Journal of Agricultural Technology 2013 Vol. 9(4): 943-951
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Cladosporiod on monocotyledon plant from Thailand

Songsuda Plakthongdee[1], Sararat Monklung[2], Ratchadawan Cheewangkoon[1] and Chaiwat To–anun[1*]

[1]Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand , [2]Crop Production Technology Program, Faculty of Animal Science and Agricultural Technology, Silpakorn University, Phetchaburi Information Technology Campus, Phetchaburi 76120, Thailand

Songsuda Plakthongdee, Sararat Monklung, Ratchadawan Cheewangkoon and Chaiwat To–anun (2013) Cladosporiod on monocotyledon plant from Thailand. Journal of Agricultural Technology 9(4):943-951.

During our studies on the diverse cladosporoid species occurring on monocotyledon plants, many species of Cladosporium previously unreported in Thailand were encountered. In this paper, we report on six species of Cladosporium from various monocotyledon plants. Based on analyses of the internal transcribed spacers (ITS1, 5.8S nrDNA, ITS2) and the translation elongation factor 1–α gene (EF–1α), the isolates are members of *Cladosporium cladosporiodes* complex. Based on the cultural and micromorphological characteristics, they were identified as *C. cladosporiodes*, *C. cucumerinum*, *C. perangusum*, *C. pseudocladosporiodes*, *C. tenuissimum* and *C. xylophilum*. These six species represent new records in Thailand and world new host records.

Keys word: Cladosporioid, Cladosporium, monocotyledon

Introduction

Cladosporioid (Cladosporium–like) hyphomycetes are common, widespread fungi. The genus *Cladosporium* is one of the largest, most heterogeneous genera of hyphomycetes, comprising more than 772 names (Dugan *et al*., 2004), and including endophytic, fungicolous, human pathogenic, phytopathogenic and saprobic species.

As plant pathogens, cladosporioid hyphomycetes cause severe impact on the growth of various economically important plants (Agrios, 2005). Since crop losses caused by fungal diseases pose a serious threat to global food/ security, it became apparent that the threat to agriculture from the deliberate release of pathogens, such as *Cladosporium*, should not be underestimated. Nonetheless, the information of those phytopathogenic fungi in Thailand are quite limited

* Corresponding author: To–anun, C.; e-mail: chaiwat.toanun@gmail.com; ratcha.222@gmail.com

and, therefore, still causing many difficulties for mycologist, plant pathologist, quarantine, and other scientific societies in Thailand to identify until species level. Almost no information, regarding this group of fungi and its distribution to the host plants specific in Thailand, are provided. Presently, only eight species of *Cladosporium* have been reported, namely, *Cladosporium cladosporioides* (Bensch *et al*., 2010; Lumyong *et al*., 2003), *C. colocasiae* (Manoch *et al*., 1986), *C. musae* (Lumyong *et al*., 2003), *C. oxysporum* (Lumyong *et al*., 2003), *C. perangustum* (Bensch *et al*., 2010), *C. sphaerospermum* (Sakayaroj *et al*., 2010), *C. subuliforme* (Bensch *et al*., 2010) and *C. tenuissimum* (Bensch *et al*., 2010; Tokumasu *et al*., 1990). Therefore, survey and research on diversity of other cladosporoid fungi in Thailand, and its distribution to the host plants are urgently needed.

The aims of this study are to provide a comprehensive database and a literature guide for the identification of cladosporiod fungi which are beneficial to support for the identification, detection, tracking, and risk assessment of this group of fungi.

Materials and methods

Isolates

Symptomatic monocotyledon leaves were collected at various locations in Chiang Mai, Thailand from 2011 to 2012. Symptomatic leaves were observed under stereomicroscope and incubated in moist chambers. Leaves were inspected daily for microfungi. Single–conidial isolates were obtained using techniques of Crous *et al*. (1991) and Crous (1998). Isolates were inoculated onto 2% potato–dextrose agar (PDA) at 25 °C to promote sporulation. All cultures obtained in this study were maintained in the culture collection of Plant Pathology Department, Chiang Mai University.

Morphology

Microscopic observations of the isolates were made from colonies cultivated for 7 d in 25 °C. Preparations from cultured fungal colonies were mounted on glass slides with clear lactic acid for microscopic examination. The study of conidial development and abranching patterns of conidial chains was done using the technique f Bench *et al*. (2010). Conidial terminology was followed from Schubert *et al*. (2007).

