

Phylogeny of Chaetothyriaceae in northern Thailand including three new species

Putarak Chomnunti

Thida Win Ko Ko

Ekachai Chuksatirote

Institute of Excellence in Fungal Research, School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand; King Saud University, College of Science, Botany and Microbiology Department, P.O. Box 2455, Riyadh 1145, Saudi Arabia

Kevin D. Hyde¹

Lei Cai

Key Laboratory of Systematic Mycology & Lichenology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, P.R. China

E.B. Gareth Jones

BIOTEC Bioresources Technology Unit, National Center for Genetic Engineering and Biotechnology, 113 Thailand Science Park, Pathayothin Road, Khlong Luang, Pathum Thani, Bangkok 12120, Thailand

Rampai Kodsueb

Faculty of Science and Technology, Pibulsongkram Rajabhat University, Phisanulok 65000, Thailand

Bahkali Ali Hassan

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

Hang Chen

Chinese Academy of Forestry, Bailongshi, Kunming City, Yunnan Province 650224, P.R. China

Abstract: In a recent study unusual taxa of epiphyllous ascomycota belonging to Chaetothyriaceae (Eurotiomycetes) were collected in northern Thailand. This family is poorly understood due to morphological confusion and lack of phylogenetic studies. This paper deals with three new species, *Ceratomyrium thailandicum*, *Chaetothyrium brischofiicola* and *Phaeosaccardinula ficus*, which are fully described and illustrated. A DNA sequence analyses of LSU and ITS rDNA genes shows that the new species cluster in the Chaetothyriaceae. This paper adds six sequences for Chaetothyriaceae to GenBank, providing much needed data for the family.

Key words: *Ceratomyrium*, Chaetothyriaceae, *Chaetothyrium*, LSU, *Phaeosaccardinula*, phylogeny

INTRODUCTION

The Chaetothyriaceae are typical of capnodiaceous Dothideomycetes because they form on the surface of leaves and resemble typical sooty molds (Batista and Ciferri 1962). Species of Chaetothyriaceae are mostly epiphytes, colonizing the surface of living leaves with mycelium appressed to the host cuticle without penetrating host tissues (Batista and Ciferri 1962, von Arx and Müller 1975). Ascomata are surrounded by a thin pellicle of superficial mycelium forming black sooty mold-like areas on leaves that are easily detached from the cuticle (Batista and Ciferri 1962). However the ecology of many species of Chaetothyriaceae is poorly studied and it is unclear whether they are saprotrophic or biotrophic (Barr 1987). Members of Chaetothyriaceae often are confused with capnodiaceous sooty molds due to their similar morphology and habitat preferences, however these fungi are never associated with insects such as several Capnodiaceae (Hansford 1946). Sooty molds are a general taxonomic term for capnodiaceous and/or chaetothyriaceous fungi; common genera from both these groups often are found growing together in sooty mold complexes in plant exudates or the sugary honeydew secreted by insects, for example *Aithaloderma* (*Leptoxylum*), *Aureobasidium*, *Capnodium*, *Cladosporium*, *Microxyphium*, *Podosyphium*, *Scorias* and *Trichomerium* (*Tripaspermum*) (Thaung 2006).

Studies on Chaetothyriaceae were conducted mainly by Hansford (1946), Batista and Ciferri (1962), von Arx and Müller (1975) and Hughes (1976), and few studies have been undertaken since. Members of Chaetothyriaceae are primarily tropical species characterized by dark mycelium forming as a loose net of hyphae over the substrate, and they produce ascomata beneath a mycelial pellicle with or without setae (Batista and Ciferri 1962, Hughes 1976, Pereira et al. 2009). The family is poorly circumscribed and most work comprised brief descriptions with line drawings (e.g. Hansford 1946, Batista and Ciferri 1962). The arrangement of genera often seem subjective because individual authors emphasize certain characters, such as spore septation, presence of ascomata setae and mycelium color (Batista and Ciferri 1962, Hughes 1976). Batista and Ciferri (1962) considered the family Chaetothyriaceae to be the type family in order Chaetothyriales. This group share a number of centrum characters with members of the Dothideomycetes, such as the presence of bitunicate

asci, periphysoids and periphysate ostioles. Eriksson (1982) placed the Chaetothyriaceae together with Herpotrichiellaceae in the order Dothideales. Barr (1987) however placed Chaetothyriaceae in the order Chaetothyriales and incorporated eight families. The majority of early systematic studies on Chaetothyriaceae were based on morphological data, and their taxonomic placement remains unclear (Eriksson 1999).

Without sequence data it is difficult to distinguish which taxa belong in Capnodiaceae or Chaetothyriaceae, unless species are identified to genus and thus placed in appropriate orders. Consequently it is important to restudy these poorly known groups with modern molecular techniques (Eriksson 2006). A number of studies (Spatafora et al. 1995, Berbee 1996, Winka et al. 1998, Haase et al. 1999, Untereiner 2000, Badali et al. 2008, Gueidan et al. 2008) made an effort to provide some data relating to the phylogenetic position of this group. Based on the nucleotides data of 18S rDNA gene some authors (Spatafora et al. 1995, Berbee 1996, Winka et al. 1998, Haase et al. 1999) suggested that Chaetothyriales is more closely related to the Eurotiales than to Dothideales, Pleosporales or any other ascostromatic ascomycetes. Winka et al. (1998) transferred Chaetothyriales to the class Eurotiomycetes, and she assumed that the order comprised three families, Chaetothyriaceae, Herpotrichiellaceae and Coccodiniaceae. A combination of protein-coding gene and ribosomal operon data show the black yeasts of the Chaetothyriales align within a subclass, the Chaetothyrionomycetidae (Miadlikowska and Lutzoni 2004), which is sister to the Eurotiomycetidae (Eurotiomycetes) (Lutzoni et al. 2004, Reeb et al. 2004). The most recent study of Schoch et al. (2006) confirmed removal of Chaetothyriales from Dothideomycetes and strongly support their placement in the Eurotiomycetes. Moreover the information from the small subunit rDNA gene showed that *Ceramothyrium linnaeae* (Chaetothyriaceae) appeared to be more closely related to the Herpotrichiellaceae (Winka et al. 1998). The combined ITS rDNA, EF1 and TUB phylogeny showed that a chaetothyriaceous species, *Ceramothyrium carniolicum*, is closely related with the plant-associated *Cladophialophora* species (Herpotrichiellaceae), however the human pathogenic group of *Cladophialophora* clustered with the remaining Herpotrichiellaceae (Badali et al. 2008). In addition *Ceramothyrium carniolicum* clustered together with rock-inhabiting *Capronia peltigerae* (Herpotrichiellaceae) following analysis of combined nuLSU-nucSSU-mitSSU-RPB1 sequences (Untereiner et al. 2011). Nevertheless the phylogenetic position of Chaetothyriaceae and their relationships with other members of Chaetothyriales are still unclear (Untereiner 2000, Badali et al. 2008, Gueidan et al. 2008) because many species of Chaetothyriales are poorly

known and the data from molecular studies of these organisms are still limited.

At present the order Chaetothyriales includes two families of non-lichenized ascomycetes, the Chaetothyriaceae and Herpotrichiellaceae (Geiser et al. 2006, Kirk et al. 2008). Thirteen genera are included in Chaetothyriaceae (Kirk et al. 2008). Anamorphs are known only for two of these genera; *Merismella* Syd. is the anamorph of *Chaetothyrium* Speg. and *Stanhughesia* Constant. is the anamorph of *Ceramothyrium* Bat. & Maia (Hyde et al. 2011). Other chaetothyriaceous anamorphs without known teleomorphs are *Vonarxia* Bat. 1960 and *Cyphellophora* G.A. de Vries 1962.

