reference cultures and DNA data for morphologically similar species. Further collections from other countries are needed to help resolve the taxonomy of some species complexes occurring on various plant hosts in Thailand.

Key words: biodiversity, cercosporoid hyphomycetes, Mycosphaerellaceae, phylogeny

Introduction

Species of *Cercospora* (Mycosphaerellaceae, Capnodiales) commonly occur associated with leaf and fruit spots on a range of cultivated and wild plants worldwide (Crous & Braun 2003, Groenewald *et al.* 2013, Amaradasa *et al.* 2014, Bakhshi *et al.* 2015b). To date there have been several studies focused on these fungi in Thailand, and more than 500 cercosporoid species have been identified (Giatgong 1980, Sontirat *et al*. 1980, Petcharat & Kanjanamaneesathian 1989, Braun *et al*. 2006, Meeboon *et al*. 2007a, 2007b, 2007c, 2008, Nakashima *et al*. 2007, Phengsintham *et al*. 2013). However, almost all these studies have thus far relied exclusively on morphological data, and very few records are supported by cultures and DNA data. The first application of DNA phylogenetic analysis (ITS) to distinguish *Cercospora* species from Thailand was published by To-anun *et al*. (2010, 2011). In other studies multi-locus DNA data proved highly effective to distinguish among species of cercosporoid fungi (Groenewald *et al*. 2013, Crous *et al.* 2013, Bakhshi *et al.* 2015a, 2015b). The same approach also proved successful to study other, related, cercosporoid genera from Thailand (Hunter *et al*. 2006, Cheewangkoon *et al*. 2008). To date, however, most cercosporoid records from Thailand cannot be substantiated based on a lack of cultures and DNA data. The main objective of the present study was therefore to confirm the identification of different *Cercospora* spp. associated with various plant diseases from Northern Thailand, and to resolve their taxonomy and DNA phylogeny.

Accepted by Eric McKenzie: 24 Aug. 2015; published: 30 Oct. 2015

Materials and methods

Isolates

Specimens with disease symptoms were collected in the field and taken to the laboratory for fungal isolation. Leaves were examined directly using a dissecting microscope to observe *Cercospora* conidiophore fascicles, or when insufficiently developed, incubated in moist chambers for 1–2 d to induce sporulation. Single conidium colonies were established on Petri dishes containing 2% malt extract agar (MEA) as described by Crous *et al*. (2009). Reference strains are maintained at the working collection of P.W. Crous (CPC), with representative isolates deposited in the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands (Table 1).

DNA extraction, amplification and sequencing

Genomic DNA was extracted from fungal mycelium growing on MEA, placed in a 2-ml Eppendorf tube with 600 µl hexadecyltrimethyl ammonium bromide (CTAB) extraction buffer (500 µl of TES Buffer (100 mM Tris pH 8; 10 mM EDTA pH 8; 2% SDS), 140 µl of 5 M NaCl and 65 µl of 10% CTAB solution) and mixed well (protocol modified from Möller *et al.* 1992). To break the cells, the tube was placed in a boiling water bath for 3 min, after which it was chilled directly on ice for 10 min. Four hundred microliters of chloroform:isoamyl alcohol (24:1) were added and mixed properly by inversion, and centrifuged at 14,000 rpm for 5 min at room temperature to separate the phases. The upper phase was carefully collected and transferred to a new 2 ml tube. An equal volume of cold 5 M ammonium acetate was added and the gDNA precipitated with 600 µl of cold isopropanol and inverted. After 15 min incubation on ice, the solution was centrifuged at 14,000 rpm for 5 min and the supernatant discarded. The pellet was washed with 70% ethanol, air-dried and resuspended in 100 µl of TE buffer.

All isolates were sequenced for two genomic loci, namely the internal transcribed spacer region with intervening 5.8S nrRNA gene (ITS) and partial calmodulin gene (*cmdA*). The primer ITS5 (White *et al*. 1990) or V9G (de Hoog & Gerrits van den Ende 1998) and ITS4 (White *et al*. 1990) were used to amplify the ITS and the primer set CAL-228F and CAL-737R (Carbone & Kohn 1999) or CAL2Rd (Groenewald *et al*. 2013) for *cmdA*. The reaction mixture had a total volume of 12.5 µl containing 1 µl diluted DNA, 1× PCR buffer, 2 mM $MgCl_2$, 25 µM of each dNTPs, 1 µM of each primer, and 0.5 U *Taq* DNA polymerase (GoTaq, Promega). The amplification reactions were done on a 2720 Thermal Cycler (Applied Biosystems). PCR amplification conditions for ITS were set as follows: an initial denaturation temperature of 94°C for 5 min, followed by 35 cycles of denaturation temperature of 94°C for 45 s, primer annealing at 48°C for 45 s, primer extension at 72°C for 2 min and a final extension step at 72°C for 7 min. PCR amplification conditions for *cmdA* were set as follows: an initial denaturation temperature of 94°C for 3 min, followed by 35 cycles of denaturation temperature of 94°C for 30 s, primer annealing at 58°C for 40 s, primer extension at 72°C for 50 s and a final extension step at 72°C for 5 min. The PCR products were separated by electrophoresis at 100 V for 30 min on a 1% (w/v) agarose gel stained with GelRed in 1 × TAE buffer (0.4 M Tris, 0.05 M NaAc, and 0.01 M EDTA, pH 7.85) and visualized under UV light.

The resulting fragments were sequenced in both directions with the various PCR primers using a BigDye® Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems, Foster City, CA) and analysed on an ABI Prism 3100 DNA Sequencer (Perkin-Elmer, Norwalk, CN).

Phylogenetic analyses

A consensus sequence was computed from the forward and reverse sequences using SeqMan from the Lasergene package (DNASTAR, Madison, Wisconsin). The consensus sequence was added to the alignment using MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/index.html; Katoh & Standley 2013) and manually improved in MEGA v. 5 (Tamura *et al*. 2011). MrModeltest v. 2.3 (Nylander 2004) was used to determine the best nucleotide substitution model setting for each locus.

The phylogenetic analyses of sequence data were performed in MrBayes v. 3.2.1 (Ronquist *et al*. 2012). The optimal substitution model for each locus, as recommended by MrModeltest, was implemented. The heating parameter was set at 0.3 and the Markov Chain Monte Carlo (MCMC) analysis of four chains was started in parallel from a random tree topology and lasted until the average standard deviation of split frequencies reached 0.01. Trees were saved each 1,000 generations and the resulting phylogenetic tree was printed with Geneious v. 5.5.4 (Drummond *et al*. 2011). New sequences generated in this study were submitted to GenBank (accession numbers listed in Table 1) and the alignment and phylogenetic tree to TreeBASE (ID 17818; www.treebase.org).

TABLE 1. Names, accession numbers and collection details of isolates studied.

Species and Culture accession number(s)[1]	Host name and family or isolation source	Country	Collector(s)	GenBank accession numbers (ITS, *cmdA*)[2]
Cercospora agavicola				
CBS 117292; CPC 11774 (ex-type)	*Agave tequilana* var. *azul* (Agavaceae)	Mexico: Penjamo	V. Ayala-Escobar & Ma. de Jesús Yáñez-Morales	AY647237; AY966899
Cercospora althaeina				
CBS 248.67; CPC 5117 (ex-type)	*Althaea rosea* (Malvaceae)	Romania: Fundulea	O. Constantinescu	JX143530; JX142792
Cercospora apii				
CBS 132683; CPC 16663	*Moluccella laevis* (Lamiaceae)	Zimbabwe	S. Dimbi	JX143531; JX142793
CBS 252.67; CPC 5084	*Plantago lanceolata* (Plantaginaceae)	Romania: Domnesti	O. Constantinescu	DQ233318; DQ233394
CPC 5260	*Glebionis coronaria* (≡ *Chrysanthemum coronarium*, Asteraceae)	New Zealand: Auckland	C.F. Hill	JX143533; JX142795
Cercospora apii complex				
CPC 23816	*Apium graveolens* (Apiaceae)	Thailand: Mae Wang	K. Wongsopa	KT193650; KT193710
CPC 24837	—	Thailand	S. Seekanha	KT193651; KT193711
Cercospora apiicola				
CBS 116457; CPC 10267 (ex-type)	*Apium* sp. (Apiaceae)	Venezuela: Caripe	N. Pons	AY840536; AY840434
Cercospora armoraciae				
CBS 250.67; CPC 5088 (ex-type)	*Armoracia rusticana* (= *A. lapathifolia*, Brassicaceae)	Romania: Fundulea	O. Constantinescu	JX143545; JX142807
CBS 555.71; IMI 161117; CPC 5082	*Coronilla varia* (Fabaceae)	Romania: Hagieni	O. Constantinescu	JX143550; JX142812
Cercospora beticola				
CBS 116454; CPC 11558	*Beta vulgaris* (Chenopodiaceae)	Germany	S. Mittler	AY840526; AY840424

...*Continued on next page*

TABLE 1. (Continued)

Species and Culture accession number(s)[1]	Host name and family or isolation source	Country	Collector(s)	GenBank accession numbers (ITS, *cmdA*)[2]
CBS 116456; CPC 11557 (ex-type)	*Beta vulgaris* (Chenopodiaceae)	Italy: Ravenna	V. Rossi	AY840527; AY840425
CBS 117.47	*Beta vulgaris* (Chenopodiaceae)	Czech Republic	G.E. Bunschoten	DQ233322; DQ233400
CPC 23815	*Beta vulgaris* (Chenopodiaceae)	Thailand: Chiang Mai	K. Wongsopa	KT193652; KT193712
CPC 24825	*Apium graveolens* (Apiaceae)	Thailand: Mae Rim	S. Seekanha	KT193653; KT193713
Cercospora cf. *brunkii*				
CBS 132657; CPC 11598	*Geranium thunbergii* (≡ *G. nepalense* var. *thunbergii*, Geraniaceae)	South Korea: Namyangju	H.D. Shin	JX143559; JX142821
Cercospora capsici				
CBS 118712	Lesions on calyx attached to fruit	Fiji	P. Tyler	GU214653; JX142830
MUCC 574; MUCNS 810; MAFF 238227	*Capsicum annuum* (Solanaceae)	Japan: Chiba	S. Uematsu	JX143569; JX142833
CPC 22000	*Capsicum frutescens* (Solanaceae)	Thailand: San Sai	J. Nguanhom	KT193654; KT193714
CPC 22007	*Capsicum frutescens* (Solanaceae)	Thailand: Mae Klang Loung	J. Nguanhom	KT193655; KT193715
CPC 22008	*Capsicum frutescens* (Solanaceae)	Thailand: Chiang Dao	J. Nguanhom	KT193656; KT193716
CPC 22009	*Capsicum frutescens* (Solanaceae)	Thailand: Li, Lamphun	J. Nguanhom	KT193657; KT193717
CPC 22011	*Capsicum frutescens* (Solanaceae)	Thailand: Li, Lamphun	J. Nguanhom	KT193658; KT193718
CPC 22012	*Capsicum frutescens* (Solanaceae)	Thailand: Li, Lamphun	J. Nguanhom	KT193659; KT193719
CPC 22013	*Capsicum frutescens* (Solanaceae)	Thailand: Li, Lamphun	J. Nguanhom	KT193660; KT193720
CPC 22015	*Capsicum annuum* var. *acuminatum* (Solanaceae)	Thailand: Kalayaniwattana	J. Nguanhom	KT193661; KT193721
Cercospora celosiae				
CBS 132600; CPC 10660	*Celosia argentea* var. *cristata* (≡ *C. cristata*, Amaranthaceae)	South Korea: Chuncheon	H.D. Shin	JX143570; JX142834
Cercospora chenopodii				