DNA isolation, amplification and sequence analysis

Fungal DNA extraction, PCR amplification and sequence analysis was patterned after Cheewangkoon *et al*. (2008). The Primers V9G (Hoog & Gerrits van den Ende, 1998) and LR5 (Vilgalys and Hester, 1990) were used to amplify part of the nuclear rDNA operon spanning the 3′ end of the 18S rRNA gene (SSU), the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region (ITS2) and the 5′ end of the 28S rRNA gene (LSU). The primers EF1–728F and EF1–986R (Carbone and Kohn, 1999) were used to amplify the translation elongation factor 1–α gene (EF–1α).

The generated sequences were compared with other fungal DNA sequences from NCBI's GenBank sequence database using a blast search; sequences with high similarity were added to the alignments. The additional GenBank sequences were manually aligned using Sequence Alignment Editor v. 2.0a11 (Rambaut, 2002). The phylogenetic analyses of the aligned sequence data were performed using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford, 2003) with the heuristic search option using the 'treebisection–reconstruction' (TBR) algorithm. All sites were treated as unordered and unweighted, with gaps treated as missing data. The strength of the internal branches of the resulting trees was tested with bootstrap analyses using 1000 replications (Felsenstein, 1985). Tree scores, including tree length, CI, RI, and RC, were also calculated.

Results and discussions

Phylogenetic analysis

The resulted alignment containing 62 taxa (including the outgroup taxon) and 412, 264 characters (including alignment gaps) were used in the ITS and TEF partitions, respectively. A total of 174 equally parsimonious trees with 710 steps (CI = 0.4873, RI =0.6951, RC = 0.3388) were generated by the parsimony ratchet analysis. One of the best trees is shown in Fig. 1.

The phylogenetic trees (Fig 1.) show that the Cladosporium species in this study segregate into 6 distinct clades, residing in the *C. cladosporiodes* complex clade. All represented species consisted of multiple strains are clustering in clades with bootstrap support values ranging from 60% (*C. cladosporiodes*) to 94% (*C. cucumerinum*).

Taxonomy

Cladosporium cladosporioides (Fresen.) G.A. de Vries. Fig. 2A.

Colonies on PDA were grey–olivaceous to dull green or olivaceous–grey. Conidiophores were straight to slightly flexuose, medium brown, (43–)51–69(–92) × (2.5–)3–3.5(–4) µm. Conidiogenous cells were integrated, usually terminal, pale brown, bearing branched conidial chains, (16–)24–26(29) × (2–)2.5–3(–3.5) µm. Ramoconidia were present, 1-2 cell, pale brown, with dimensions of (15–)18–27(–30)×(2.5–)–3–3.5(–4) µm. Conidia were spherical to subpherical, smooth–walled, and the length and width of conidia were (3–) 3.5–4(–4.5) × (2–)2.5–3(–3.5) µm.

Notes: Morphology of the fungus agreed well with the description of *C. cladosporiodes* (Bensch *et al*., 2010). The species is morphologically closely related to *C. tenuissimum*, *C. cucumerinum* and *C. vignae* but differ in their pathogenicity to specific host plants (Bench *et al*., 2010). The pathogenicity test was not determined in this study. *C. cladosporiodes* was reported from Thailand on *Areca* sp. (Bensch *et al.* 2010; 2012) and *Musa acuminata* (Lamyong *et al.* 2003). This study presented new its host plant including *Calamus siamensis*, *Hyophorbe verschaffeltii* and *Keriodoxa elegans*.

Cladosporium cucumerinum Ellis & Arthur Fig. 2B.

Conidiophores were straight to slightly flexuose, medium brown, (45–)61–75(–98) × (2–)2.5–3(–3.5) µm. Conidiogenous cells were integrated, usually terminal, pale brown, bearing branched conidial chains, (10–)12–22(36) × (2–)2.5–3(–3.5) µm. Ramoconidia were present, 1-2 cell, pale brown, with dimensions of (15.5–)18–x19(–24.5) × (2.5–)–3–3.5(–4) µm. Conidia were spherical to subpherical, smooth–walled, and the length and width of conidia were (2–)2.5–3(–3.5) × (1.5–)2–2.5(–3) µm.