The purpose of this study is to represent the taxonomic placement of Chaetothyriaceae species from northern Thailand with morphology and phylogeny, and we describe three species that are new to science.

MATERIALS AND METHODS

Isolates and morphology.—Sites were visited throughout the provinces of Chiang Mai and Chiang Rai in northern Thailand, and leaves of various plants with sooty mold-like colonization were collected. Material was returned to the laboratory in plastic bags. Sections of ascomata were made free hand and mounted in lactophenol; morphological characteristics of material mounted in water were observed with a Nikon80i DIC microscope (measurements made with Tarosoft image framework). Melzer's reagent was used to test the amyloidity of the apical ring, and India ink was used to reveal the mucilaginous sheath. Initial isolation used singles spores to obtain pure cultures (Choi et al. 1999), and isolates were grown on 2% Difco potato dextrose agar (PDA) at 28 C, 12 h light/12 h dark for routine maintenance. Colony color and characteristics were assessed after 4 wk, and the material was used for molecular study. The holotypes are deposited at the Mae Fah Luang University (MFLU) Herbarium, Chiang Rai, Thailand. Cultures are deposited at Mae Fah Luang University Culture Collection (MFLUCC) and BIOTEC Culture Collection (BCC).

DNA isolation, amplification and sequencing.—DNA was extracted from mycelium with Biospin Fungus Genomic DNA Extraction Kit (BioFlux®) according to the manufacturer's protocol for maximum yield. Partial 28S rDNA regions were amplified with primers LR0R and LR6 (Vilgalys and Hester 1990). Internal transcribed spacers (ITS) region including 5.8S of the nuclear ribosomal DNA were amplified with primers ITS4 and ITS5 (White et al. 1990). Amplification reaction mixtures contained 50 ng template DNA, PCR Master Mix 1X, 0.5 µM each primer in a 25 µL volume, 50 U Taq DNA polymerase, 400 µM each dNTP, 3 mM MgCl₂. Amplification was performed in a GeneAmp PCR System under these conditions: initial denaturation at 94 C for 5 min, followed by 35 cycles each consisting of 1 min denaturation at 94 C, annealing for 30 s at 55 C and extension at 72 C for 1 min and a final extension at 72 C for 5 min. To check homogeneity and size of amplicons PCR products were run on 1.5% agarose gels

(BIO-RAD) containing ethidium bromide as the staining agent. DNA sequencing reaction was performed with BigDye Terminator 3.1 cycle sequencing kit (Applied Biosystems, Foster City, California). Electrophoresis of purified sequencing product was performed on ABI-PRISM3730 DNA Analyzer (Applied Biosystems).

Sequence alignments and phylogenetic analysis.—DNA sequences were aligned with BioEdit (Hall 1999) and Clustal X 1.83 (Thompson et al. 1997) with available sequences of the Capnodiaceae, Chaetothyriaceae, Herpotrichiellaceae and Verrucariaceae from GenBank. Details of sequences are provided herein (TABLE I). *Teratosphaeria suberosa* was selected as outgroup. Phylogenetic analysis were performed with PAUP* 4.0b10 (Swofford 2002) for each gene separately. Maximum parsimony (MP) analyses were performed with the heuristic search option on and 1000 random taxa addition with a stepwise starting tree, tree bisection and reconnection (TBR) as the branch-swapping algorithm. Gaps were treated as missing data, and ambiguously aligned regions were excluded from analyses. Clade stability was estimated in a bootstrap (BT) analysis with 1000 replicates (Hillis and Bull 1993). Other measures were consistency index (CI), retention index (RI) and homoplasy index (HI).

Bayesian analyses employing a Markov chain Monte Carlo method were performed in MrBayes 3.0b4 (Ronquist and Huelsenbeck 2003) for combined gene dataset. The most suitable substitution models for the respective datasets were selected with MrModeltest 2.2 (Nylander 2004). The Bayesian analyses were conducted with the Markov chains run 1 000 000 generations and trees were sampled every 100 generations (Cai et al. 2006). Trees were visualized in TreeView (Page 1996). Sequences generated in this study have been deposited in GenBank (HQ895835, HQ895836, HQ895837) and alignments in TreeBASE (TB2:S11868).

RESULTS

Phylogenetic analysis.—The aligned LSU rDNA sequences of approximately 860 bases were obtained from isolates provided herein (TABLE I). We excluded 18 base pairs that did not align well from the analysis. Of the remaining 834 characters used in analysis 592 were constant, 35 were variable and parsimony uninformative and 207 were parsimony informative. A heuristic search found 100 equally parsimonious trees with the length (TL) of 603 steps (CI = 0.574, RI = 0.844, RC = 0.485, HI = 0.426). The overall topology of the 50% majority rule consensus tree of 10 000 trees sampled during the Bayesian analysis was similar to the MP tree. The MP tree is illustrated herein (FIG. 1) with bootstrap support above branches and Bayesian values marked below branches. The phylogenetic hypothesis (FIG. 1) highly supports three monophyletic groups, Capnodiaceae (Capnodiales), Verrucariaceae (Verrucariales) and Herpotrichiellaceae/Chaetothyriaceae (Chaetothyriales). The sequences of the three new species described here

cluster within the Chaetothyriaceae clade that includes *Ceramothyrium carniolicum*, *Cyphellophora eugeniae*, *Cyphellophora hylomeconis* and *Vonarxia vagans*. They also are unrelated to sequences of species of Capnodiaceae (Capnodiales) (FIG. 1). The placement of *Ceramothyrium thailandicum* and *Phaeosaccardinula ficus* in this lineage received bootstrap support of 68% and 82% and Bayesian posterior probabilities of 0.96 and 1.00, while *C. thailandicum* clusters in a subclade with *C. carniolicum* with significant support of 0.96 Bayesian posterior probabilities. The aligned ITS rDNA sequences of approximately 600 bases were obtained from isolates listed herein (TABLE I) as well. We aligned ITS rDNA sequences to confirm that the result of three new specimens were closely related to Chaetothyriaceae. We excluded 196 base pairs that did not align well in the analysis. Of the remaining 410 characters 216 were constant, 34 were variable and parsimony uninformative and 160 were parsimony informative. A heuristic search found 100 equally parsimonious trees with the length (TL) of 527 steps (CI = 0.583, RI = 0.655, RC = 0.382, HI = 0.417). The overall topology of the 50% majority rule consensus tree of 10 000 trees sampled during the Bayesian analysis was similar to the MP tree. The MP tree is illustrated (FIG. 2) with bootstrap support above the branches. The sequences of the three new species described here cluster in Chaetothyriaceae (FIG. 2).

Ceramothyrium thailandicum Chomnunti & K.D.

Hyde sp. nov.

FIG. 3

Mycobank MB519683

Ascomata 200–255 µm diam × 100–160 µm alta, superficialia, uno vel dispersi, brunnea ad pallida brunnea et tenuiter viridula. Peridium 12–19 µm latum, hyalinum, brunneum ad atra brunneum in basi texturae angularis, cum compressa texturae prismatica in latus. Asci 70–96 × 39–53 µm, 8-spori bitunicati, clavati vel pyriformes, denique pedicellati, masime evanescens. Ascospores 24.7–35.5 × 5.7–8.7 µm, 3–5-seriata, hyalinae, cylindric-clavatae, 7–9 transversus septatae, constricta in septatae, circum mucilagina vaginae.

Etymology: in reference to its occurrence in Thailand.