...*Continued on next page*

TABLE 1. (Continued)

Species and Culture accession number(s)[1]	Host name and family or isolation source	Country	Collector(s)	GenBank accession numbers (ITS, *cmdA*)[2]
CBS 132620; CPC 14237	*Chenopodium* cf. *album* (Chenopodiaceae)	France: Ardeche	P.W. Crous	JX143571; JX142835
Cercospora cf. *citrulina*				
CBS 119395; CPC 12682	*Musa* sp. (Musaceae)	Bangladesh: Western	I. Buddenhagen	EU514222; JX142843
CBS 132669; CPC 12683	*Musa* sp. (Musaceae)	Bangladesh: Western	I. Buddenhagen	EU514223; JX142844
CPC 20714	*Cyathula prostrata* (Amaranthaceae)	Thailand: Hang Dong	J. Nguanhom	KT193662; KT193722
CPC 20740	*Momordica charantia* (Cucurbitaceae)	Thailand: Ban Ti, Lamphun	J. Nguanhom	KT193663; KT193723
CPC 23937	*Coccinia grandis* (Cucurbitaceae)	Thailand: Doi Saket	J. Nguanhom	KT193664; KT193724
CPC 24842	*Coccinia grandis* (Cucurbitaceae)	Thailand: Chiang Mai	S. Seekanha	KT193665; KT193725
MUCC 576; MUCNS 300; MAFF 237913	*Citrullus lanatus* (Cucurbitaceae)	Japan: Okinawa	T. Kobayashion *et al.*	JX143579; JX142845
MUCC 577; MUCNS 254; MAFF 238205	*Momordica charantia* (Cucurbitaceae)	Japan: Kagoshima	E. Imaizumi & C. Nomi	JX143580; JX142846
Cercospora corchori				
MUCC 585; MUCNS 72; MAFF 238191 (ex-type)	*Corchorus olitorius* (Tiliaceae)	Japan: Shimane	T. Mikami	JX143584; JX142850
Cercospora cyperacearum				
CPC 22014	*Solanum mammosum* (Solanaceae)	Thailand: Li, Lamphun	J. Nguanhom	KT193666; KT193726
CPC 23918 (ex-type)	*Cyperus alternifolius* (Cyperaceae)	Thailand: Kun Chang Kien	S. Seekanha	KT193667; KT193727
CPC 24811	—	Thailand	S. Seekanha	KT193668; KT193728
Cercospora cyperina				
CPC 23919 (ex-type)	*Cyperus alternifolius* (Cyperaceae)	Thailand: Kun Chang Kien	S. Seekanha	KT193669; KT193729
Cercospora delaireae				
CBS 132595; CPC 10455; GV2 PPRI number: C558 (ex-type)	*Delairea odorata* (= *Senecio mikanioides*, Asteraceae)	South Africa: Long Tom Pass	S. Neser	JX143587; JX142853
Cercospora euphorbiae-sieboldianae				
CBS 113306 (ex-type)	*Euphorbia sieboldiana* (Euphorbiaceae)	South Korea: Samcheok	H.D. Shin	JX143593; JX142859

...Continued on next page

TABLE 1. (Continued)

Species and Culture accession number(s)[1]	Host name and family or isolation source	Country	Collector(s)	GenBank accession numbers (ITS, *cmdA*)[2]
Cercospora fagopyri				
CBS 132623; CPC 14541 (ex-type)	*Fagopyrum esculentum* (Polygonaceae)	South Korea: Yangpyeong	H.D. Shin	JX143594; JX142860
Cercospora cf. *flagellaris*				
CBS 113127; RC3766; TX-18	*Eichhornia crassipes* (Pontederiaceae)	USA: Texas	D. Tessmann & R. Charudattan	DQ835075; DQ835148
CBS 132637; CPC 10079	*Trachelium* sp. (Campanulaceae)	Israel	E. Tzul-Abad	JX143600; JX142866
Cercospora glycinicola				
CPC 23911 (ex-type)	*Glycine max* (Fabaceae)	Thailand: Mae Hia	J. Nguanhom	KT193670; KT193730
CPC 23912	*Glycine max* (Fabaceae)	Thailand: Mae Hia	J. Nguanhom	KT193671; KT193731
Cercospora cf. *helianthicola*				
MUCC 716	*Helianthus tuberosus* (Asteraceae)	Japan: Wakayama	C. Nakashima & I. Araki	JX143615; JX142882
Cercospora cf. *ipomoeae*				
CBS 132639; CPC 10102	*Persicaria thunbergii* (Polygonaceae)	South Korea: Pocheon	H.D. Shin	JX143616; JX142883
CBS 132652; CPC 10833	*Ipomoea nil* (= *I. hederacea*, Convolvulaceae)	South Korea: Chuncheon	H.D. Shin	JX143617; JX142884
Cercospora kikuchii				
CBS 128.27; CPC 5068 (ex-type)	*Glycine soja* (Fabaceae)	Japan	T. Matsumoto	DQ835070; DQ835134
Cercospora lactucae-sativae				
CBS 132604; CPC 10728	*Ixeris chinensis* subsp. *strigosa* (≡ *Ixeris strigosa*, Asteraceae)	South Korea: Chuncheon	H.D. Shin	JX143621; JX142888
CPC 10082	*Ixeris chinensis* subsp. *strigosa* (≡ *Ixeris strigosa*, Asteraceae)	South Korea: Chuncheon	H.D. Shin	JX143622; JX142889
CPC 20719	*Lactuca sativa* var. *longifolia* (Asteraceae)	Thailand: Chiang Mai	J. Nguanhom	KT193672; KT193732
CPC 23817	*Lactuca sativa* (Asteraceae)	Thailand: Chiang Mai	K. Wongsopa	KT193673; KT193733
CPC 23818	*Lactuca sativa* (Asteraceae)	Thailand: Chiang Mai	K. Wongsopa	KT193674; KT193734

...*Continued on next page*

TABLE 1. (Continued)

Species and Culture accession number(s)[1]	Host name and family or isolation source	Country	Collector(s)	GenBank accession numbers (ITS, *cmdA*)[2]
CPC 23819	*Lactuca sativa* (Asteraceae)	Thailand: Chiang Mai	K. Wongsopa	KT193675; KT193735
CPC 24838	—	Thailand: Chiang Mai	S. Seekanha	KT193676; KT193736
MUCC 570; MUCNS 463; MAFF 238209	*Lactuca sativa* (Asteraceae)	Japan: Chiba	C. Nakashima	JX143623; JX142890
Cercospora cf. *malloti*				
CPC 20729	*Melampodium divaricatum* (Asteraceae)	Thailand: Mae On	J. Nguanhom	KT193677; KT193737
CPC 20737	*Asystasia salicifolia* (Acanthaceae)	Thailand: Mae Hia	J. Nguanhom	KT193678; KT193738
CPC 22010	*Physalis peruviana* (Solanaceae)	Thailand: Li, Lamphun	J. Nguanhom	KT193679; KT193739
CPC 22023	*Nicotiana tabacum* (Solanaceae)	Thailand: Phu Phing Palace	J. Nguanhom	KT193680; KT193740
CPC 22024	*Phlox drummondii* (Polemoniaceae)	Thailand: Phu Phing Palace	J. Nguanhom	KT193681; KT193741
CPC 23821	*Brassica alboglabra* (Brassicaceae)	Thailand: Chiang Mai	K. Wongsopa	KT193682; KT193742
CPC 23826	*Codiaeum variegatum* (Euphorbiaceae)	Thailand: Chiang Rai	K. Wongsopa	KT193683; KT193743
CPC 23828	*Jatropha integerrima* (Euphorbiaceae)	Thailand: Chiang Mai	K. Wongsopa	KT193684; KT193744
CPC 23834	*Abelmoschus esculentus* (Malvaceae)	Thailand: Chiang Mai	K. Wongsopa	KT193685; KT193745
CPC 23835	*Abelmoschus esculentus* (Malvaceae)	Thailand: Chiang Mai	K. Wongsopa	KT193686; KT193746
CPC 23920	*Plantago major* (Plantaginaceae)	Thailand: Kun Chang Kien	J. Nguanhom	KT193687; KT193747
CPC 24820	*Eupatorium odoratum* (Asteraceae)	Thailand: Mae Hia	S. Seekanha	KT193688; KT193748
CPC 24822	—	Thailand: Suthep-Pui	S. Seekanha	KT193689; KT193749
CPC 24827	*Musa sapientum* (Musaceae)	Thailand: Sa Moeng	S. Seekanha	KT193690; KT193750
CPC 24828	*Musa sapientum* (Musaceae)	Thailand: Sa Moeng	S. Seekanha	KT193691; KT193751
CPC 24845	—	Thailand	S. Seekanha	KT193692; KT193752
MUCC 575; MUCNS 582; MAFF 237872	*Cucumis melo* (Cucurbitaceae)	Japan: Okinawa	K. Uehara	JX143625; JX142892
MUCC 787	*Mallotus japonicus* (Euphorbiaceae)	Japan: Okinawa	C. Nakashima & T. Akashi	JX143626; JX142893

...Continued on next page

TABLE 1. (Continued)

Species and Culture accession number(s)[1]	Host name and family or isolation source	Country	Collector(s)	GenBank accession numbers (ITS, *cmdA*)[2]
Cercospora mercurialis				
CBS 550.71 (ex-type)	*Mercurialis perennis* (Euphorbiaceae)	Romania: Cheia	O. Constantinescu	JX143628; JX142895
Cercospora cf. *mikaniicola*				
CPC 20741	*Mikania cordata* (Asteraceae)	Thailand: Mae Hia	J. Nguanhom	KT193693; KT193753
CPC 22030	*Mikania cordata* (Asteraceae)	Thailand: Lamphun	J. Nguanhom	KT193694; KT193754
CPC 22031	*Mikania cordata* (Asteraceae)	Thailand: Lamphun	J. Nguanhom	KT193695; KT193755
CPC 23908	*Mikania cordata* (Asteraceae)	Thailand: Sa Moeng	J. Nguanhom	KT193696; KT193756
CPC 23909	*Mikania cordata* (Asteraceae)	Thailand: Sa Moeng	J. Nguanhom	KT193697; KT193757
Cercospora musigena				
CPC 24809 (ex-type)	*Musa* sp. (Musaceae)	Thailand: Fang, Chiang Mai	S. Seekanha	KT193698; KT193758
CPC 24831	*Musa* sp. (Musaceae)	Thailand: Fang, Chiang Mai	S. Seekanha	KT193699; KT193759
Cercospora cf. *nicotianae*				
CBS 131.32; CPC 5076	*Nicotiana tabacum* (Solanaceae)	Indonesia: Medan	H. Diddens & A. Jaarsveld	DQ835073; DQ835146
CBS 132632; CPC 15918	*Glycine max* (Fabaceae)	Mexico: Tamaulipas	Ma. de Jesús Yáñez-Morales	JX143631; JX142898
CBS 570.69; CPC 5075	*Nicotiana tabacum* (Solanaceae)	Nigeria	S.O. Alasoadura	DQ835074; DQ835147
CPC 20715	*Nicotiana tabacum* (Solanaceae)	Thailand: Mae Tang	J. Nguanhom	KT193700; KT193760
CPC 20730	*Houttuynia cordata* (Saururaceae)	Thailand: Mae Hia	J. Nguanhom	KT193701; KT193761
CPC 22006	*Nicotiana tabacum* (Solanaceae)	Thailand: Wiang Pa Pao	J. Nguanhom	KT193702; KT193762
CPC 22019	*Petunia hybrida* (Solanaceae)	Thailand: Chiang Mai Univ.	J. Nguanhom	KT193703; KT193763
Cercospora olivascens				
CBS 253.67; IMI 124975; CPC 5085 (ex-type)	*Aristolochia clematitis* (Aristolochiaceae)	Romania: Cazanele Dunarii	O. Constantinescu	JX143632; JX142899