Notes: *Cladosporium cucumerinum* has similar conidiophore and conidium morphology which closely to *C. cladosporioides* and *C. vignae*. (Bensch et al., 2010). This study is the first report of *C. cucumerinum* from Thailand and also new host plants including *Saccharum officinarum*, *Zea maize*, *Equisetum debile*, *Phoenix loureiri* and *Livistona chinensis*.

Cladosporium perangustum Bensch, Crous & U. Braun Fig 2C.

Colonies on PDA were grey–olivaceous to dull green or olivaceous–grey. Conidiophores were straight to slightly flexuose, medium brown, (43–)51–69(–

92) × (2.5–)3–3.5(–4) µm. Conidiogenous cells were integrated, usually terminal, pale brown, bearing branched conidial chains, (16–)24–26(29) × (2–) 2.5–3(–3.5) µm. Ramoconidia were present, 1-2 cell, pale brown, with dimensions of (15–)18–27(–30) × (2.5–)–3–3.5(–4) µm. Conidia were spherical to subpherical, smooth–walled, and the length and width of conidia were (3–) 3.5–4(–4.5) × (2–)2.5–3(–3.5) µm.

Notes: *Cladosporium perangustum* has been reported from Thailand on Acacia sp. (Bensch et al. 2010; 2012). This study provides the report of *C. cucumerinum* on new host plants including *Equisetum debile* and *Hymenocallis littoralis*.

Cladosporium pseudocladosporioides Bensch, Crous & U. Braun Fig 2D.

Colonies on PDA were grey–olivaceous to dull green or olivaceous–grey. Conidiophores were straight to slightly flexuose, medium brown, (20–)28–95(–112) × (2.5–)3–3.5(–4) µm. Conidiogenous cells were integrated, usually terminal, pale brown, bearing branched conidial chains, (10–)12–36(44) × (2–)2.5–3(–3.5) µm. Ramoconidia were present, 1-2 cell, pale brown, with dimensions of (8–)9–12(–13) × (2–)2.5–3(–3.5) µm. Conidia were spherical to subpherical, smooth–walled, and the length and width of conidia were (6–)7–8(–9) × (2–)2.5–3(–3.5) µm.

Notes: *Cladosporium pseudocladosporioides* has never been reported in Thailand. This study is the first in Thailand to report on this fungus which also has new host plants including *Cymbopogon citratus*, *Cyperus rotundus* and *Dactyloctenium aegyptium*.

Cladosporium tenuissimum Cooke Fig. 2E.

Colonies on PDA were grey–olivaceous to dull green or olivaceous–grey. Conidiophores were straight to slightly flexuose, medium brown, (43–)45–105(–127) × (2–)2.5–3(–3.5) µm. Conidiogenous cells were integrated, usually terminal, pale brown, bearing branched conidial chains, (19–)23–33(41) × (1–)1.5–2(–2.5) µm. Ramoconidia were present, 1-2 cell, pale brown, with dimensions of (10.5–)12–18(–19) × (3–)–3.5–4.5 (–5) µm. Conidia were spherical to subpherical, smooth–walled, and the length and width of conidia were (4–)4.5–5.5(–6) × (2–)2.5– 3(–3.5) µm.

Notes: *Cladosporiodes tenuissimum* was reported from Thailand on *Acasia mangium*. (Bensch *et al.* 2010; 2012) and *Pinus khasya* (Tokumasu *et al.* 1990). This study presented new host plant including *Chamaedorea erumpeus* and *Cocos nucifera*.

Cladosporium xylophilum Bensch, Shabunin, Crous & U. Braun Fig. 2F.

Colonies on PDA were grey–olivaceous to dull green or olivaceous–grey. Conidiophores were straight to slightly flexuose, medium brown, (60–)72–86(–110) × (2–)2.5–3(3.5) μm. Conidiogenous cells were integrated, usually terminal, pale brown, bearing branched conidial chains, (20–)22–24(26) × (1–)1.5–2(–2.5) μm. Ramoconidia were present, 1-2 cell, pale brown, with dimensions of (18–)20–33(–36) × (2–)–2.5–3(–3.5) μm. Conidia were spherical to subpherical, smooth–walled, and the length and width of conidia were (3–)3.5–4(–4.5) × (1–)1.5–2(–2.5) μm.