Habit on living leaves of *Lagerstroemia* sp., covering the upper leaf surface with dark mycelium without penetrating host tissues, leaves remaining green and healthy. Mycelial mat subiculum-like, comprising hyphae that are mostly narrow, 3.1–4.6 µm wide ($\bar{x}$ = 3.7 µm, n = 20); brown or brownish green at margin, slightly constricted at the septa, dense, radiating outward, anastomosing at their tips with cells of the hyphal network. Ascomata 200–255 µm diam, 100–160 µm high ($\bar{x}$ = 255 × 128 µm,

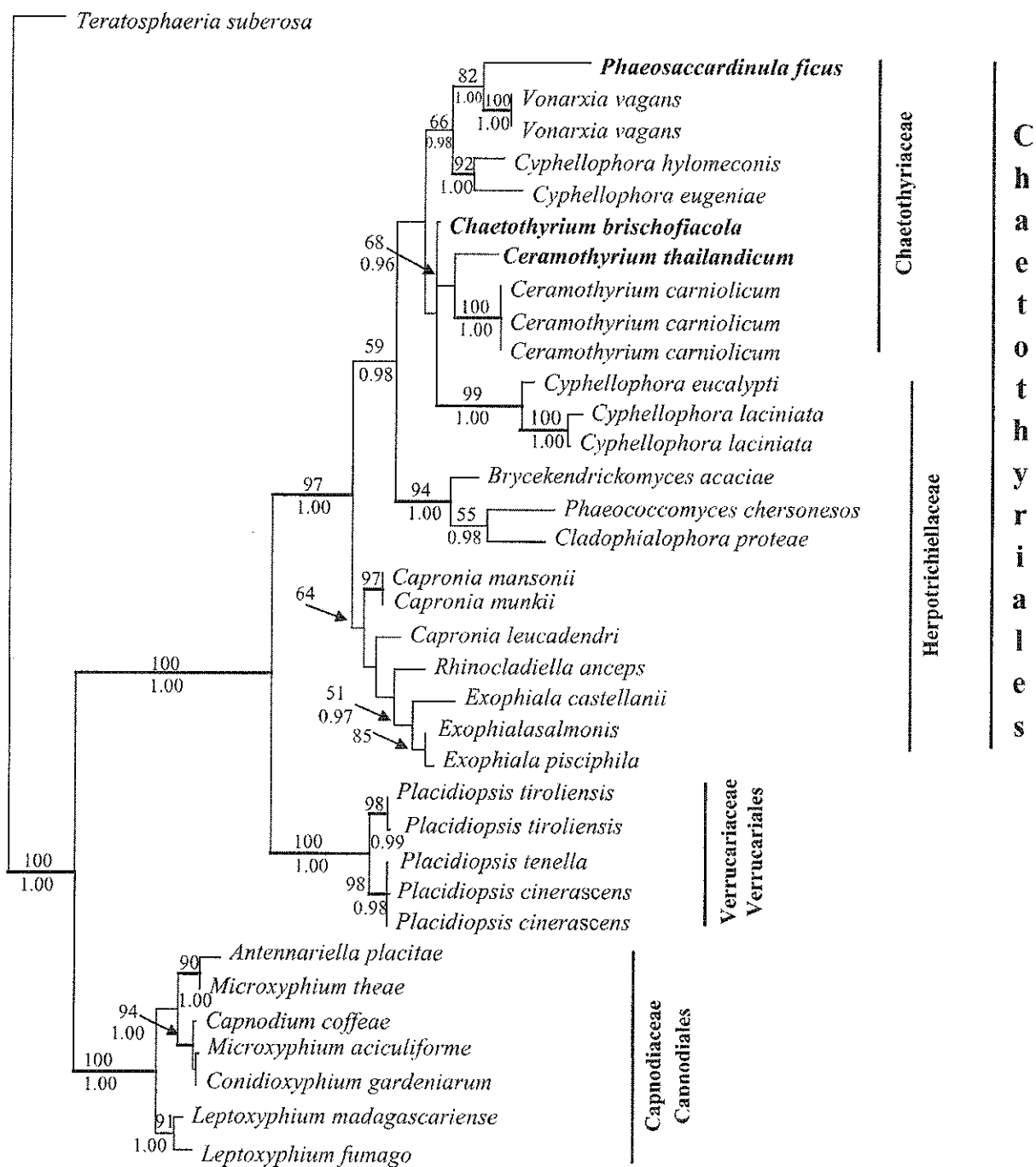
TABLE 1. LSU rDNA sequences included in this analysis

Species	Strain number/ voucher	Host	References	GenBank accession number ^a	
				LSU	ITS
<i>Antennariella placitae</i>	CBS 124785	<i>Eucalyptus placita</i>	Cheewangkoon et al. (2009)	GQ303299	GQ303268
<i>Brycekendrickomyces acaciae</i>	CBS 124104	<i>Acacia auriculiformis</i>	Crous et al. (2009a)	FJ839641	FJ839606
<i>Capnodium coffeae</i>	CBS 147.52	<i>Coffea robusta</i>	Schoch et al. (2006)	DQ247800	AJ244239
<i>Capronia leucadendri</i>	CBS 122672	<i>Leucadendron</i> sp.	Marincowitz et al. (2008)	EU552108	—
<i>Capronia mansonii</i>	CBS 101.67	<i>Populus tremula</i>	Untereiner et al. (1999)	AY004338	AF050247
<i>Capronia munkii</i>	DAOM 216390	<i>Populus tremuloides</i>	Gueidan et al. (2006)	EF413604	EF050250
<i>Ceramothyrium carniolicum</i>	CBS 175.95	<i>Pyrola rotundifolia</i>	Lumbsch et al. (2000)	AY004339	—
<i>Ceramothyrium carniolicum</i>	CBS 175.95	<i>Pyrola rotundifolia</i>	Geiser et al. (2006)	EF413628	—
<i>Ceramothyrium carniolicum</i>	CBS 175.95	<i>Pyrola rotundifolia</i>	Gueidan et al. (2008)	FJ358232	—
<i>Ceramothyrium thailandicum</i>	MFLU (CC) 10-0079	<i>Lagerstroemia</i> sp.	This study	HQ895835	HQ895838
<i>Chaetothyrium brischofiacola</i>	MFLU (CC) 10-0083	<i>Brischofia javanica</i>	This study	HQ895836	HQ895839
<i>Cladophialophora proteae</i>	CBS 111667	<i>Protea cynaroides</i>	Crous et al. (2007)	EU035411	EU035411
<i>Conidioxyphium gardeniarum</i>	CPC 14327	<i>Gardenia jasminoides</i>	Schoch et al. (2009)	GU301807	—
<i>Cyphellophora eucalypti</i>	CBS 124764	<i>Eucalyptus</i> sp.	Cheewangkoon et al. (2009)	GQ303305	GQ303274
<i>Cyphellophora eugeniae</i>	CBS 124105	<i>Eugenia uniflora</i>	Crous et al. (2009a)	FJ839652	FJ839617
<i>Cyphellophora hylomeconis</i>	CBS 113311	<i>Helomeco velane</i>	Crous et al. (2007)	EU035415	—
<i>Cyphellophora laciniata</i>	CBS 190.61	<i>Homo sapiens</i>	Crous et al. (2007)	FJ358239	—
<i>Cyphellophora laciniata</i>	CBS 190.61	<i>Homo sapiens</i>	Crous et al. (2007)	EU035416	EU035416
<i>Exophiala castellanii</i>	CBS 158.58	<i>Homo sapiens</i>	Gueidan et al. (2008)	FJ358241	—
<i>Exophiala pisciphila</i>	CBS 537.73	<i>Ictalurus punctatus</i>	Gueidan et al. (2008)	DQ823101	DQ826739
<i>Exophiala salmonis</i>	CBS157.67/ CBS120274	<i>Salmo clarkii</i>	Rakeman et al. (2005)	AY213702	JF747138
<i>Leptoxypium fumago</i>	CBS 123.26	<i>Hibiscus tiliaceus</i>	Schoch et al. (2009)	GU214430	—
<i>Leptoxypium madagascariense</i>	CBS 124766	<i>Eucalyptus camaldulensis</i>	Cheewangkoon (2009)	GQ303308	GQ303277
<i>Microxyphium aciculiforme</i>	CBS 892.73	<i>Polyscias guilfoylei</i>	Schoch et al. (2009)	GU301847	—
<i>Microxyphium theae</i>	CBS 202.30	<i>Thea sinensis</i>	Schoch et al. (2009)	GU301849	—
<i>Phaeococcomyces chersonesos</i>	IMI 389175	Marble	Bogomolova and Minter (2003)	AJ507323	—
<i>Phaeosaccardinula ficus</i>	MFLU (CC) 10-0080	<i>Ficus</i> sp.	This study	HQ895837	HQ895840
<i>Placidiosis cinerascens</i>	MA 16304	—	Prieto et al. (2010)	GQ344571	—
<i>Placidiosis cinerascens</i>	MA 16308	—	Prieto et al. (2010)	GQ344570	—
<i>Placidiosis tenella</i>	LI 281352	—	Prieto et al. (2010)	GQ344575	GQ344616
<i>Placidiosis tirolensis</i>	LI281349	on soil	Prieto et al. (2010)	GQ344581	GQ344618
<i>Placidiosis tirolensis</i>	LI 281351	on soil	Prieto et al. (2010)	GQ344580	—
<i>Rhinocladiella anceps</i>	CBS 157.54	<i>Diatrype tremellophora</i>	Arzanlou et al. (2007)	EU041861	EU041804
<i>Teratosphaeria suberosa</i>	CPC 11032	<i>Eucalyptus dunni</i>	Crous et al. (2009c)	GQ852718	DQ303044
<i>Vonarxia vagans</i>	CBS 123533	<i>Stenocalyx uniflorus</i>	Crous et al. (2009a)	FJ839672	FJ839636
<i>Vonarxia vagans</i>	CPC 15152	<i>Stenocalyx uniflorus</i>	Crous et al. (2009a)	FJ839673	FJ839637

