

Two new *Kirschsteiniothelia* species with *Dendryphiopsis* anamorphs
cluster in *Kirschsteiniotheliaceae* fam. nov.

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Abstract: Two new *Kirschsteiniothelia* species are proposed in this study; both were collected on decaying wood from Chiang Mai and Chiang Rai provinces in northern Thailand. The taxa were isolated and the morphological characters are described and illustrated. ITS, LSU and SSU combined

sequence analysis showed taxa of *Kirschsteiniothelia* separating into three lineages: (i) *K. elaterascus* grouped within Morosphaeriaceae (Pleosporales); (ii) *K. maritima* clustered with *Mytilinidion* spp. as a sister group in the Mytiliniaceae clade; and (iii) the two new *Kirschsteiniothelia* species, which produce *Dendryphiopsis* anamorphs in culture, clustered with *K. aethiops* (the generic type) and the anamorph *D. atra*. The new family Kirschsteiniotheliaceae is introduced to accommodate taxa grouping with *K. aethiops*. *K. elaterascus* is transferred to Morosphaeria (Morosphaeriaceae) and a new genus *Halokirschsteiniothelia* is introduced to accommodate *K. maritima* (Mytiliniaceae).

Key words: Dothideomycetes, Kirschsteiniotheliaceae, new species, phylogeny

INTRODUCTION

Kirschsteiniothelia was introduced by Hawksworth (1985a) and is represented by the type *K. aethiops* based on *Sphaeria aethiops* Berk. & M.A. Curtis. The genus is characterized by superficial to semi-immersed, hemispherical or subglobose, dark brown to black ascomata, cylindrical-clavate asci that develop among numerous pseudoparaphyses, and mostly one-septate (in some species two-septate), ellipsoidal, dark-brown ascospores; there are presently 18 species recorded in *Index Fungorum* and seven estimated species in Kirk et al. (2008).

Kirschsteiniothelia D. Hawksw. is a genus of the Dothideomycetes (Hawksworth 1985a), although its ordinal and familial placements are uncertain and it is currently classified as Dothideomycetes incertae sedis in *Index Fungorum* and in Lumbsch and Huhndorf (2010). In MycoBank (Crous et al. 2004, Robert et al. 2005) this genus is placed in the Pleosporaceae, while its known hyphomycete anamorphs are referred to the Pleomassariaceae (Hyde et al. 2011). Thus placement of the genus is uncertain. Schoch et al. (2006) analyzed molecular data for *K. aethiops*, which did not cluster close to Pleosporaceae, and it was suggested that this genus should be transferred to a separate family. In a recent molecular phylogenetic analysis of the Dothideomycetes *K. elaterascus* Shearer clustered in the same clade as *Morosphaeria* (Morosphaeriaceae) while *K. maritima* (Linder) D. Hawksw. clustered in the Mytiliniaceae clade, as a sister group of *Mytilinidion* (Schoch et al. 2009, Suetrong et al. 2009).

Submitted 19 Mar 2011; accepted for publication 30 Oct 2011.

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The anamorph of *Kirschsteiniothelia* has been listed as *Dendryphiopsis atra* (Corda) S. Hughes (Kirk et al. 2008), a connection proven by morphology and molecular data (Schoch et al. 2006, Hernandez et al. 2008). The aim of the present study is to establish the taxonomic placement of *Kirschsteiniothelia* with morphological and phylogenetic characters and to describe two new species collected in Thailand.

MATERIALS AND METHODS

Sample collection, specimen examination, isolation and photography of fungi.—Collections of Dothideomycetes were made in northern Thailand and two species of *Kirschsteiniothelia* were collected from Chiang Mai and Chiang Rai provinces Jun–Oct 2009. Samples were examined under a Motic SMZ 168 series microscope. Ascomata were sectioned by hand and images captured with an Olympus SZH10 stereo-microscope by a Nikon COOLPIX 4500 digital camera. Micromorphological structures were photographed with a Nikon ECLIPSE 80i compound microscope with a Cannon 450D digital camera and measured with Tarosoft® Image Frame Work 0.9.7. Images were processed with Adobe Photoshop CS3 Extended 10.0 (Adobe Systems Inc, USA). Type material is deposited in the MFLU herbarium and cultures are deposited at MFLUCC and IFRDC culture collections.

Single-spore isolates were made on water agar (WA) or malt extract agar (MEA, Difco Laboratories, Detroit, Michigan) with 0.5 g/L chloramphenicol (antibiotic) added (Choi et al. 1999). Germinating spores were transferred to MEA and incubated at room temperature 7 d. Isolates were grown 2–3 mo when morphological characters in culture, such as mycelium, color, shape and texture, were recorded. The growth rate was measured for 7 d old colonies on MEA; part of culture replicates were used for DNA analysis.