Notes: *Cladosporiodes xylophilum* was reported from only Europe and North America (Bensch *et al.* 2012). This study is the first report from Asia and with a new host plant, *Elaeis guineensis*.

Table 1. Isolates of *Cladosporium* used for DNA analysis and morphological studies.

Species	Accession number	Host
Cladosporium cladosporiodes	AP013	*Hyophorbe verschaffeltii*
	AP014	*Keriodoxa elegans*
	AP023	*Calamus siamensis*
C. cucumerinum	AP021	*Phoenix loureiri*
	AP031	*Zea maize*
	AP030	*Saccharum officinarum*
	AP033	*Equisetum debile*
	AP034	*Livistona chinensis*
C. perangusum	AP001	*Hymenocallis littoralis*
	AP022	*Cymbopogon citratus*
	AP027	*Equisetum debile*
C. psedocladosporiodes	AP024	*Bambusa tulda*
	AP025	*Cyperus rotundus*
	AP026	*Dactyloctenium aegyptium*
	AP004	*Chamaedorea erumpeus*
C. tennuissimum	AP005	*Chamaedorea erumpeus*
	AP006	*Cocos nucifera*
	AP007	*Cocos nucifera*
	AP008	*Cocos nucifera*
	AP009	*Elaeis guineensis*
	AP017	*Livistona chinensis*