^aLSU: 28S rDNA. New sequences generated are in bold. ATCC: American Type Culture Collection, Virginia, USA. CBS: CBS Fungal Biodiversity Centre, Utrecht, The Netherlands; CPC: Culture collection of P.W. Crous, housed at CBS. DAOM: Canadian Collection of Fungus Cultures. IMI: the CABI Bioscience Culture Collection. LI: the Oberösterreichisches Landesmuseum of Linz. MA: Herbarium, Real Jardín Botánico, Spain. MFLU: Mae Fah Luang University.

n = 5); superficial, solitary or scattered, pale brown to greenish brown, setae absent, in vertical section globose to subglobose, somewhat flattened when dry, covered by a subiculum or layer, composed of a few hyaline to subhyaline, septate hyphae, with a

circumferential space around the maturing ascomata, which results from rising of the ascomata. Toward the upper part of the ascomata wall the pellicle is tightly packed. Peridium 12–19 µm wide ($\bar{x}$ = 15.3 µm, n = 20); hyaline, cells brown to dark brown at the base of



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FIG. 1. Phylogenetic relationships among 7, 5 and 23 members of Capnodiales, Verrucariales and Chaetothyriales respectively, inferred by maximum parsimony and Bayesian analyses of LSU rDNA sequences. MP bootstrap values $\geq 50\%$ and Bayesian values ≥ 0.95 are shown above and below the branches respectively. The specimens isolated in this study are in boldface.

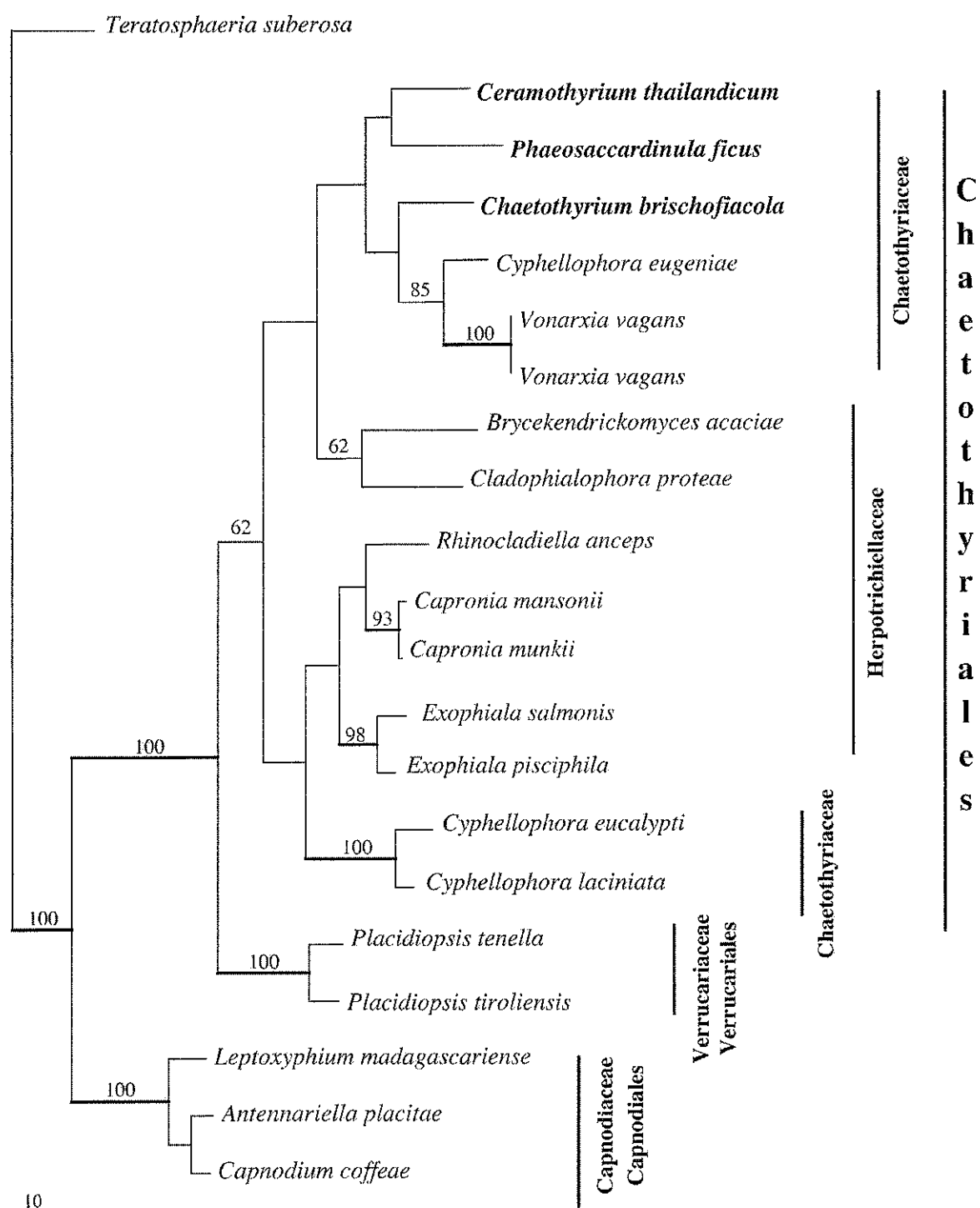


FIG. 2. Phylogenetic relationships among Capnodiales, Verrucariales and Chaetothyriales respectively, inferred by maximum parsimony of ITS rDNA sequences. MP bootstrap values $\geq 50\%$ are shown above. The specimens isolated in this study are in boldface.