...Continued on next page

TABLE 1. (Continued)

Species and Culture accession number(s)[1]	Host name and family or isolation source	Country	Collector(s)	GenBank accession numbers (ITS, *cmdA*)[2]
Cercospora cf. *physalidis*				
CBS 765.79	*Solanum tuberosum* (Solanaceae)	Peru	L.J. Turkensteen	JX143633; JX142900
Cercospora pileicola				
CBS 132607; CPC 10749 (ex-type)	*Pilea pumila* (= *P. mongolica*, Urticaceae)	South Korea: Dongducheon	H.D. Shin	JX143634; JX142901
Cercospora polygonacea				
CBS 132614; CPC 11318	*Persicaria longiseta* (≡ *P. blumei*, Polygonaceae)	South Korea: Cheongju	H.D. Shin	JX143637; JX142904
Cercospora punctiformis				
CBS 132626; CPC 14606	*Cynanchum wilfordii* (Asclepiadaceae)	South Korea: Bonghwa	H.D. Shin	JX143638; JX142905
Cercospora cf. *resedae*				
CBS 118793	*Reseda odorata* (Resedaceae)	New Zealand: Auckland	C.F. Hill	JX143639; JX142906
CBS 257.67; CPC 5057	*Helianthemum* sp. (Cistaceae)	Romania: Bucuresti	O. Constantinescu	DQ233319; DQ233395
Cercospora rumicis				
CPC 5439	*Rumex sanguineus* (Polygonaceae)	New Zealand: Manurewa	C.F. Hill	JX143648; JX142915
Cercospora senecionis-walkeri				
CBS 132636; CPC 19196	*Senecio walkeri* (Asteraceae)	Laos	P. Phengsintham	JX143649; JX142916
Cercospora sojina				
CBS 132615; CPC 11353 (ex-type)	*Glycine soja* (Fabaceae)	South Korea: Hongcheon	H.D. Shin	JX143659; JX142927
Cercospora sp.				
CPC 23905	*Crassocephalum crepidioides* (Asteraceae)	Thailand: Hang Dong	J. Nguanhom	KT193704; KT193764
CPC 23906	*Crassocephalum crepidioides* (Asteraceae)	Thailand: Hang Dong	J. Nguanhom	KT193705; KT193765
Cercospora sp. F				
CBS 132618; CPC 12062	*Zea mays* (Poaceae)	South Africa	P. Caldwell	DQ185071; DQ185107

...Continued on next page

TABLE 1. (Continued)

Species and Culture accession number(s)[1]	Host name and family or isolation source	Country	Collector(s)	GenBank accession numbers (ITS, *cmdA*)[2]
Cercospora sp. R				
CBS 114644	*Myoporum laetum* (Myoporaceae)	New Zealand: Grey Lynn	C.F. Hill	JX143732; JX142999
Cercospora sp. S				
CBS 132599; CPC 10656	*Crepidiastrum denticulatum* (≡ *Youngia denticulata*, Asteraceae)	South Korea: Yangpyeong	H.D. Shin	JX143733; JX143000
Cercospora vignigena				
CBS 132611; CPC 10812 (ex-type)	*Vigna unguiculata* (= *V. sinensis*, Fabaceae)	South Korea: Jeongeup	H.D. Shin	JX143734; JX143001
Cercospora violae				
CBS 251.67; CPC 5079 (ex-type)	*Viola tricolor* (Violaceae)	Romania: Cazanele Dunarii	O. Constantinescu	JX143737; JX143004
CPC 5368	*Viola odorata* (Violaceae)	New Zealand	C.F. Hill	JX143738; JX143005
MUCC 129	*Viola* sp. (Violaceae)	Japan: Kochi	J. Nishikawa	JX143739; JX143006
Cercospora cf. *zinniae*				
CBS 132624; CPC 14549	*Zinnia elegans* (Asteraceae)	South Korea: Yangpyeong	H.D. Shin	JX143756; JX143026
CBS 132676; CPC 15075	—	Brazil: Valverde	A.C. Alfenas	JX143757; JX143027
CPC 22027	*Zinnia elegans* (Asteraceae)	Thailand: Lamphun	J. Nguanhom	KT193706; KT193766
CPC 22040	*Zinnia elegans* (Asteraceae)	Thailand: Doi Pui	J. Nguanhom	KT193707; KT193767
CPC 22041	*Zinnia elegans* (Asteraceae)	Thailand: Doi Pui	J. Nguanhom	KT193708; KT193768
CPC 23910	*Zinnia elegans* (Asteraceae)	Thailand: Sa Moeng	J. Nguanhom	KT193709; KT193769
MUCC 131	*Zinnia elegans* (Asteraceae)	Japan: Shizuoka	J. Nishikawa	JX143758; JX143028
MUCC 572; MUCNS 215; MAFF 237718	*Zinnia elegans* (Asteraceae)	Japan: Chiba	S. Uematsu	JX143759; JX143029

[1] CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; MAFF: Ministry of Agticulture, Forestry and Fisheries, Tsukuba, Ibaraki, Japan; MUCC: Culture Collection, Laboratory of Plant Pathology, Mie University, Tsu, Mie Prefecture, Japan; MUCNS: Active cultures & specimens of Chiharu Nakashima, housed at Mie University; PPRI: Plant Protection Research Institute, Pretoria, South Africa.

[2] ITS: internal transcribed spacers and intervening 5.8S nrDNA; *cmdA*: partial calmodulin gene.

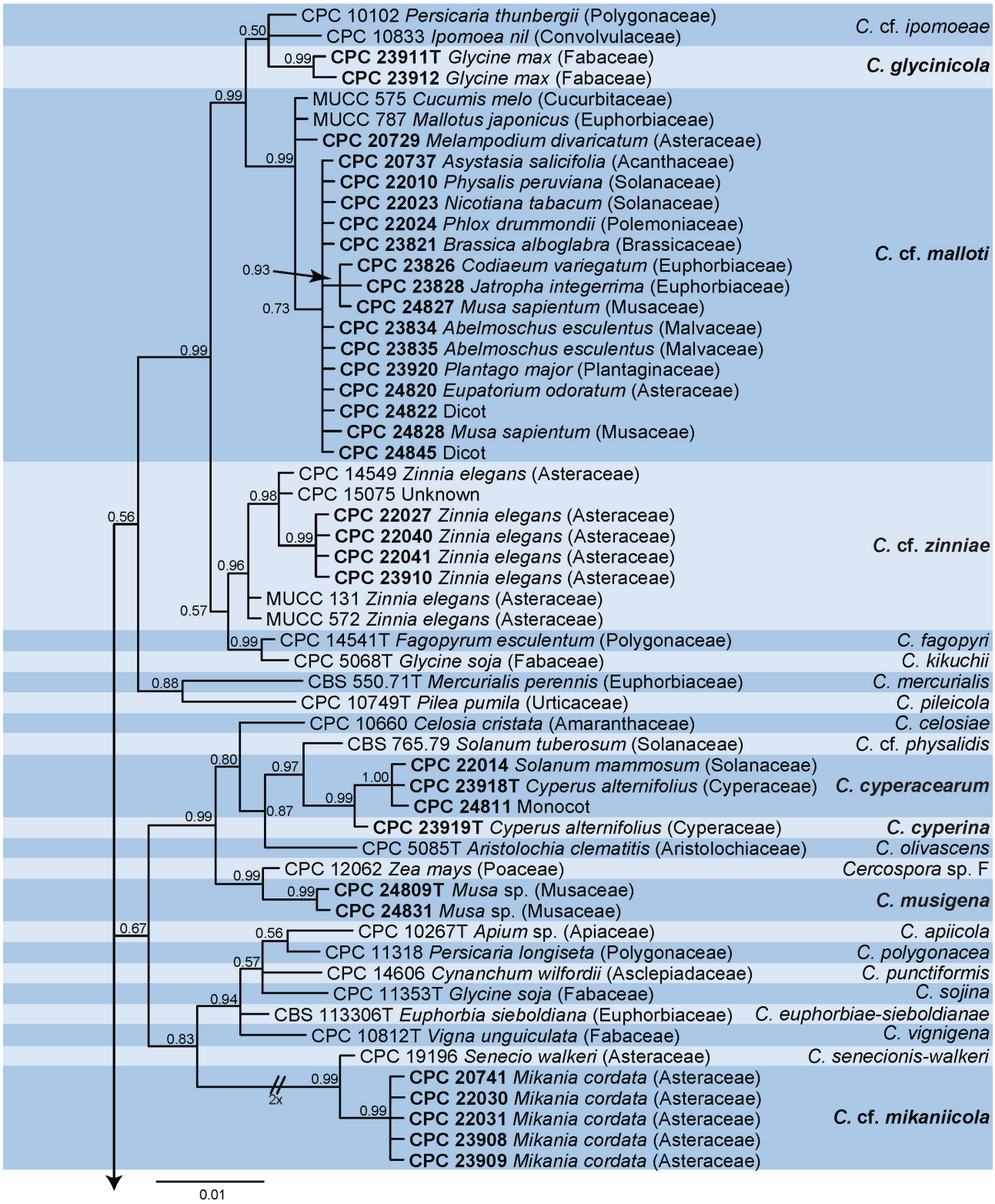

FIGURE 1. The Bayesian 50% majority rule consensus tree derived from the combined ITS/*cmdA* alignment. Bayesian posterior probabilities support values for the respective nodes are displayed in the tree. The scale bar indicates 0.01 expected changes per site and species are delimited by blocks of different colours. Strain accession numbers from Thailand and names of species containing Thai strains are printed in bold face. The tree was rooted to *Septoria provencialis* CPC 12226 (ITS GenBank DQ303096, *cmdA* GenBank JX143030).