DNA extraction, PCR amplification and DNA sequencing.—Fungal mycelium was scraped from surface colonies grown on MEA 1 mo. DNA was extracted with a Biospin Fungus Genomic DNA Extraction Kit (BioFlux®, China) following the manufacturer's instructions (Hangzhou, P.R. China); genomic DNA products were contained in 100–200 µL buffer. PCR amplification of the internal transcribed spacers (ITS), the small subunit rDNA (SSU) and the large subunit rDNA (LSU) were carried out with primers ITS5/ITS4, NS1/NS4 and LROR/LR5 (White et al. 1990, Vilgalys and Hester 1990). Amplification reaction mixtures contained 50 ng template DNA, PCR Master Mix 1 ×, 0.5 µM of each primer in a 25 µL volume, 50 U Taq DNA polymerase 400 µM of each dNTP, 3 mM MgCl₂. PCR conditions used initial denaturation at 95 °C for 2 min, 94 °C 1 min, 50 °C 1 min and 72 °C 1 min and 35 cycles 94 °C 1 min, followed by a final extension step of 72 °C for 10 min. PCR products were visualized by staining with red gel on 1% agarose gel electrophoresis for purity, and sent for sequencing by Shanghai Sangon Biological Engineering Technology & Services Co Ltd (Shanghai, P.R. China).

Phylogenetic analyses.—A BLAST query was performed to find the possible similarity of the generated *Kirschsteiniothe-*

lia sequences in the GenBank database. Analysis of combined ITS, SSU and LSU sequences data were aligned with BioEdit (Hall 1999) and Clustal X 2 (Larkin et al. 2007) with available sequences of the orders Capnodiales, Jahnulales, Mytilinidiales and Pleosporales (Dothideomycetes) obtained from the GenBank (TABLE I) and *Schisma-tonna decolorans* (Arthoniomycetes) as an outgroup.

The aligned dataset was analyzed with PAUP* 4.0b10 (Swofford 2002). Maximum parsimony (MP) analyses on different settings (unweighted, weighted [step matrix] and reweighted) produced the same topology for all analyses. Stepwise additions of sequence used 1000 replicates of random and tree-bisection-reconnection (TBR) branch-swapping algorithm, with MAXTREES set at 1000. The parsimony tree scores including tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) also were calculated. Bootstrap support for branches were based on 1000 MP replicates with a single sequence addition replicates for each bootstrap replicate. The phylogenetic tree was visualized with TreeView (Page 1996).

Models of nucleotide substitution for each genes were determined with MrModeltest 2.3 (Nylander 2004). GTR + I + G evolutionary model was selected (Ronquist and Huelsenbeck 2003). The Metropolis-coupled Markov chain Monte Carlo (MCMCMC, Rannala and Yang 1996) algorithm was used to estimate posterior probabilities (PP). Six Markov chains were run from a random starting tree for 1 000 000 generations and trees sampled every 100 generations. The first 2000 trees were discarded as burn-in before convergence of the four chains. The remaining trees were used to construct 50% majority rule consensus tree and to calculate posterior probabilities (PP) with those equal or more than 0.95 given below each branch (FIG 2). The consensus tree obtained for the Bayesian analyses confirmed the tree topology obtained from parsimony analysis.

RESULTS

Phylogenetic analysis.—The phylogenetic tree (FIG. 1) was constructed with 260 Dothideomycete taxa, mostly from Schoch et al. (2009), plus *Kirschsteiniothelia* species sequenced in this study and out-group taxa and is based on combined genes (ITS, LSU and SSU). This yielded a maximum parsimony (MP) tree length of 8982 steps (CI = 0.242, RI = 0.697, RC = 0.169, HI = 0.758). Clades with greater than or equal to 50% bootstrap support are shown on the upper nodes. In the MP analysis (FIG. 1) *Kirschsteiniothelia aethiops*, *K. lignicola*, *K. emarceis* and *Dendryphiopsis atra* formed a monophyletic group that we designate below as a new family, *Kirschsteiniotheliaceae*, within the Jahnulales.

The ITS, LSU and SSU sequences of 30 taxa (TABLE I) were combined and aligned to establish the phylogeny of *Kirschsteiniothelia* species, especially their ordinal and familial positions. The combined

TABLE I. GenBank accession numbers of taxa in combined genes used in this study and newly generated sequences are indicated in bold