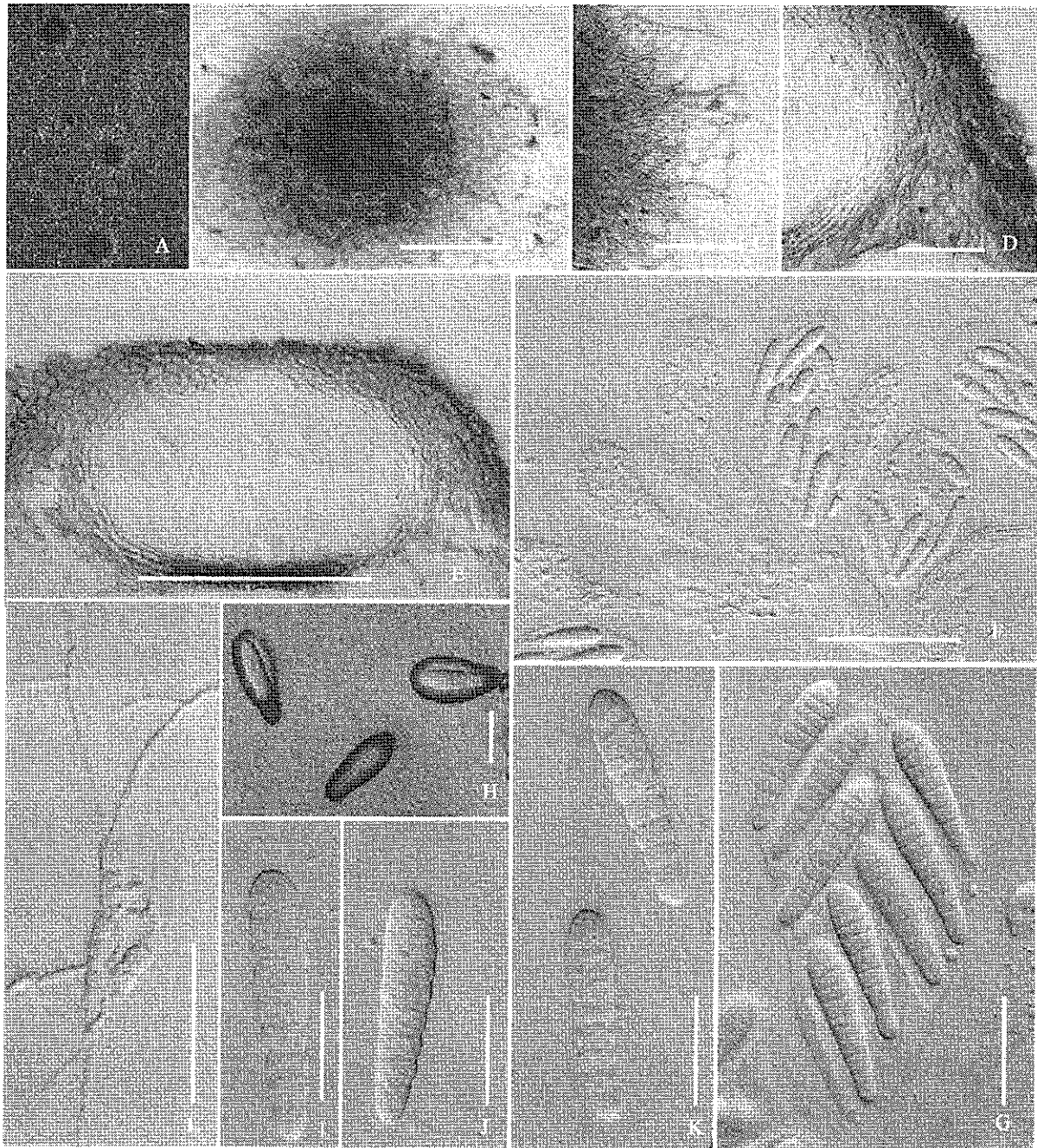


FIG. 3. *Ceramothyrium thailandicum* (holotype). A. Habit of ascomata on the host surface. B. Ascomata covered by a subiculum or layer. C. Margin of ascoma with hyphae attaching it to the host surface. D. Vertical section through ascomata. Note the wedge of hyphal tissue between the lower part of ascoma and the host surface. E. Section of ascoma. Note the peridium that comprises textura angularis at the outside and flattened cells toward the inner region. The ascoma is covered with a hyphal mat that is also pseudoparachymatous, and there is a space between the lower part of the ascoma and the hyphal covering. F. H. Bitunicate asci. G. Ascospores in Indian ink revealing the mucilaginous sheath. I-K. Ascospores that are cylindro-clavate with 7-9 septa. L. Germinating ascospores with germ tubes growing from apex and base and occasionally center. Bars: A, D = 100 µm; B, C, E, K = 50 µm; F-J = 20 µm.

textura angularis with compressed textura prismatica at the sides. Hamathecium of cellular, hyaline, pseudoparaphyses. Asci $70\text{--}96 \times 39\text{--}53 \mu\text{m}$ ($\bar{x} = 82 \times 44 \mu\text{m}$, $n = 15$), eight-spored, bitunicate, clavate or pyriform, short pedicellate, mostly evanescent. Ascospores $24.7\text{--}35.5 \times 5.7\text{--}8.7 \mu\text{m}$ ($\bar{x} = 30.3 \times 6.8 \mu\text{m}$, $n = 30$), overlapping 3–5-seriate, hyaline, cylindro-clavate, with 7–9 transverse septa, constricted at the septa, smooth-walled; surrounded by a 3–5 μm wide mucilaginous sheath; germinating mostly from apex and base of ascospores, a few also germinating from the central cells.

Culture characteristics: Colonies slow growing, reaching 2 cm diam after 20 d on PDA, colony erumpent, surface dull, velvety with a folded, radiating, wavy margin, shiny, brownish gray, and olivaceous at margin, grayish sparse aerial mycelium outer region, reverse dark, sunken to the surface of media (FIG. 6D). No anamorph was produced in PDA after 60 d incubation.

Material examined: THAILAND, Chiang Mai Province, Doi Suthep, medicinal garden, on living leaves of *Lagerstroemia* sp. (Lythraceae), 5 Nov 2009, Putarak Chomnunti, DPC043, (HOLOTYPE, MFLU10-0008) ex-type living culture MFLU (CC) 10-0079, and BCC same locality and host plant; same locality and host plant, 18 Sep 2010, Putarak Chomnunti (PARATYPE, MFLU11-0005); same locality and host plant, 10 Oct 2010, Liu Jian Kui, (PARATYPE, MFLU11-0006).

Notes: Batista and Maia (1956), and Hughes (1976) described several species of *Ceramothyrium* that lack setae. Species of *Ceramothyrium* are characterized by ascomata that are covered by a subiculum or layer, with a circumferential space around the maturing ascomata. In species of *Phaeosaccardinula* ascomata are formed beneath a layer that attaches the ascomata to the host surface. Ascospores of *Phaeosaccardinula* are also muriform, but this is never the case in *Ceramothyrium*. The genus presently includes 20 species (Kirk et al. 2008). *Ceramothyrium thailandicum* is distinct by having mostly eight-septate, clavate ascospores with a distinct mucilaginous sheath and the number of septa (Hansford 1946, Batista and Ciferri 1962). Species with clavate ascospores include *C. boedijnii*, *C. europaeum* and *C. calycanthi*. *Ceramothyrium thailandicum* differs from *C. boedijnii* and *C. europaeum* in the size of ascomata, asci and ascospores, the former having larger ascomata, longer asci and ascospores with 7–9 septa and the latter two have three and two septa respectively. *Ceramothyrium thailandicum* differs from *C. calycanthi* by smaller asci and ascomata with thicker peridium (35–40 versus 12–19 μm) composed of subglobose and polygonal texture while *C. thailandicum* has a textura prismatica.