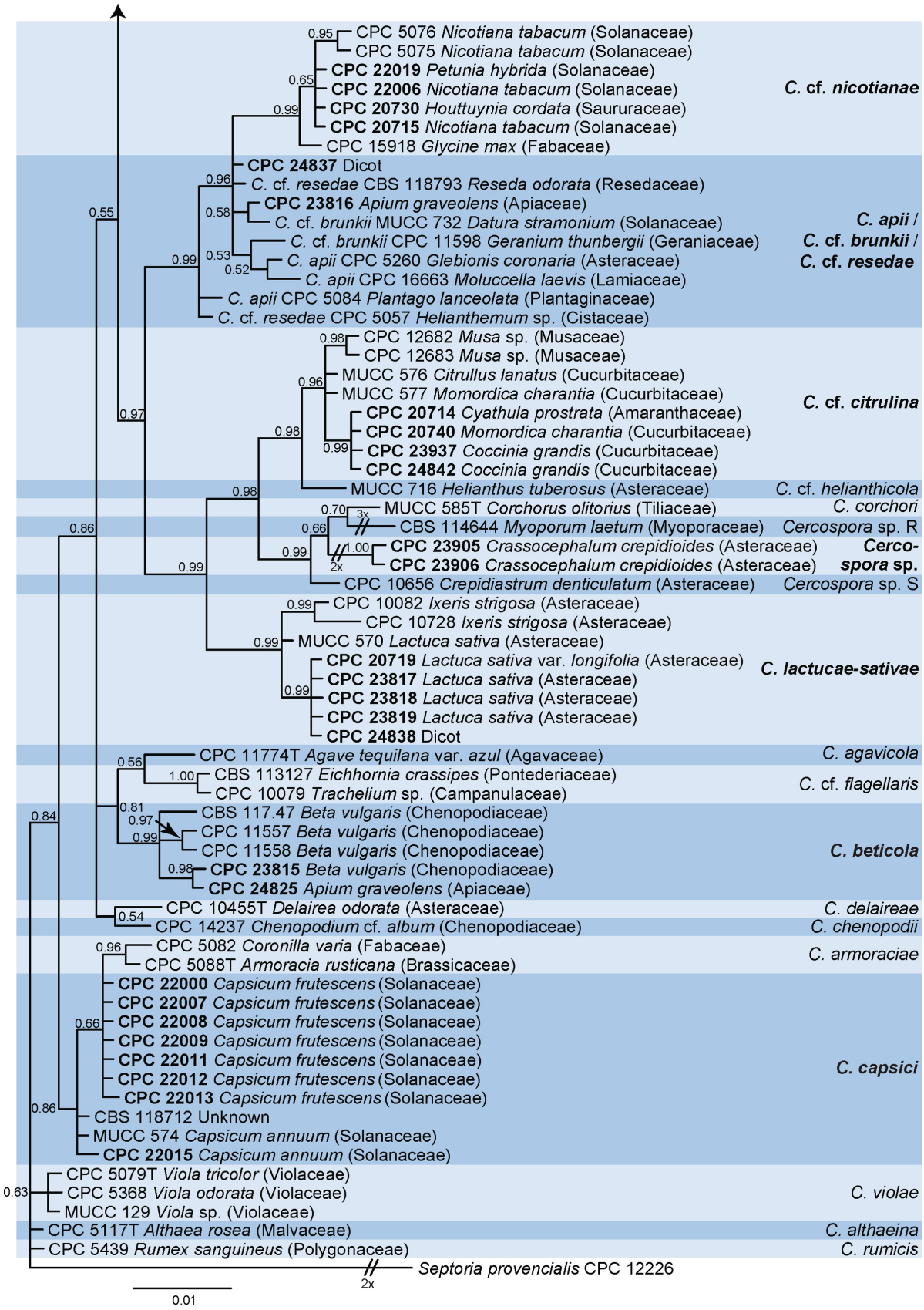

FIGURE 1. (Continued) The Bayesian 50% majority rule consensus tree derived from the combined ITS/*cmdA* alignment. Bayesian posterior probabilities support values for the respective nodes are displayed in the tree. The scale bar indicates 0.01 expected changes per site and species are delimited by blocks of different colours. Strain accession numbers from Thailand and names of species containing Thai strains are printed in bold face. The tree was rooted to *Septoria provencialis* CPC 12226 (ITS GenBank DQ303096, *cmdA* GenBank JX143030).

Taxonomy

Morphological descriptions of *Cercospora* spp. were based on structures from herbarium material. Fungal structures were mounted in lactic acid and examined using a Nikon Eclipse 80i compound microscope (×1000), with 30 measurements taken for each structure, the 95% confidence intervals were determined, and extreme values given in parentheses. Colony colours on MEA, potato dextrose agar (PDA) and oatmeal agar (OA) (recipes according to Crous *et al.* 2009) were determined after 2 wk at 25°C in the dark in duplicate. The mycological colour charts of Rayner (1970) were used to define colours of the fungal colonies. Nomenclatural novelties and descriptions were deposited in MycoBank (www. MycoBank.org; Crous *et al.* 2004). The naming system employed by Groenewald *et al.* (2013) was used to simplify comparison between the studies.

Results

Phylogenetic analysis

DNA data from the ITS and *cmdA* regions were combined in a MrBayes analysis. The sequence alignment consisted of 121 ingroup sequences and *Septoria provencialis* (CPC 12226) was used as outgroup. A combined dataset of a total of 731 characters was used in the phylogenetic analysis (470 and 261 characters for ITS and *cmdA*, respectively). Based on MrModeltest, a MrBayes analysis was conducted on the combined dataset using a symmetrical model (SYM) substitution model with equal rates. The dataset had fixed (equal) base frequencies implemented for ITS and had dirichlet base frequencies with gamma rates implemented for *cmdA* by using HKY+G model. A total of 731 characters were used for the Bayesian analysis; these contained 156 and 171 unique site patterns for ITS and *cmdA*, respectively. A total of 5,168 trees were saved, of which the last 3,876 were used to calculate the tree presented in Fig. 1.

Taxonomy

Several taxa collected in the present study were found to be morphologically and phylogenetically distinct from presently known species. The phylogenetic analyses based on the Bayesian analysis resolved a total of 15 *Cercospora* lineages from Thailand, with two clades representing undefined *Cercospora* species complexes (*sensu* Groenewald *et al*. 2013). The species representing novel taxa are treated below.

Cercospora glycinicola Cheew., Crous & U. Braun, *sp. nov.* (Fig. 2). MycoBank MB812918

Type:—THAILAND. Chiang Mai: on *Glycine max* (Fabaceae), 29 Mar. 2013, *S. Seekanha* (holotype CBS H-22289, culture ex-type CPC 23911 = CBS 140164, CPC 23912).

Leaf spots amphigenous, subcircular to irregular, pale brown, surrounded by a darker brown margin, 1−3 mm diam. Mycelium internal. *Caespituli* amphigenous, punctiform, brown. *Stromata* brown, intraepidermal or substomatal, 32.5−57.5 µm diam. *Conidiophores* in moderately dense fascicles (4−25), straight or sinuous to geniculate due to sympodial proliferation, unbranched, brown, paler toward the apex, 30−113 × 4−6 µm, 1−4-septate. *Conidiogenous cells* proliferating sympodially, integrated, terminal or conidiophores reduced to conidiogenous cells, 28−60 µm long; conidiogenous loci conspicuous, apical and formed on shoulders caused by geniculation, lateral, multi-local, loci distinctly thickened, darkened, 3–4 µm diam. *Conidia* solitary, obclavate-cylindrical, hyaline, obtuse at the apex, truncate to slightly obconically truncate at the base, 1−3-septate, 23−83 × 3−5 µm; hila thickened and darkened, 2.5–3 µm diam.

Culture characteristics:—Colonies spreading, with moderate to dense, felty aerial mycelium, entire to undulate margin, reaching 42 mm diam after 2 wk. On MEA white, with somewhat greyish pink exudates. On PDA white to slightly grey, with diffuse red pigment in agar surrounding colony. On OA white to slightly grey, with diffuse red pigment in agar.

Etymology:—Named after its *Glycine*-inhabiting habit.

Notes:—*Cercospora glycinicola* is morphologically close to *C. sojina* (Shin & Kim 2001) but distinct in having shorter conidiophores (30–113 µm, *versus* 40–200 µm) and above all narrower conidia with few septa (3–5 µm wide, 1–3-septate, *versus* 4–8 µm wide, 3–7-septate). Phylogenetically it is also distinct from species presently known from DNA sequence data, including *C. sojina*. However, although these two isolates originate from the same lesion, they were morphologically different. Isolate CPC 23911 had more geniculate conidiophores, shorter conidiogenous cells

(28−38 µm) and slightly shorter conidia (23−68 µm). In contrast, isolate CPC 23912 had straight conidiophores, longer conidiogenous cells (45−60 µm) and somewhat acicular conidia. However, phylogenetically the two isolates only differed via one nucleotide position in *cmdA*.

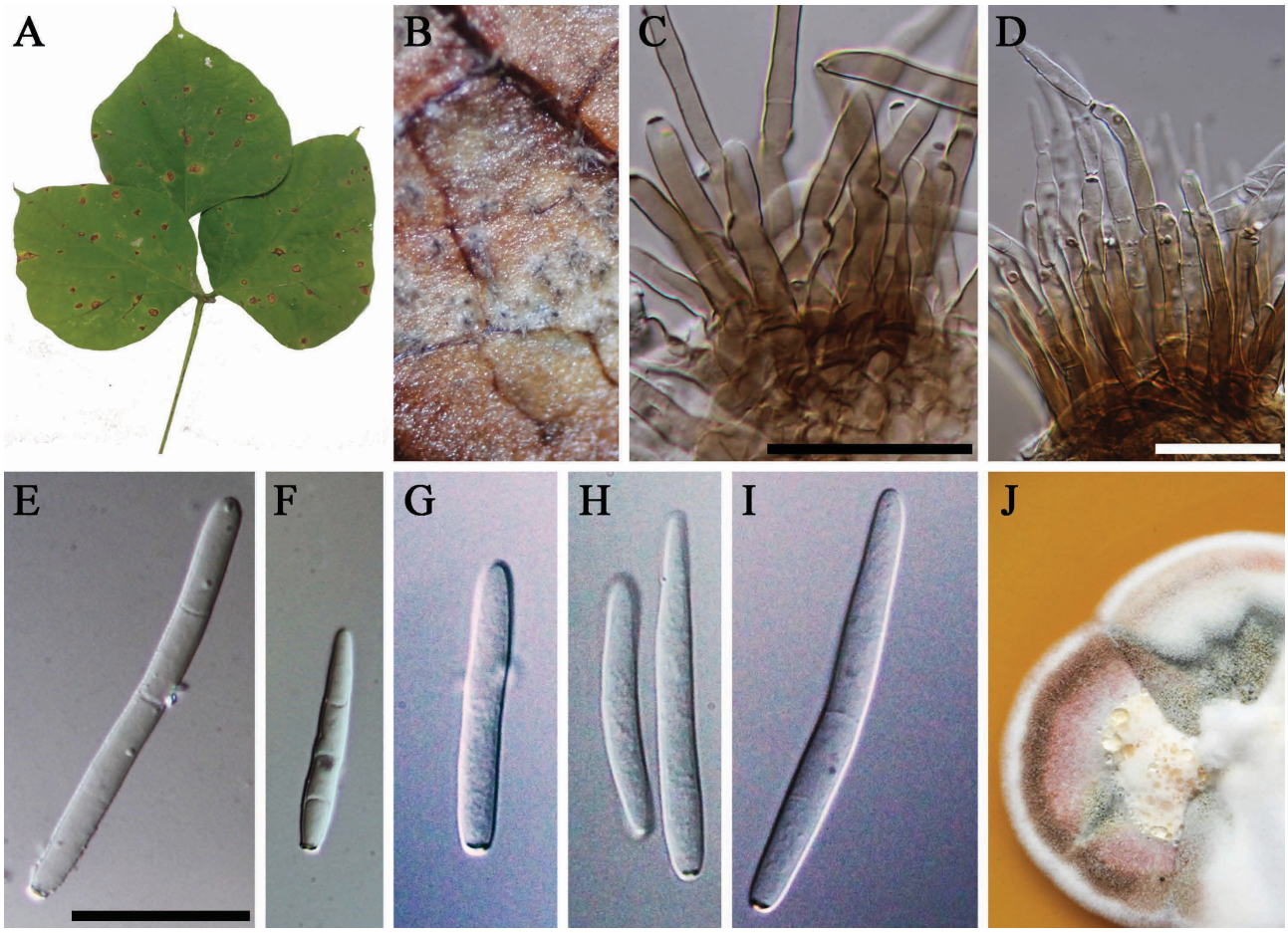

FIGURE 2. *Cercospora glycinicola* (CBS H-22289). A. Leaf spot; B. Close-up of leaf spot; C, D. Conidiophores and conidiogenous cells; E−I. Conidia; J. Colony on MEA.—Scale bars: C−D = 40 µm; E−I = 50 µm.