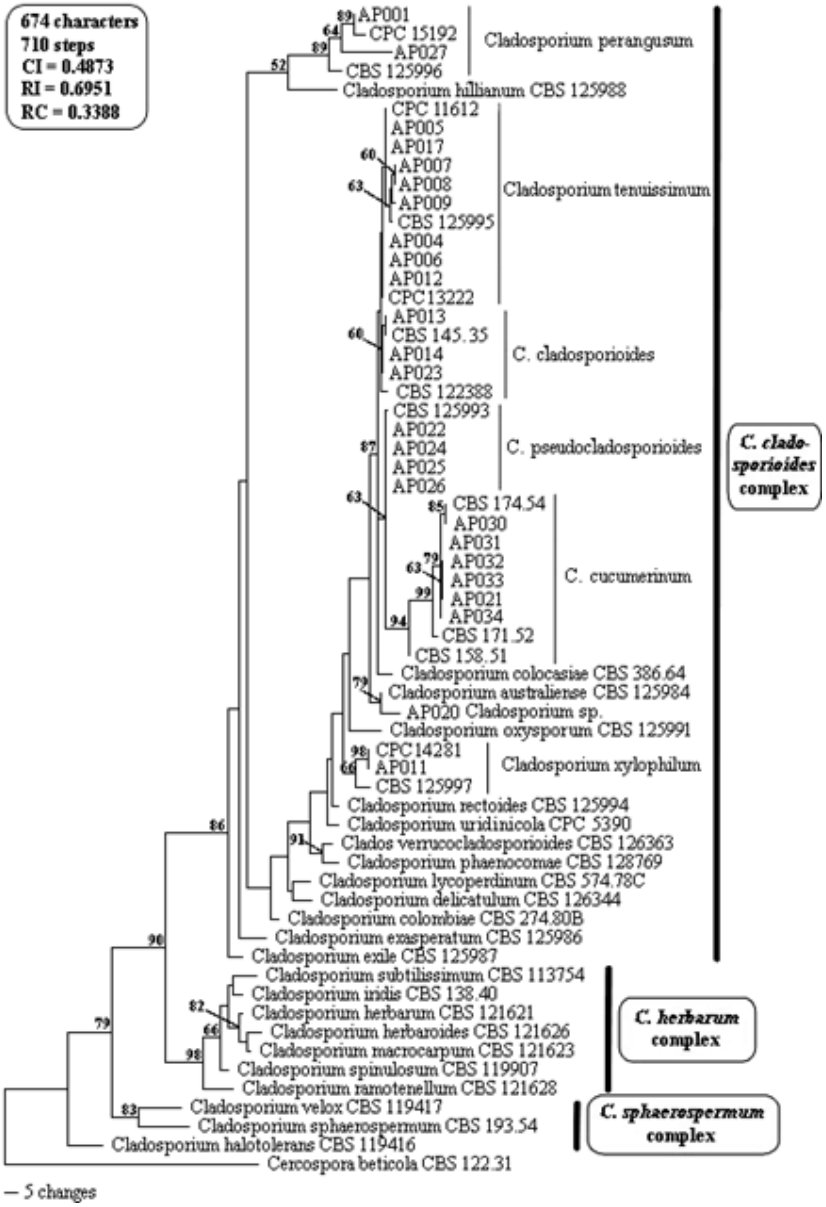

Fig. 1. One of 174 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the combined sequence alignment (ITS and EF). The scale bar shows five changes, and bootstrap support values from 1 000 replicates are shown at the nodes. The tree was rooted to sequences of *Cercospora beticola* (CBS112.31).

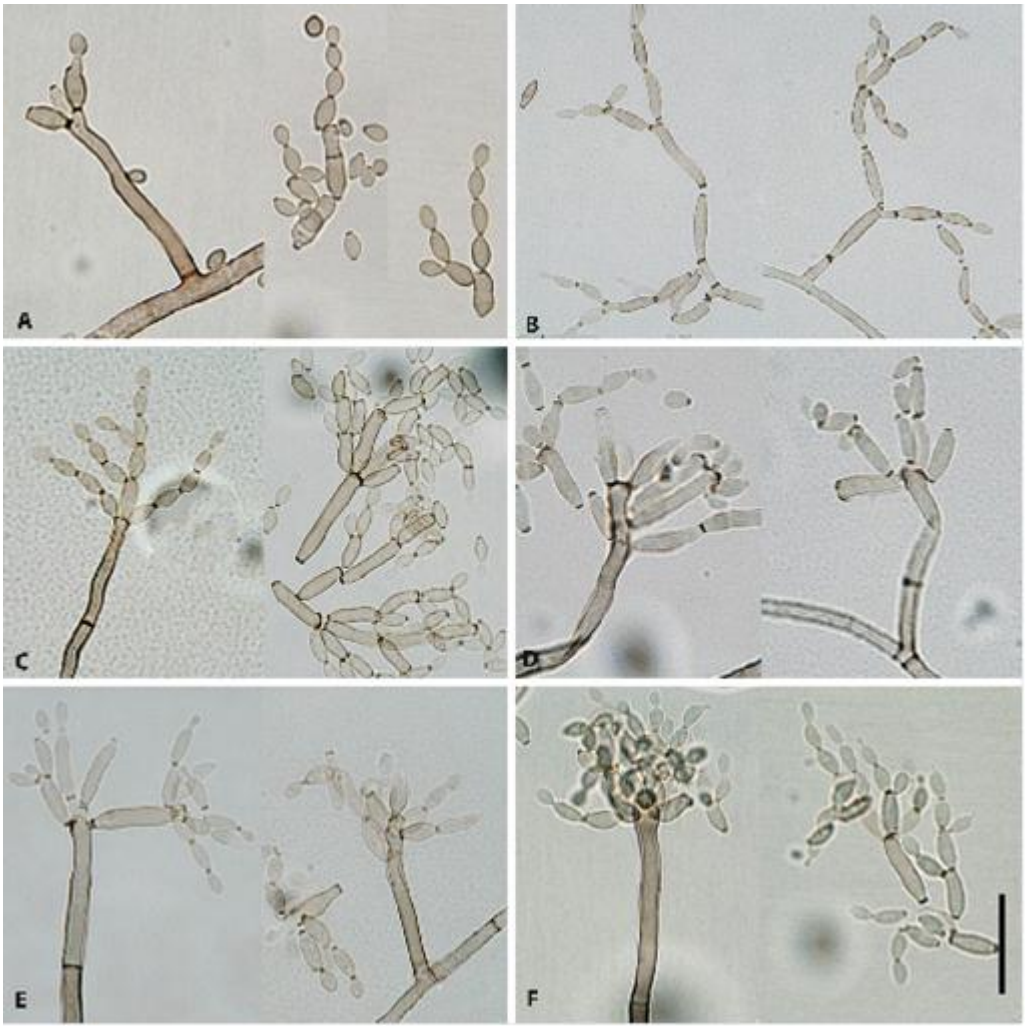

Fig. 2. *Cladosporium* spp. Conidiophores, conidial chains and connidia. *C. cladosporiodes* (A), *C. cucumerinum* (B), *C. perangusum* (C), *C. psedocladosporiodes* (D), *C. tennuissimum* (E) and *C. xylophilum* (F). Scale = 20 (A, C–F), 10 (B) μm.