***Chaetothyrium brischoficola* Chomnunti & K.D.**

Hyde sp. nov.

FIG. 4

MycoBank MB519828

Ascomata 130–145 μm diam $\times$ 89–101 μm alta, cupulata in siccitate, subglobose, brunnea, cum setae. Asci 58–74 $\times$ 21–45 μm , 8-spore, bitunicati, clavati vel ellipsoidei, breve pedicellati. Ascosporeae 17–27 $\times$ 5.3–9.7 μm , 3–4-seriate, oboviodae, hyalinae, 4–6 transversus septatae, 2–4 longitudinalis septatae.

Etymology: from the host *Brischofia* and Latin *cola*, meaning “dwelling on”, in reference to the occurrence on the host.

Fungus appearing as black dots scattered on the upper surface of *Brischofia javanica* leaves and also dark mycelium cover the surface of leaves that become blackened, mycelium superficial, pale brown to hyaline, hyphae reticulate-branched, constricted, pale brown to hyaline 3.4–5.5 μm thick ($\bar{x} = 4.5 \mu\text{m}$, $n = 20$). Ascomata developing beneath a brown layer attaching it to the leaf surface, cupulate on drying; ostiole or papilla not apparent and method of spore release not established, in vertical section subglobose, 130–145 μm diam, 89–101 μm high ($\bar{x} = 140 \times 97 \mu\text{m}$, $n = 5$), brown to dark brown, scattered Ascomatal setae 10–12, scattered, dark brown at base, brownish at the tip, erect, rigid 47–68 $\times$ 5–6 μm ($\bar{x} = 60 \times 5 \mu\text{m}$, $n = 20$). Peridium membranous, 10–14 μm thick ($\bar{x} = 12 \mu\text{m}$, $n = 20$) pseudoparenchymatous, composed of textura angularis and textura prismatica in the outer region, becoming light brown to hyaline and flattened in the inner region. Asci 58–74 $\times$ 21–45 μm ($\bar{x} = 65 \times 31 \mu\text{m}$, $n = 10$), eight-spored, bitunicate, clavate to ellipsoid, short pedicellate or sessile with J-apical ring 4.9–7.1 $\times$ 2.3–3.6 μm ($\bar{x} = 5.8 \times 3 \mu\text{m}$, $n = 10$). Ascospores 17–27 $\times$ 5.3–9.7 μm ($\bar{x} = 6.6 \times 20 \mu\text{m}$, $n = 10$), 3–4-seriate, obovoid, hyaline, with 4–6 transverse septa, some with 2–4 longitudinal septa, constricted at the septum germinating from apical and central cells.

Culture characteristics: Colonies on PDA growing to 15 cm diam after 20 d, flattened and surface brown to pale brown, spreading smooth margins with sparse aerial mycelium, olivaceous green at margin, reverse iron gray to brown (FIG. 6A, B). No anamorph was produced in PDA after 60 d incubation.

Material examined: THAILAND, Chiang Rai Province, Khunkorn water fall, on living leaf of *Brischofia javanica* (Phyllanthaceae), 18 Dec 2009, Putarak Chomnunti, DPC047, (HOLOTYPE, MFLU10-0012) ex-type living culture MFLU(CC)10-0083 and BCC same locality and host plant species; same locality and host plant species, 30 Aug 2010, Liu Jian Kui (PARATYPE, MFLU11-0009); same locality and host plant species, 21 Sep 2010, Putarak Chomnunti (PARATYPE, MFLU11-0010).

Notes: The genus *Chaetothyrium* Speg. is characterized by having setae on the ascomata as well as

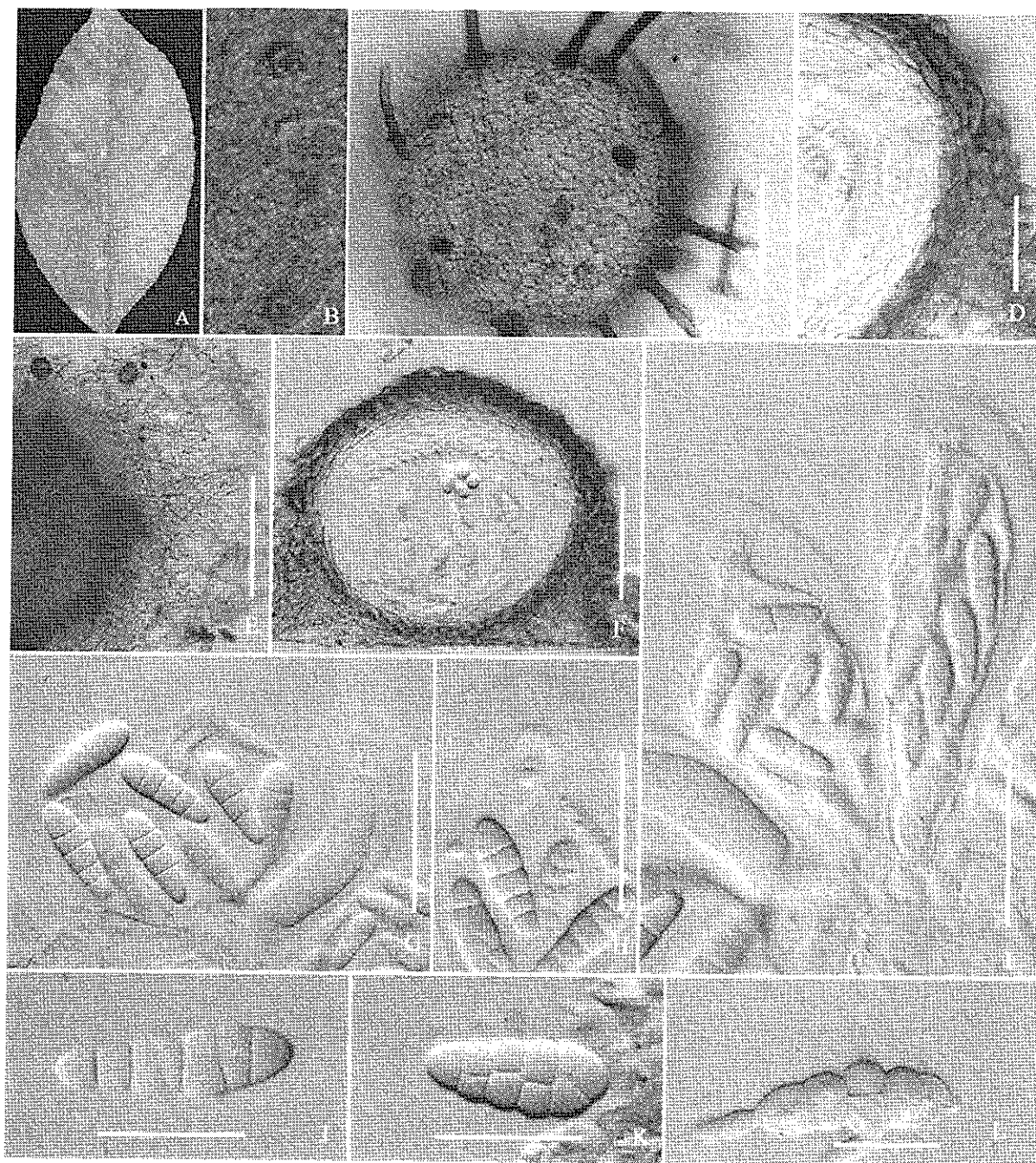


FIG. 4. *Chaetothyrium brischoficola* (holotype). A. Appearance of leaves colonized by the fungus. B. Cupulate ascomata on living leaves of *Brischofia javanica*. C. Globose ascoma with black setae. D. Section of peridium that comprises brown-walled cells of *textura angularis* at the outer region and light brown to hyaline flattened cells in the inner region. E. Ascomata removed from leaf showing mycelial network attaching ascoma to the leaf surface. F. Vertical section of ascoma. Note the wedge of mycelial tissue attaching the ascoma to the host surface. G–I. Subglobose asci with apical ring. J–K. Ascospores with transverse and longitudinal septa. L. Germinating ascospore. Germ tubes grow from apical and central cells. Bars: C–F = 50 μ m; G, H, I, J, K, L = 20 μ m.