Cercospora cyperacearum Cheew., Crous & U. Braun, *sp. nov.* (Fig. 3). MycoBank MB812919

Type:—THAILAND. Chiang Mai: on leaves of *Cyperus alternifolius* (Cyperaceae), 12 May 2013, *S. Seekanha* (holotype CBS H-22290, culture ex-type CPC 23918 = CBS 140165).

Other specimens examined:—THAILAND. Chiang Mai: on unknown monocot, 12 May 2013, *S. Seekanha*, CPC 24811; Lamphun on leaves of *Solanum mammosum* (Solanaceae), 9 Dec. 2010, *J. Nguanhom*, CPC 22014.

Leaf spots amphigenous, pale brown to brown, margin indefinite, elongated to irregular. *Caespituli* amphigenous, punctiform, brown. *Stromata* substomatal to intraepidermal, brown, 38–63 µm high, 18–38 µm wide. *Conidiophores* fasciculate, pale olivaceous, paler and narrower towards the apex, unbranched, main portion straight, subcylindrical, only conidiogenous cells distinctly geniculate, 18−68 × 4−5 µm. *Conidiogenous cells* proliferating sympodially 5−9 times, integrated, terminal, 18–35 µm long, conidiogenous loci conspicuous, thickened and darkened, apical and lateral, circumspersed, 1−3 µm diam. *Conidia* solitary, hyaline, thin-walled, smooth, obclavate-cylindrical, subacute to acute at the apex, truncate at the base, 40−63 × 2−2.5 µm, indistinctly 1–6-septate, hila slightly thickened, darkened and refractive, 1−3 µm diam.

Culture characteristics:—Colonies spreading, with dense aerial mycelium, reaching 40 mm diam after 2 wk. On MEA surface green-glaucous with pink pigment surrounding colony with undulate margins; reverse fucous-black. On PDA surface smoke grey with pink at the margin, entire margin; reverse fucous-black. On OA surface dense mycelium, with sparse entire margin, pure olivaceous-grey with diffuse livid red pigment surrounding colony; reverse dark vinaceous with lavender grey at the centre.

Etymology:—Epithet derived from the host genus, *Cyperus*.

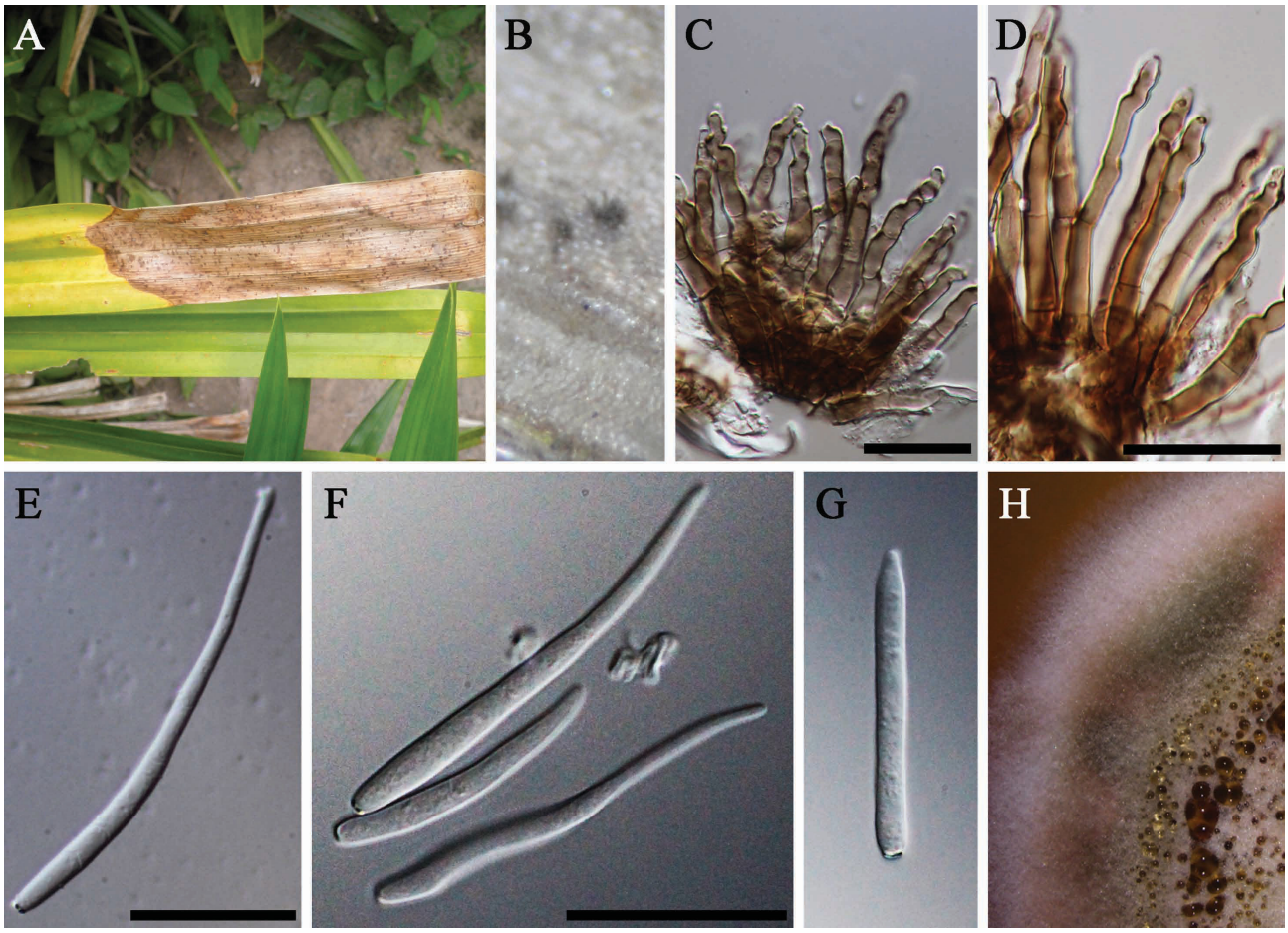

FIGURE 3. *Cercospora cyperacearum* (CBS H-22290). A. Leaf spot; B. Close-up of leaf spot; C, D. Conidiophores and conidiogenous cells; E−G. Conidia; H. Colony on MEA.—Scale bars: C−G = 40 µm.

Notes:—*Cercospora cyperacearum* is undoubtedly plurivorous, as it is known from DNA sequences retrieved from unrelated hosts, including dicots and monocots, rendering a final conclusion impossible. The occurrence on additional hosts cannot be excluded and is probable, i.e., previously described *Cercospora* species might be involved. The *Cercospora* species described from *Cyperus* spp. are morphologically distinct (Braun *et al.* 2014). *Cercospora cyperigena* U. Braun & Crous has much shorter, 0–1-septate conidiophores, 5–20 × 2–5 µm, and *C. cyperi* Sawada has small stromata, 10–25 µm diam, smaller conidiogenous loci, (1–)1.5–2(–2.5) µm diam, and broader conidia, (2–)2.5–5(–5.5) µm (Braun *et al.* 2014). *Cercospora cyperacearum* on *Cyperus alternifolius* in Thailand is characterised by forming large stromata and narrow conidia, and agrees well with the description of *Cercospora ugandensis* in Vasudeva (1963) based on Indian material on *Cyperus* sp., which is, however, not in agreement with the original description of this species that has been reduced to synonymy with *C. cyperi* in Braun *et al.* (2014). The characters of conidiophores and conidia of *Cercospora* spp. on *Solanum* are not in agreement with the material on *Solanum mammosum*. *C. solanicola* and *C. melongenae* are *C. apii*-like, i.e., with consistently acicular conidia, and were reduced to synonymy with *C. physalidis s. lat.* in Braun & Mel'nik (1997), which was considered to be part of the *C. apii s. lat.* complex in Crous & Braun (2003). These species are characterised by having long, pluriseptate conidiophores to 200 µm, and conidia to 300 × 2.5–5 µm. The conidiophores in *C. solani* agree well with those of *C. cyperacearum* but the lesions are indistinct and the acicular conidia are 3.5–6 µm wide (Chupp 1954; type material examined: Thüm., Mycoth. univ. 2070, HAL). *C. solanigena* (Bhartiya *et al*. 2000), described from India on *Solanum melongena*, resembles *C. cyperacearum*. However, the stromata are smaller, 10–30 µm diam, and the conidiophores are 16–100 × 3–5 µm, 1–6-septate, with conidia being cylindrical-obclavate to acicular (based on the original illustrations), 15–85 × 2–5 µm, 1–5-septate.

Cercospora cyperina Nguanhom, Crous & U. Braun, *sp. nov.* (Fig. 4). MycoBank MB812920

Type:—THAILAND. Chiang Mai: on leaves of *Cyperus alternifolius* (Cyperaceae), 12 May 2013, *S. Seekanha* (holotype CBS H-22291, culture ex-type CPC 23919 = CBS 140166).