Acknowledgements

This work was financially supported by the Thailand Research Fund (DBG5380011 and MRG5580163).

References

Agrios, G.N. (2005). Plant Pathology. 5th ed. Academic Press, New York. pp. 922.

Bensch, S., Stjernman, M., Hasselquist, D., Ostman, O., Hansson, B., Westerdahl, H. and Pinheiro, R.T. (2010). Host specificity in avian blood parasites: a study of Plasmodium and Haemoproteus mitochondrial DNA amplified from birds. Proceedings of the Royal Society of London Series B–Biological Sciences 267:1583–1589.

Carbone, I., and Kohn, L.M. (1999). A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91:553-556.

Cheewangkoon, R., Crous, P.W., Hyde, K.D., Groenewald, J.Z. and To–anan, C. (2008). Species of *Mycosphaerella* and related anamorphs on *Eucalyptus* leaves from Thailand. Persoonia 21:77–91.

Crous, P.W., Wingfield, M.J, Park, R.F. (1991). *Mycosphaerella nubilosa* a synonym of M. molleriana. Mycological Research 95:628–632.

Crous, P.W. (1998). *Mycosphaerella* spp. and their anamorphs associated with leaf spot diseases of *Eucalyptus*. Mycologia Memoir 21:1–170

Dugan, F.M., Schubert, K., and Braun, U. (2004). Check–list of *Cladosporium* names. Schlechtendalia 11:1–103.

Felsenstein, J. (1985). Confidence limitsnon phylogenies: an approach using the bootstrap. Evolution 39:783–791.

Hoog, G.S. de, Gerrits, van den Ende A.H.G. (1998). Molecular diagnostics of clinical strains of filamentous basidiomycetes. Mycoses 41:183–189.

Lumyong, P., Photita, W., McKenzie, E.H.C., Hyde, K.D. and Lumyong, S. (2003). Saprobic fungi on dead wild banana. Mycotaxon 85:345–346.

Manoch, L., Tokumasu, S. and Tubaki, K. (1986). A preliminary survey of microfungal flora of pine leaf litter in Thailand. Transactions of the Mycological Society of Japan 27:159–165.

Rambaut, A. (2002). Sequence Alignment Editor. Version 2.0 Department of Zoology, University of Oxford, Oxford, United Kingdom.

Sakayaroj, J., Preedanon, S., Supaphon, O., Jones, E.B.G., and Phongpaichit, S. (2010). Phylogenetic diversity of endophyte assemblages associated with the tropical seagrass Enhalus acoroides in Thailand. Fungal Divers 42:27–45.

Schubert, K., Groenewald, J.Z., Braun, U., Dijksterhuis, J., Starink, M.S., Hill, C.F., Zalar, P., Hoog, G.S. de, and Crous, P.W. (2007). Biodiversity in the *Cladosporium herbarum* complex (*Davidiellaceae*, Capnodiales), with standardisation of methods for *Cladosporium* taxonomy and diagnostics. Studies in Mycology 58:105–156.

Swofford, D.L. (2003). PAUP*. Phylogenetic analysis using parsimony (*and their methods). Version 4. Sinauer Associates, Sunderland, Massachusetts

Tokumasu, S., Sugiyama, M., and Tubaki, K. (1990). Taxonomy of Mortierella ramanniana and related species. Abstracts Fourth International Mycological Congress IMC 4, Regensburg IA–58/3, pp. 58.

Bensch, K., Groenewald, J.Z., Dijksterhuis, J., Starink–Willemse, M., Andersen, B., Summerell, B.A., Shin, H.–D., Dugan, F.M., Schroers, H. –J., Braun, U., and Crous, P.W. (2010). Species and ecological diversity within the Cladosporium cladosporioides complex (Davidiellaceae, Capnodiales). Stud. Mycol. 67:1–96.

Bensch, K., Braun, U., Groenewald, J.Z., and Crous, P.W. (2012). The genus Cladosporium. Studies in Mycology 72:1-401.

Vilgalys, R. and Hester, M. (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. Journal of Bacteriology 172:4238-4246.

(Received 23 April 2013; accepted 30 June 2013)