scattered on the host around the ascomata (Batista and Ciferri 1962). The genus includes 26 species (Kirk et al. 2008). *C. brischoficola* differs from other species by having obovoid ascospores with transverse septa and occasionally longitudinal septa. In this respect the species might be better placed in *Treubiomyces*, which has cylindrical oblong, muriform ascospores (Batista 1962, Hughes 1976). Many of the ascospores in our species however lacked longitudinal septa and are obovoid. Because this species might be borderline between *Chaetothyrium* and *Treubiomyces* we place it in former genus, the type of the family. *C. brischoficola* differs from other species in the genus in having a distinct apical ring and ascospores that occasionally have longitudinal septa. We examined type material of specimens of *Chaetothyrium* at K (i.e. *C. boedijnii* Hansf., *C. capense* [Doidge] Hansf., *C. concinnum* Syd., *C. fusisporum* L.R. Fraser., *C. mangiferae* Bat. and I.H. Lima., *C. permixtum* Syd., *C. roseum* Hansf., *C. sorsorensis* Hansf. and Thirum. and *C. ugandense* Hansf.) and none of these species has an apical ring in the ascus. However we do not think that these characters warrant the introduction of a new genus at this time so this taxon is included in *Chaetothyrium*. Future studies should include more species of *Chaetothyrium* to verify whether the genus is a monophyletic group or if the species with longitudinal septa and an ascus ring are phylogenetically distant.

***Phaeosaccardinula ficus* Chomnunti & K.D. Hyde**
sp. nov.

FIG. 5

Mycobank MB519829

Ascomata scattered, cupulata in siccitate, 188–226 μm diam $\times$ 116–193 μm alta, superficialia, globosa, nigra, setae nulla, cum medius ostiolum 30–36 $\times$ 25–31 μm . Asci 120–185 $\times$ 49–64 μm , bitunicati, puriformes ad clavati et paene oboviodei ad ellipsoideae, sessile vel denique pedicellati. Ascospores 33–56 $\times$ 10–17 μm , 2–4-seriatae, hyalinae, oblonga-ellipsoideae, muriformes, 7–8 transversus septatae and 6–8 longitudinalis septatae, constricta in septae.

Etymology: in reference to its occurrence on *Ficus* sp.

Mycelium forming sparse covering on host surface, hyphae brownish and pale brown, constricted at the septa, 3.7–5.4 μm wide ($\bar{x}$ = 4.4 μm , n = 20). Ascomata scattered on upper surface of living leaves of *Ficus* sp., cupulate when dry, 188–226 μm diam, 116–193 μm high ($\bar{x}$ = 209 $\times$ 174 μm , n = 10), superficial, globose, dull black, lacking setae, with a central ostiole that has a white center, forming beneath a layer that attaches the ascomata to the host surface. Ostiole 30–36 $\times$ 25–31 μm ($\bar{x}$ = 33 $\times$ 28 μm , n = 5); present in mature ascomata. Peridium 20–32 μm wide ($\bar{x}$ = 27 μm , n = 30), with 2–3 strata with the spine on the surface, glabrous, comprising

textura globulosa at the outside to textura angularis and flattened inwardly hyaline of textura prismatica. Pseudoparaphyses hyaline, nonseptate. Asci 120–185 $\times$ 49–64 μm ($\bar{x}$ = 148 $\times$ 55 μm , n = 10), eight-spored, bitunicate, pyriform to clavate and almost obovoid to ellipsoid, sessile or short pedicellate, early evanescent, lacking an ocular chamber. Ascospores 33–56 $\times$ 10–17 μm ($\bar{x}$ = 41 $\times$ 13 μm , n = 50), overlapping 2–4-seriate, hyaline, olivaceous green at the septa of mature ascospores, oblong-ellipsoid, muriform, with 7–8 transversal septa and 6–8 longitudinal septa, constricted at the septum, with mucilaginous sheath (absent in some mature ascospores) germ tubes developing from numerous cells.

Culture characteristics: Colonies on PDA erumpent, growing slowly, 2 cm diam after 20 d, spreading, with folded, velvety, wavy margin and water droplets forming on the surface, with dark mycelium, olivaceous green at margin, white to grayish sparse aerial mycelium covering colony, reverse dark, sunken (FIG. 6C). No anamorph was produced on PDA after 60 d incubation.

Material examined: THAILAND, Chiang Rai Province, Baan Du, on living leaf of *Ficus* sp. (Moraceae), 6 Nov 2009, K.D. Hyde, DPC044, (HOLOTYPE, MFLU10-0009) ex-type living culture in MFLU(CC)10-0080 and BCC same locality and host plant; same locality and host plant, 25 Jan 2011, Putarak Chomnunti (PARATYPE, MFLU11-0007, MFLU11-0008).

Notes: The genus *Phaeosaccardinula* contains 14 species. (Kirk et al. 2008) and is characterized by the presence of superficial, globose or flattened-globose, dark, non-setulose ascomata, and muriform, hyaline to brownish ascospores. The specimen found on *Ficus* sp. is most similar to *P. dictyosporum* and *P. javanica*. *P. javanica* can be distinguished from *P. ficus* by the larger ascomata (300 $\times$ 250 μm) and smaller ascospores (30–35 $\times$ 8–15 μm). *P. dictyosporum* is different from *P. ficus* due to a tessellate scutellum (750 μm) with a clypeus-like margin (Eriksson and Jingzhu 1985) and relatively longer and wider ascospores (35–61 $\times$ 16–24 μm). Mature ascospores of *P. ficus* are olivaceous green at the septa and have a mucilaginous sheath, while ascospores of *P. javanica* are ellipsoid to fusoid with rounded ends, without a mucilaginous sheath (Hansford 1946).

DISCUSSION

In the present study we provide detailed descriptions of three new species of Chaetothyriaceae illustrated with photomicrographs and refer to their taxonomic placement as inferred from LSU sequence analysis. *Ceratomyrium thailandicum*, *Chaetothyrium brischoficola* and *Phaeosaccardinula ficus* are chaetothyriaceous