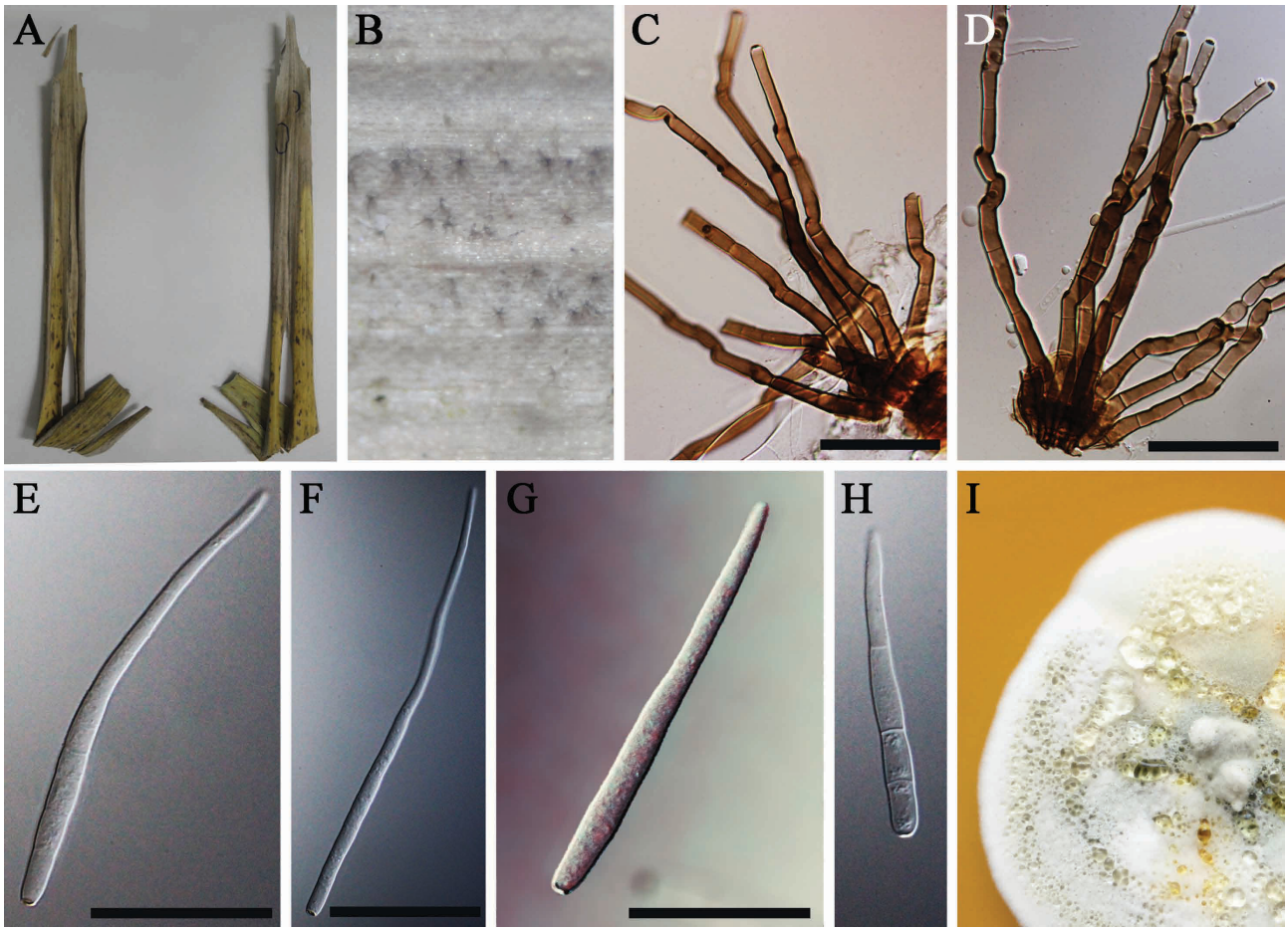

FIGURE 4. *Cercospora cyperina* (CBS H-22291). A. Leaf spot; B. Close-up of leaf spot; C, D. Conidiophores and conidiogenous cells; E−H. Conidia; I. Colony on MEA.—Scale bars: C−D = 40 µm; E−H = 50 µm, and G applies to H.

Leaf spots amphigenous, pale brown to brown, margin indefinite, elongated to irregular. *Mycelium* internal. *Caespituli* amphigenous, punctiform, brown. *Stromata* none or composed of 2−3 brown cells. *Conidiophores* loosely fasciculate, with fascicles of 2−9 conidiophores, brown, paler towards the apex, cylindrical to geniculate, somewhat constricted at septa, branched, 2−6-septate, 73−138 × 5−6 µm. *Conidiogenous cells* proliferating sympodially 1−6 times, integrated, terminal and intercalary, 20–38 µm long; conidiogenous loci conspicuous, thickened and darkened, multi-local, formed apical or on shoulders caused by proliferation, 2.5−4 µm diam. *Conidia* solitary, hyaline, thin-walled, smooth, acicular to somewhat obclavate, subobtuse at the apex, truncate to slightly obconically truncate at the base, 55−155 × 2.5−4 µm, indistinctly 15–20-septate, hila slightly thickened, darkened and refractive, 2.5–3 µm diam.

Culture characteristics:—Colonies spreading, low convex, with sparse to moderate aerial mycelium, entire margin and folded surface, reaching 44 mm diam after 2 wk. On MEA surface whitish with patches of greyish rose; reverse olivaceous-black. On PDA surface whitish, pale grey at the centre with diffuse red pigment surrounding the colony; reverse olivaceous-black. On OA whitish with patches of pale grey, but red at the margin; reverse olivaceous-grey.

Etymology:—Epithet derived from the host genus, *Cyperus*.

Notes:—*Cercospora cyperina* is morphologically close to *C. cyperi* Sawada (Braun *et al*. 2014) but distinct by having longer, distinctly geniculate conidiophores with constrictions and much larger conidiogenous loci, 3–4 µm diam [conidiophores (10–)20–90 µm long, without constrictions, loci (1–)1.5–2(–2.5) µm diam in *C. cyperi*]. This taxon was also supported as a new species based on its distinct phylogenetic position. In the combined tree (Fig. 1), it is sister to *C. cyperacearum* and thus separate from other species occurring on *Cyperus*.

Cercospora musigena Nguanhom, Crous & U. Braun, *sp. nov.* (Fig. 5). MycoBank MB812921

Type:—THAILAND. Chiang Mai: on leaves of *Musa* sp. (Musaceae), 27 May 2013, *S. Seekanha* (holotype CBS H-22292, culture ex-type CPC 24809 = CBS 140167, CPC 24831).

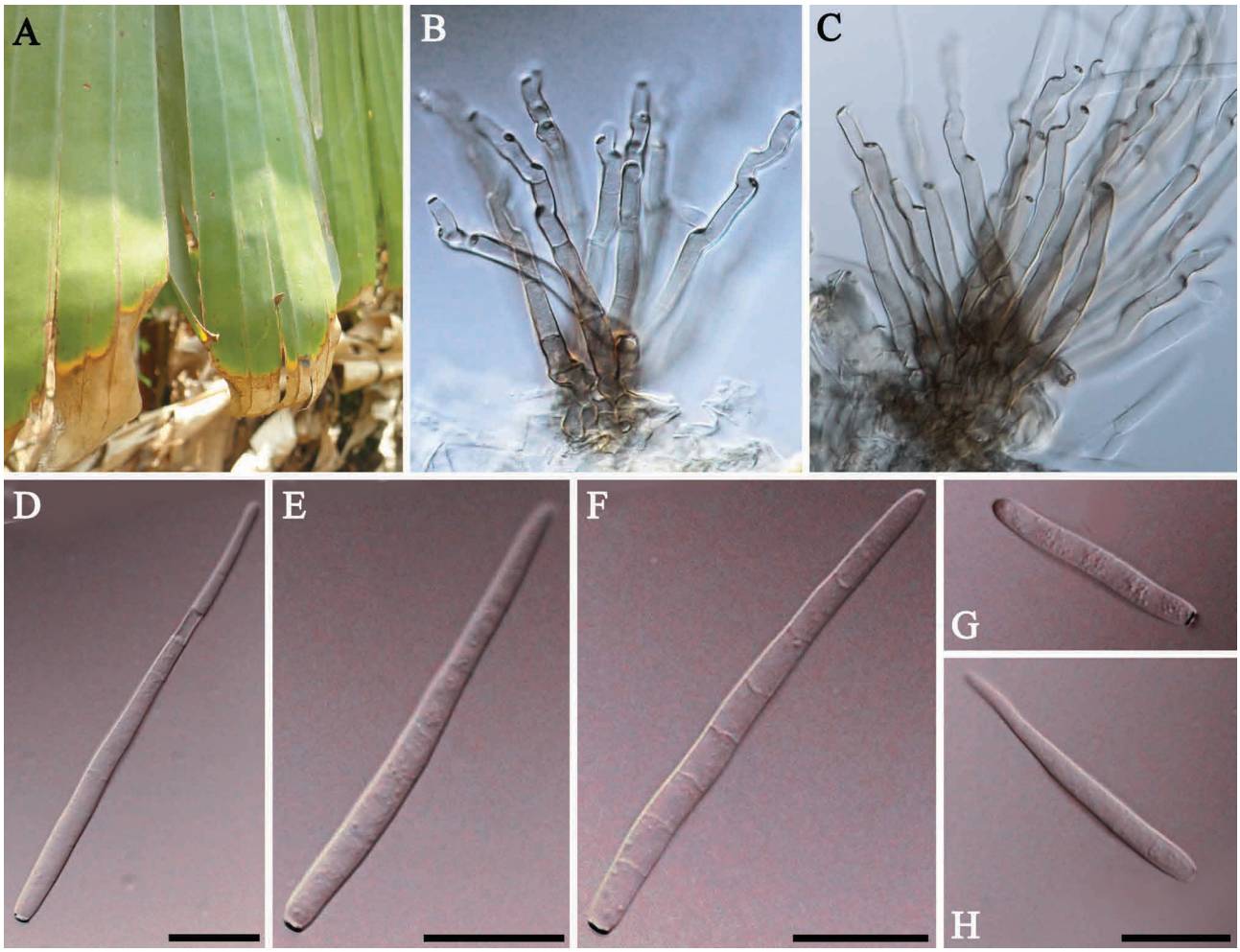

FIGURE 5. *Cercospora musigena* (CBS H-22292). A. Leaf spot; B, C. Conidiophores and conidiogenous cells; D–H. Conidia.—Scale bars: B–C = 50 µm; D–H = 30 µm.

Leaf spots irregular, pale brown along the leaf margins, often surrounded by a yellow halo. *Caespituli* amphigenous, punctiform, brown. *Stromata* intraepidermal to subepidermal, brown, 30–45 µm diam. *Conidiophores* in moderately large fascicles (11–20 per fascicle), erumpent through the cuticle, brown, paler toward the apex, 3–5-septate, cylindrical, 1–3 times geniculate in upper part, tapering to flat-tipped loci, branched, 30–75 × 4–5 µm. *Conidiogenous cells* proliferating sympodially 1–4 times, integrated, terminal, rarely intercalary; conidiogenous loci distinct, apical or formed on shoulders due to sympodial proliferation, thickened and darkened, protruding, 2–3 µm diam. *Conidia* solitary, hyaline, straight to mildly curved, acicular, truncate at the base, obtuse at the apex, thin-walled, smooth, 15–130 × 3–5 µm, 2–20-septate, hila thickened, darkened, 2–2.5 µm diam.

Culture characteristics:—Colonies spreading, flat, with sparse to dense aerial mycelium, even margin, reaching 48 mm diam after 2 wk. On MEA surface pale purplish grey, with rosy buff outer region; reverse sepia. On PDA surface vinaceous-buff, with red diffuse pigment surrounding culture; reverse bay. On OA surface whitish, with patches of grey; reverse chestnut, with rust in outer region.

Etymology:—Named after the host from which it was isolated, *Musa* sp.

Notes:—*Cercospora musigena* is similar to *C. hayi* Calp. by its acicular conidia with truncate bases, being part of the *C. apii* complex (Braun *et al.* 2014). In *C. hayi*, however, there are fewer conidiogenous loci per conidiogenous cell, and the conidial tips are acute to subacute (Calpouzos 1955). *C. apii s. lat.* (including *C. hayi*) on *Musa* spp. is genetically heterogeneous. Sequences retrieved from *C. apii*-like cultures isolated from banana clustered in three

different clades (Groenewald *et al.* 2013). However, the identity of the name *C. hayi* is still unresolved and will need to be clarified by means of epitypification (Braun *et al*. 2014).

***Cercospora* sp. (**Fig. 6).

Specimen examined:—THAILAND. Chiang Mai: on leaves of *Crassocephalum crepidioides* (Asteraceae), 29 Mar. 2013, *J. Nguanhom* (specimen CBS H-22293, culture CPC 23905, CPC 23906 = CBS 140168).

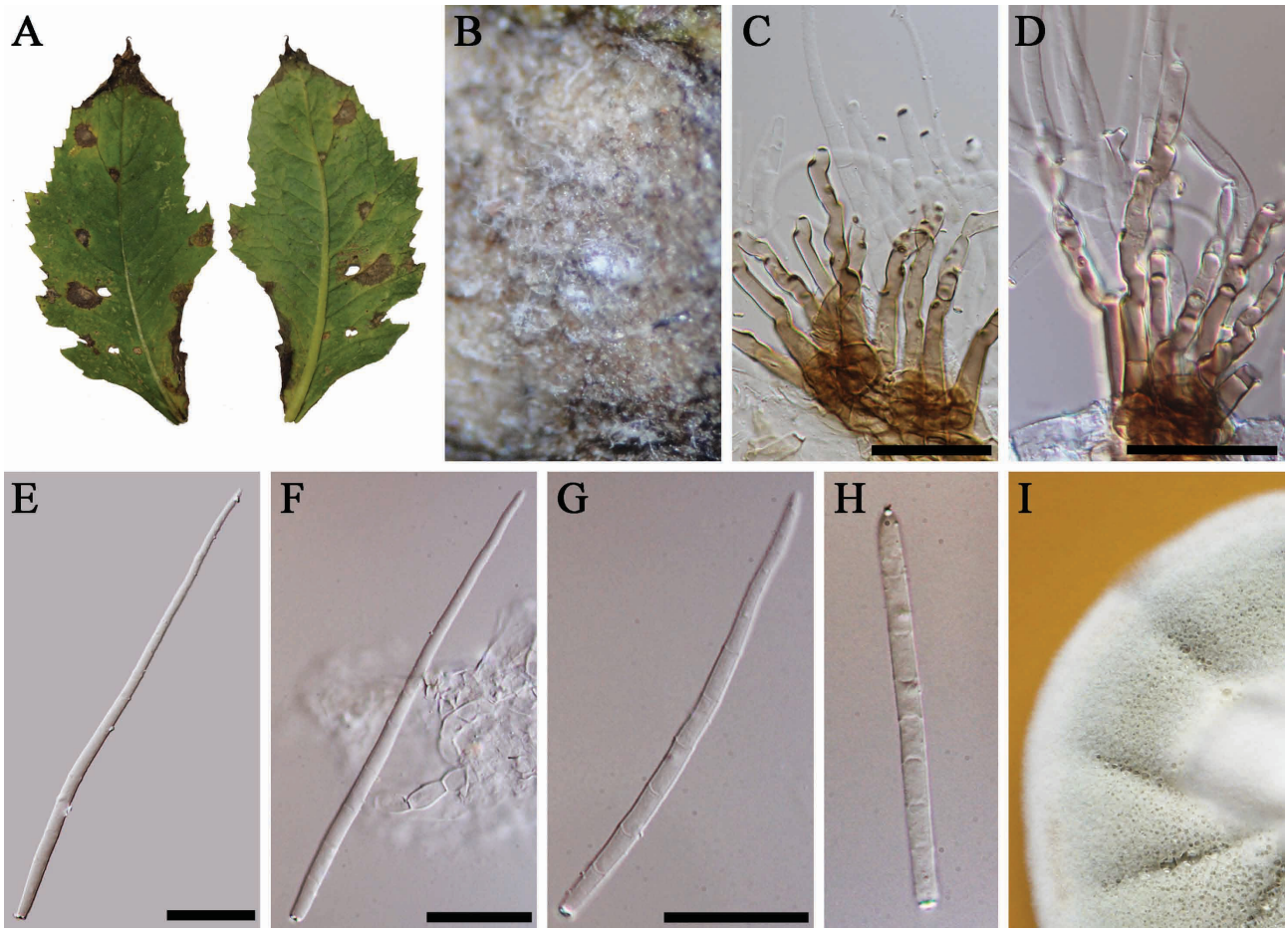

FIGURE 6. *Cercospora* sp. (CBS H-22293). A. Leaf spot; B. Close-up of leaf spot; C, D. Conidiophores and conidiogenous cells; E−H. Conidia; I = colony on MEA.—Scale bars: C−D = 40 µm; E−H = 50 µm, G applies to H.

Leaf spots amphigenous, circular to irregular, dark brown with pale brown centre, 3−5 mm diam. *Caespituli* amphigenous, punctiform, brown. *Stromata* medium in size, substomatal or intraepidermal, brown, 27.5–37.5 µm diam. *Conidiophores* in moderately large fascicles, arising from stromata, through stomata or erumpent, cylindrical to strongly geniculate, brown, paler towards the apex, unbranched, rarely constricted near the apex, 50−133 × 5−6 µm. *Conidiogenous cells* proliferating sympodially 2−12 times, integrated, terminal, 20−75 µm long; loci conspicuous, apical or on shoulders formed by geniculation, thickened and darkened, 2–3 µm diam. *Conidia* solitary, acicular, shorter ones subcylindrical, hyaline, smooth, thin-walled, straight to curved, apices subacute to obtuse, base truncate, indistinctly 3−12-septate, 27.5−180 × 2−5 µm, hila thickened and darkened, 2–3 µm wide; microcyclic conidiation observed.

Culture characteristics:—Colonies spreading, flat, with sparse to moderate aerial mycelium, folded surface and even margins, reaching 45 mm after 2 wk. On MEA pale grey, with white centres, whitish at the margin; olivaceous-grey in reverse. On PDA whitish grey; reverse olivaceous-grey. On OA whitish grey (due to aerial mycelium); reverse blackish.

Notes:—The genus *Crassicephalum* is close to *Senecio* and allied genera and in subtribe *Senecioninae* in tribe *Senecioneae*. The cercosporoid fungus on *Crassocephalum crepidioides* is morphologically indistinguishable from *Cercospora apii s. lat*., by having acicular conidia with truncate bases. *C. senecionis* Ellis & Everh. is morphologically very close, except for somewhat wider conidiophores, 4–8 µm, and conidia, 3–6 µm (Braun & Mel'nik 1997). *C.*

senecionicola Davis differs in having uniformly short, non-geniculate conidiophores, 15–55 × 4–5 µm, and acicular to obclavate conidia, 2–3.5 µm wide (Chupp 1954). The Indian *C. senecionis-grahamii* Thirum. & Govindu (Thirumalachar & Govindu 1962) is morphologically barely distinguishable from material on *Crassocephalum crepidioides*. *Erechtites* is another genus belonging in subtribe *Senecininae* and confusable with *Crassocephalum*. *C. erechtitis* G.F. Atk. is a widespread species, also known from Asia (Hsieh & Goh 1990), and is morphologically also similar to material on *Crassocephalum,* being *C. apii*-like in morphology. Sequence data of the species on hosts belonging to the *Senecioninae*, which are morphologically involved in this complex, are not yet available for comparison. Furthermore, *Crassocephalum crepidioides* is an African species not native in Thailand. Therefore, an infection of this host by another *Cercospora* species, native or exotic, cannot be excluded. Hence, the present data do not allow a final conclusion and taxonomic treatment of the *Cercospora* sp. occurring on *Crassocephalum* to be made until such time as the phylogeny of related taxa has been clarified.

Discussion

Approximately 500 cercosporoid species have been reported from Thailand, including 300 species of *Cercospora*. To date these taxa have primarily been identified based on their morphology, and only a few have been studied phylogenetically (To-anun *et al*. 2010, 2011). In several phylogenetic studies, multi-gene DNA sequence data have proven highly effective to distinguish among species of *Cercospora* (Groenewald *et al*. 2005, 2006a, b, 2010, 2013, Bakhshi *et al.* 2015a, b). The same approach was followed in this study, leading to the conclusion that morphological characters and molecular techniques are complementary, and both necessary to underpin novel species of *Cercospora* from Thailand. The results obtained here provide strong support for the distinction of several *Cercospora* species based on an analysis of ITS and *cmdA* DNA sequence data.

Four new species of *Cercospora* were recognized in this study. *Cercospora glycinicola* is morphologically similar to *C. sojina*, which also occurs on *Glycine max*, but is distinct in that it has shorter conidiophores and narrower conidia. Two species were described from *Cyperus*, namely *C. cyperacearum* and *C. cyperina.* Based on a range of characters related to conidiophore length, septation, stromatal size, and conidium morphology, these species appear distinct from the taxa presently known to occur on *Cyperus* (Braun *et al.* 2014). However, *Cercospora cyperacearum* is plurivorous, and also occurs on *Solanum mammosum*, although it is morphologically distinct from the species known from *Solanum* (Braun & Mel'nik 1997, Bhartiya *et al.* 2000, Crous & Braun 2003). Further collections in the region are required to determine if *C. cyperacearum* could also be found on additional hosts, but ultimately cross-inoculation experiments would be required to determine if the different hosts are only chance occurrences, or if this is a truly plurivorous species.

Cercospora collections occurring on *Musa* have always been assigned to *C. hayi*, which was originally described from banana leaves collected in Cuba (Calpouzos 1955). However, in a recent phylogenetic study, Groenewald *et al*. (2013) showed that sequences retrieved from *C. apii*-like cultures isolated from banana leaves collected in different countries clustered in three different clades. It is therefore not surprising that the collection obtained from Thailand is distinct from these unnamed taxa, and from *C. apii s. str*. Unfortunately, the three taxa referred to by Groenewald *et al.* (2013) are sterile, and thus further collections would be called for to try and elucidate the *Cercospora* complex occurring on banana, which is a host that appears to harbour a range of unique cercosporoid fungi (Arzanlou *et al.* 2008).

In addition to these novel taxa, three *Cercospora* species were found on new hosts (based on the clades phylogenetically defined by Groenewald *et al*. 2013): *C.* cf. *citrullina* on *Cyathula prostrata* (Amaranthaceae); *C.* cf. *malloti* on *Abelmoschus esculentus* (Malvaceae), *Asystasia salicifolia* (Acanthaceae), *Brassica alboglabra* (Brassicaceae), *Codiaeum variegatum* (Euphorbiaceae), *Eupatorium odoratum* (Asteraceae), *Jatropha integerrima* (Euphorbiaceae), *Melampodium divaricatum* (Asteraceae), *Musa sapientum* (Musaceae), *Nicotiana tabacum* (Solanaceae), *Phlox drummondii* (Polemoniaceae), *Physalis peruviana* (Solanaceae), and *Plantago major* (Plantaginaceae); and *C.* cf. *nicotianae* on *Houttuynia cordata* (Saururaceae).

Results obtained in this study showed that the most common *Cercospora* sp. found in Thailand was *C.* cf. *malloti*, which occurred on a wide host range. The collected isolates of *C.* cf. *malloti* shared similar conidiophore characteristics, being thick-walled, with distinct loci formed at the apex and on the shoulders caused by conidiophore geniculation (Groenewald *et al*. 2013). However, there were also some variable characters, namely differences in conidiophore geniculation and conidium length, suggesting that either the DNA loci currently used are not sensitive enough to

distinguish all species, or that different environmental conditions and hosts to some degree influence the observed *Cercospora* phenotype. As most isolates sporulate poorly in culture (if at all), comparisons were always done on material *in vivo*. *Cercospora malloti* was originally described from *Mallotus* (Euphorbiaceae) collected in the USA. Fresh material would thus need to be recollected from this host in the USA to resolve the phylogenetic relationships of this taxon. Other than these wide host range species, some taxa also appeared to be host specific, namely *C. capsici* on *Capsicum* spp. (Solanaceae), *C.* cf. *mikaniicola* on *Mikania cordata* (Asteraceae) and *C.* cf. *zinnia* on *Zinnia elegans* (Asteraceae). To fully resolve the taxonomy of the *Cercospora* spp. occurring in Thailand, however, a global initiative is called for, as the phylogenetic position of many "common" species remains unknown, and these species, like *C. malloti*, will have to be recollected on their original hosts from their respective countries of origin. Further global studies are presently underway to try and establish a phylogenetic reference tree, collection and database for the genus *Cercospora*.