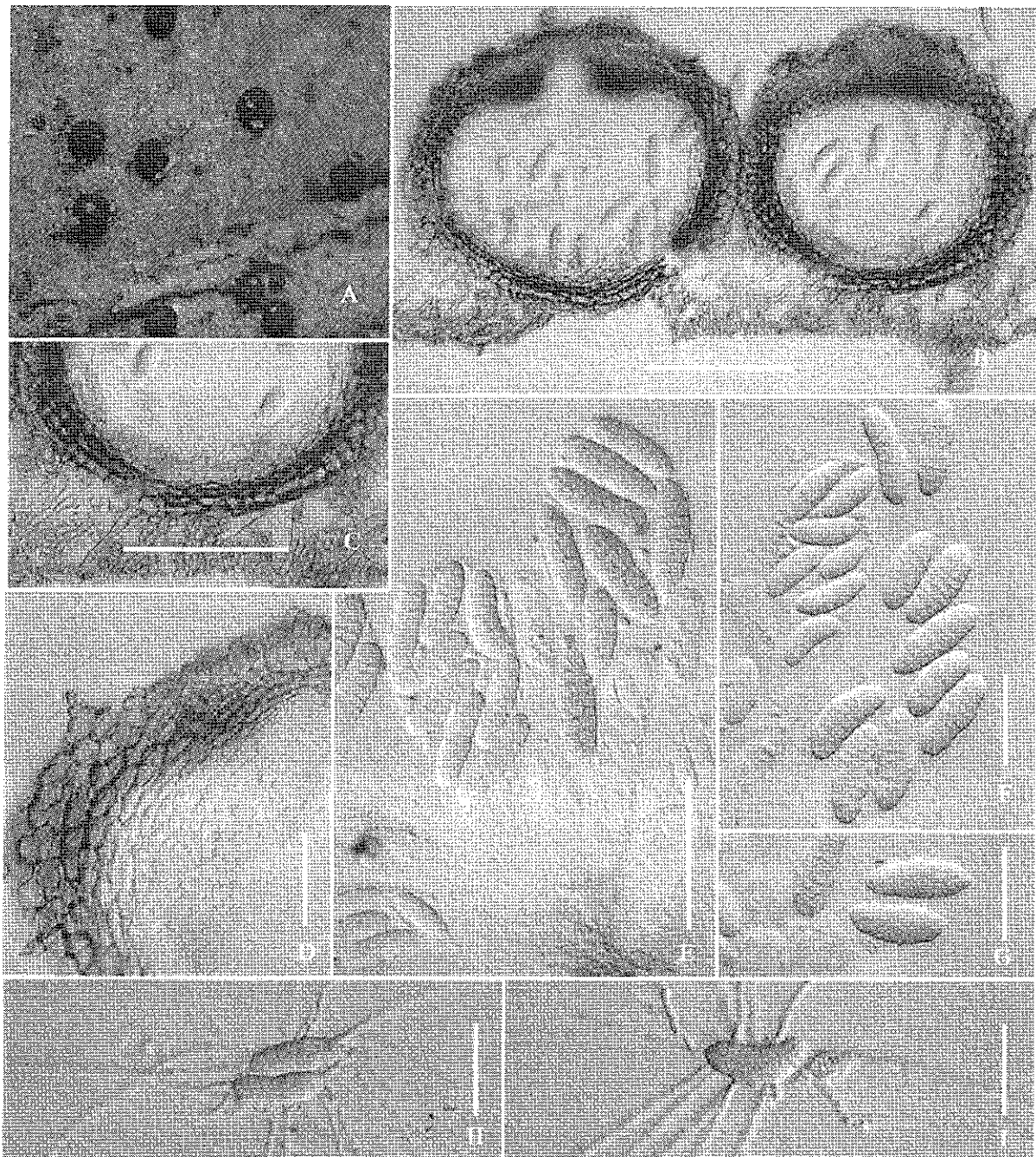


FIG. 5. *Phaeosaccardinula ficus* (holotype). A. Black ascomata scattered on living leaves of *Ficus* sp. B. Vertical section through ascomata showing ostiole. Note the subiculum-like structure of mycelia at the base of the ascomata which attach them to the plant surface. C. Hyphal network of subiculum at base of ascoma. D. Peridium comprising textura globulosa at the outside to textura angularis and flattened inward. Note the spine on the surface. E. Asci obovoid to clavate. F–G. Muriform ascospores. H–I. Germinating ascospores. Germ tubes develop from numerous cells of ascospores. Bars: B–C = 100 μ m, E = 50 μ m, D–I = 20 μ m.

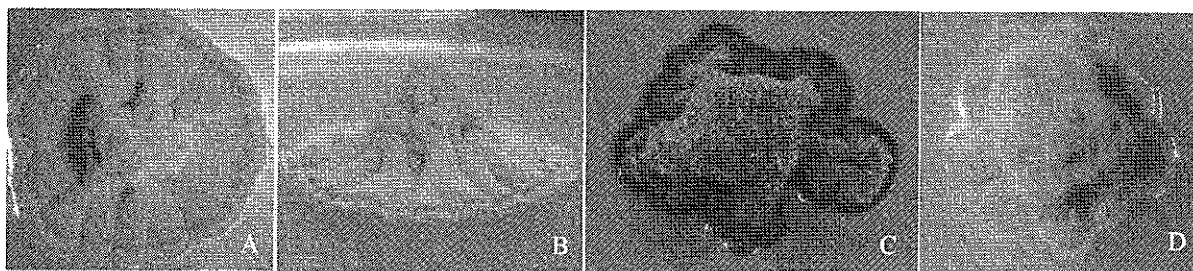


FIG. 6. Colony on PDA after 4 wk at 27 C in dark. A, B = *Chaetothyrium brischofiacola*, C = *Phaeosaccardinula ficus*, D = *Ceramothyrium thailandicum*.

sooty molds characterized by ascomata that form beneath a mycelial pellicle on the leaf surface and that is attached to the upper part of ascomata (Batista and Ciferri 1962). *Ceramothyrium thailandicum* was placed in *Ceramothyrium* because the ascomata and mycelium do not have setae and ascospores are clavate with a distinct mucilaginous sheath and numbers of septa differ from existing species (Hughes 1976, Constantinescu 1989). *Phaeosaccardinula ficus* is accommodated in *Phaeosaccardinula* characterized by ascomata and mycelium that lack setae and muriform ascospores (Batista and Ciferri 1962, Hughes 1976, Eriksson and Jingzhu 1985). Both *Chaetothyrium* and *Treubiomycetes* are characterized by ascomata with setae (Hughes 1976), however in the former genus species have obovoid ascospores with transverse septa only while in the latter genus species have cylindrical oblong, muriform ascospores (Batista and Ciferri 1962, Hughes 1976). Many of the ascospores in our species are obovoid and may have or lack longitudinal septa, and we therefore prefer to place our species in *Chaetothyrium*. A new genus, *Chaetothyriomyces*, from Brazil was introduced by Pereira et al. (2009) and this differs because it is characterized by asci with 16 ascospores.

Few rDNA sequences of Chaetothyriaceae are available in GenBank. SSU rDNA sequences were provided for *Ceramothyrium linnaeae* (Dearn.) S. Hughes by Winka et al. (1998) who showed them to be closely related to *Chaetothyrium* Speg., the type genus of Chaetothyriaceae. Phylogenetic analyses of LSU as well as ITS sequences revealed that the three new species here cluster within the Chaetothyriales (Chaetothyriaceae and Herpotrichiellaceae) and are remote from the Capnodiales (sooty molds) (FIGS. 1, 2). Verrucariales also was shown to be sister to Chaetothyriales (FIG. 1) thus confirming the results of previous morphology and molecular studies (Lutzoni et al. 2001).

The three new taxa described in the present study have phylogenetic similarity with a putative member of the Chaetothyriaceae (*Ceramothyrium carniolicum*)

and anamorphic taxa (*Vonarxia vagans*, *Cyphellophora eugeniae*, *Cyphellophora hylomeconis*) (Chaetothyriaceae; FIG. 1). Type-derived sequences of members of Chaetothyriaceae have not been used in previous phylogenetic analysis because no such sequences are available. Therefore further studies with more taxon sampling are needed to obtain a better understanding of the Chaetothyriales complex (Badali et al. 2008, Gueidan et al. 2008, Untereiner et al. 2011). As such the present study contributes six more chaetothyriaceous sequences including the first LSU and ITS rDNA sequence for the type genus *Chaetothyrium* and this should provide better data for future work on the sooty mold complex.

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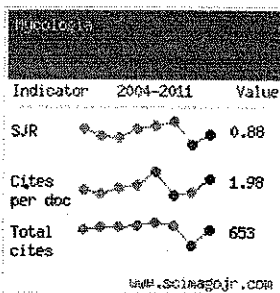
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