Acknowledgements

This work was financially supported by the Royal Golden Jubilee Ph.D. Program (PHD/0061/2551) and the Thailand Research Fund (DBG5380011 and MRG5580163). J.N. also thanks the technical staff from the CBS-KNAW Fungal Biodiversity Centre for their invaluable assistance.

References

Amaradasa, B.S., Madrid, H., Groenewald, J.Z., Crous, P.W. & Amundsen, K. (2014) *Porocercospora seminalis* gen. et comb. nov. the causal organism of buffalograss false smut. *Mycologia* 106: 77–85.
http://dx.doi.org/10.3852/13-147

Arzanlou, M., Groenewald, J.Z., Fullerton, R.A., Abeln, E.C.A., Carlier, J., Zapater, M.-F., Buddenhagen, I.W., Viljoen, A. & Crous, P.W. (2008) Multiple gene genealogies and phenotypic characters differentiate several novel species of *Mycosphaerella* and related anamorphs on banana. *Persoonia* 20: 19–37.
http://dx.doi.org/10.3767/003158508X302212

Bakhshi, M., Arzanlou, M., Babai-ahari, A., Groenewald, J.Z., Braun, U. & Crous, P.W. (2015a) Application of the consolidated species concept to *Cercospora* spp. from Iran. *Persoonia* 34: 65–86.
http://dx.doi.org/10.3767/003158515X685698

Bakhshi, M., Arzanlou, M., Babai-ahari, A., Groenewald, J.Z., Braun, U. & Crous, P.W. (2015b) Is morphology in *Cercospora* a reliable reflection of generic affinity? *Phytotaxa* 213 (1): 22–34.
http://dx.doi.org/10.11646/phytotaxa.213.1.2

Bhartiya, H.D., Dubey, R.C. & Singh, S.K. (2000) New *Cercospora* spp. associated with vegetable crops in north eastern Uttar Pradesh. *Indian Phytopathology* 53: 149–152.

Braun, U., Crous, P.W. & Nakashima, C. (2014) Cercosporoid fungi (*Mycosphaerellaceae*) 2. Species on monocots (*Acoraceae* to *Xyridaceae*, excluding *Poaceae*). *IMA Fungus* 5: 203–390.
http://dx.doi.org/10.5598/imafungus.2014.05.02.04

Braun, U. & Mel'nik, V.A. (1997) Cercosporoid fungi from Russia and adjacent countries. *Trudy Botanischeskogo Instituta Imeni V. L. Komarova, St. Petersburg* 20: 1–130.

Braun, U., Hill, C.F. & Schubert, K. (2006) New species and new records of biotrophic micromycetes from Australia, Fiji, New Zealand and Thailand. *Fungal Diversity* 22: 13–35.

Calpouzos, L. (1955) *Studies on the Sigatoka disease of banana and its fungus pathogens*. Cienfuegos, Atkins Garden and Research Laboratory.

Carbone, I. & Kohn, L.M. (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 91: 553–556.
http://dx.doi.org/10.2307/3761358

Cheewangkoon, R., Crous, P.W., Hyde, K.D., Groenewald, J.Z. & To-anan, C. (2008) Species of *Mycosphaerella* and related anamorphs on *Eucalyptus* leaves from Thailand. *Persoonia* 21: 77–91.
http://dx.doi.org/10.3767/003158508X370857

Chupp, C. (1954) *A monograph of the fungus genus Cercospora*. Ithaca, New York. Published by the author.

Crous, P.W. & Braun, U. (2003) *Mycosphaerella and its anamorphs.* 1. *Names published in Cercospora and Passalora.* [CBS Biodiversity Series no. 1.] CBS-KNAW Fungal Biodiversity Centre, Utrecht.

Crous, P.W., Gams, W., Stalpers, J.A., Robert, V. & Stegehuis, G. (2004) MycoBank: an online initiative to launch mycology into the 21st century. *Studies in Mycology* 50: 19–22.

Crous, P.W., Verkley, G.J.M., Groenewald, J.Z. & Samson, R.A. (Eds.) (2009) *Fungal biodiversity*. [CBS Laboratory Manual Series no. 1]. CBS-KNAW Fungal Biodiversity Centre, Utrecht.

Crous, P.W., Braun, U., Hunter, G.C., Wingfield, M.J., Verkley, G.J., Shin, H.-D., Nakashima, C. & Groenewald, J.Z. (2013) Phylogenetic lineages in *Pseudocercospora*. *Studies in Mycology* 75: 37–114.
http://dx.doi.org/10.3114/sim0005

de Hoog, G.S. & Gerrits van den Ende, A.H.G. (1998) Molecular diagnostics of clinical strains of filamentous *Basidiomycetes*. *Mycoses* 41: 183–189.
http://dx.doi.org/10.1111/j.1439-0507.1998.tb00321.x

Drummond, A.J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Duran, C., Field, M., Heled, J., Kearse, M., Markowitz, S., Moir, R., Stones-Havas, S., Sturrock, S., Thierer, T. & Wilson, A. (2011) Geneious v5.4. Available from: http://www.geneious.com/ (accessed 12 October 2015)

Giatgong, P. (1980) *Host index of plant diseases of Thailand*. Mycology Section, Plant Pathology and Microbiology Division, Department of Agriculture, Bangkok, Thailand.

Groenewald, J.Z., Groenewald, M., Braun, U. & Crous, P.W. (2010) *Cercospora* speciation and host range. *In*: Lartey, R.T., Weiland, J.J., Panella, L., Crous, P.W. & Windels, C.E. (Eds.) *Cercospora leaf spot of sugar beet and related species*. APS Press, Minnesota, USA, pp. 21–37.

Groenewald, J.Z., Nakashima, C., Nishikawa, J., Shin, H.-D., Park, J.-H., Jama, A.N., Groenewald, M., Braun, U. & Crous, P.W. (2013) Species concepts in *Cercospora*: spotting the weeds among the roses. *Studies in Mycology* 75: 115–170.
http://dx.doi.org/10.3114/sim0012

Groenewald, M., Groenewald, J.Z. & Crous, P.W. (2005) Distinct species exist within the *Cercospora apii* morphotype. *Phytopathology* 95: 951–959.
http://dx.doi.org/10.1094/PHYTO-95-0951

Groenewald, M., Groenewald, J.Z., Braun, U. & Crous, P.W. (2006a) Host range of *Cercospora apii* and *C. beticola*, and description of *C. apiicola*, a novel species from celery. *Mycologia* 98: 275–285.
http://dx.doi.org/10.3852/mycologia.98.2.275

Groenewald, M., Groenewald, J.Z., Harrington, T.C., Abeln, E.C.A. & Crous, P.W. (2006b) Mating type gene analysis in apparently asexual *Cercospora* species is suggestive of cryptic sex. *Fungal Genetics and Biology* 43: 813–825.
http://dx.doi.org/10.1016/j.fgb.2006.05.008

Hsieh, W.H. & Goh, T.K. (1990) *Cercospora and similar fungi from Taiwan*. Maw Chang Book Company. Taipei, Taiwan, Republic of China.

Hunter, G.C., Crous, P.W., Wingfield, B.D., Pongpanich, K. & Wingfield, M.J. (2006) *Pseudocercospora flavomarginata* sp. nov., from *Eucalyptus* leaves in Thailand. *Fungal Diversity* 22: 71–90.

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.
http://dx.doi.org/10.1093/molbev/mst010

Meeboon, J., Hidayat, I., Nakashimam, C. & To-anun, C. (2007a) *Cercospora habenariicola* sp. nov. and some new records of cercosporoid fungi from Thailand. *Mycotaxon* 99: 117–121.

Meeboon, J., Hidayat, I. & To-anun, C. (2007b) An annotated list of cercosporoid fungi in Northern Thailand. *Journal of Agricultural Technology* 3, 51–63.

Meeboon, J., Hidayat, I. & To-anun, C. (2007c) Diversity and taxonomy of cercosporoid fungi in Thailand. *In*: Soytong, K. & Hyde, K.D. (Eds.) *Proceedings of the International Conference on Integration of Science and Technology for Sustainable Development (ICIST) "Biological Diversity, Food and Agricultural Technology".* KMITL, Bangkok, Thailand, pp. 273–278.

Meeboon, J., Hidayat, I., To-anun, C. & Nakashima, C. (2008) Cercosporoid fungi from Thailand II. New species of *Cercospora* and *Passalora. Sydowia* 60: 253–260.

Möller, E.M., Bahnweg, G., Sandermann, H. & Geiger, H.H. (1992) A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acids Research* 20: 6115–6116.
http://dx.doi.org/10.1093/nar/20.22.6115

Nakashima, C., Motohashi, K., Meeboon, J. & To-anun, C. (2007) Studies on *Cercospora* and allied genera in northern Thailand. *Fungal Diversity* 26: 257–270.

Nylander, J.A.A. (2004) *MrModeltest 2.0*. Program distributed by the author, Uppsala University, Uppsala, Sweden.

Petcharat, V. & Kanjanamaneesathian, M. (1989) Species of plant pathogen *Cercospora* in Southern Thailand. *Thai Phytopathology* 9: 23–27.

Phengsintham, P., Braun, U., McKenzie, E.H.C., Chukeatirote, E., Cai, L. & Hyde, K.D. (2013) Monograph of cercosporoid fungi from Thailand. *Plant Pathology & Quarantine* 3: 67–138.

Rayner, R.W. (1970) *A mycological colour chart*. CMI and British Mycological Society, Kew, Surrey, England.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.
http://dx.doi.org/10.1093/sysbio/sys029

Shin, H.D. & Kim, J.D. (2001) *Cercospora and allied genera from Korea*. Plant Pathology of Korea 7. National Institute of Agricultural Science and Technology, Suwon.

Sontirat, P., Phitakpraiwan, P., Choonbamroong, W. & Kueprakone, U. (1980) *Plant pathogenic Cercosporae in Thailand.* Department of Agriculture, Ministry of Agriculture and Cooperative, Bangkok, Thailand.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731–2739.
http://dx.doi.org/10.1093/molbev/msr121

Thirumalachar, M.J. & Govindu, H.C. (1962) Notes on some Indian cercosporae. *Sydowia* 16: 284–288.

To-anun, C., Hidayat, I. & Meeboon, J. (2010) *Cercospora christellae*, a new cercosporoid fungus associated with weed *Christella parasitica* from northern Thailand. *Journal of Agricultural Technology* 6: 331–339.

To-anun, C., Hidayat, I. & Meeboon, J. (2011) Genus *Cercospora* in Thailand: taxonomy and phylogeny (with a dichotomous key to species). *Plant Pathology & Quarantine* 1: 11–87.

Vasudeva, R.S. (1963) *India cercosporae*. Indian Council of Agricultural Research, New Delhi.

White, T.J., Bruns, T. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylognetics. *In*: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, J.W. (Eds.) *A Guide to Molecular Methods and Applications.* Academic Press, New York, pp. 315–322.