Species	Family/order	Strain	GenBank accession number		
			LSU	SSU	ITS
<i>Capnodium coffeae</i>	Capnodiaceae/Capnodiales	CBS147.52	GU214400	DQ247808	
<i>Dendryphiopsis atra</i>	Picomassariaceae/Plecosporales	DAOM 231155	DQ678046		AJ344239
<i>Jahnula aquatica</i>	Aliquandostipitaceae/Jahnulales	R68-1	EF175655		
<i>Jahnula bipileta</i>	Aliquandostipitaceae/Jahnulales	F49-1	EF175657	EF175633	
<i>Kirschsteiniotelia aethiops</i>	Kirschsteinioteliaceae/Plecosporales	CBS109.53	AY016361	EF175635	
<i>Kirschsteiniotelia elateruscus</i>	Morospheariaceae/Plecosporales	HKUCC7769	AY787934	AY016344	
<i>Kirschsteiniotelia lignicola</i>	Kirschsteinioteliaceae/Plecosporales	MFLUCC10-0105	HQ441568	HQ441569	HQ441567
<i>Kirschsteiniotelia maritima</i>	Mytilinioteliaceae/Mytilinidiales	CBS221.60	AY849943		
<i>Kirschsteiniotelia maritima</i>	Mytilinioteliaceae/Mytilinidiales	CBS221.60	GU332303		
<i>Kirschsteiniotelia emareis</i>	Kirschsteinioteliaceae/Plecosporales	MFLUCC10-0106	HQ441571	HQ441572	HQ441570
<i>Lophiostoma arundinis</i>	Lophiostomataceae/Plecosporales	CBS621.86	DQ782384	DQ782383	AJ496633
<i>Lophiostoma macrostomum</i>	Lophiostomataceae/Plecosporales	HHUF2790	AB433273		
<i>Lophium mytilinum</i>	Mytilinioteliaceae/Mytilinidiales	CBS114111		EF596818	
<i>Massaria anomia</i>	Massariaceae/Plecosporales	M47 (WU30509)	HQ599378	HQ599453	EF596819
<i>Massaria conspurcata</i>	Massariaceae/Plecosporales	M14 (WU30519)	HQ599393	HQ599453	HQ599378
<i>Massaria inquinans</i>	Massariaceae/Plecosporales	M15 (WU30526)	HQ599400	HQ599441	HQ599393
<i>Massaria inquinans</i>	Massariaceae/Plecosporales	M19 (WU30527)	HQ599402	HQ599442	HQ599400
<i>Massaria ulmi</i>	Massariaceae/Plecosporales	M25 (WU30566)	HQ599428	HQ599444	HQ599402
<i>Massarina eburnea</i>	Massariaceae/Plecosporales	CBS473.64	GU301840	HQ599446	HQ599428
<i>Massarina igniaria</i>	Massariaceae/Plecosporales	CBS845.96	GU296170	GU296171	
<i>Morosphearia ramunclicola</i>	Morospheariaceae/Plecosporales	BCC18404	GU296171	GU296171	
<i>Morosphearia velutiaspora</i>	Morospheariaceae/Plecosporales	BCC17059	GQ925853	GQ925838	
<i>Mycosphaerella africana</i>	Mycosphaerellaceae/Capnodiaceae	CPC794	GQ925852	GQ925841	
<i>Mycosphaerella punctiformis</i>	Mycosphaerellaceae/Capnodiaceae	CBS113265	GU214433	GU214536	
<i>Mytilinidion acicola</i>	Mytilinioteliaceae/Mytilinidiales	EB0379	NG027571		EU167569
<i>Mytilinidion californicum</i>	Mytilinioteliaceae/Mytilinidiales	EB0385	GU397346	GU397362	
<i>Mytilinidion mytilinum</i>	Mytilinioteliaceae/Mytilinidiales	CBS303.34	GU323208	GU323186	
<i>Preussia funiculata</i>	Mytilinioteliaceae/Mytilinidiales	CBS659.74	FJ161184	FJ161144	HM163570
<i>Preussia terricola</i>	Sporormiaceae/Plecosporales	CBS317.65	GU301864	GU296187	
<i>Schizomatoma decolorans</i>	Roccellaceae/Arthoniales	DUKE 0047570	GQ203725		GQ203765
			AY548815	AY548809	AY548808

Abbreviations of isolates and culture collections: ATCC = American Type Culture Collection, Virginia, U.S.A.; BCC = BIOTEC Culture Collection, Bangkok, Thailand; CBS = Centraalbureau voor Schimmelfcultures, Utrecht, Netherlands; CPC = P.W. Crous; DAOM = National Mycological Herbarium, Department of Agriculture, Ottawa, Ontario, Canada; DUKE = Duke University Herbarium, Durham, North Carolina, U.S.A.; EB = Original designation for culture and dried specimens from Eric W.A. Boehm, presently deposited with CBS and BPI as designated; HHUF = Herbarium of Hiroaki University, Japan; HKUCC = The University of Hong Kong Culture Collection, Hong Kong, China; KT = K. Tanaka; MFLUCC = Mae Fah Luang University Culture Collection, Thailand; M47 = T. Graefenhain; M14, M15, M19 and M25 = H. Voglmayr. New sequences generated in this study are in bold.

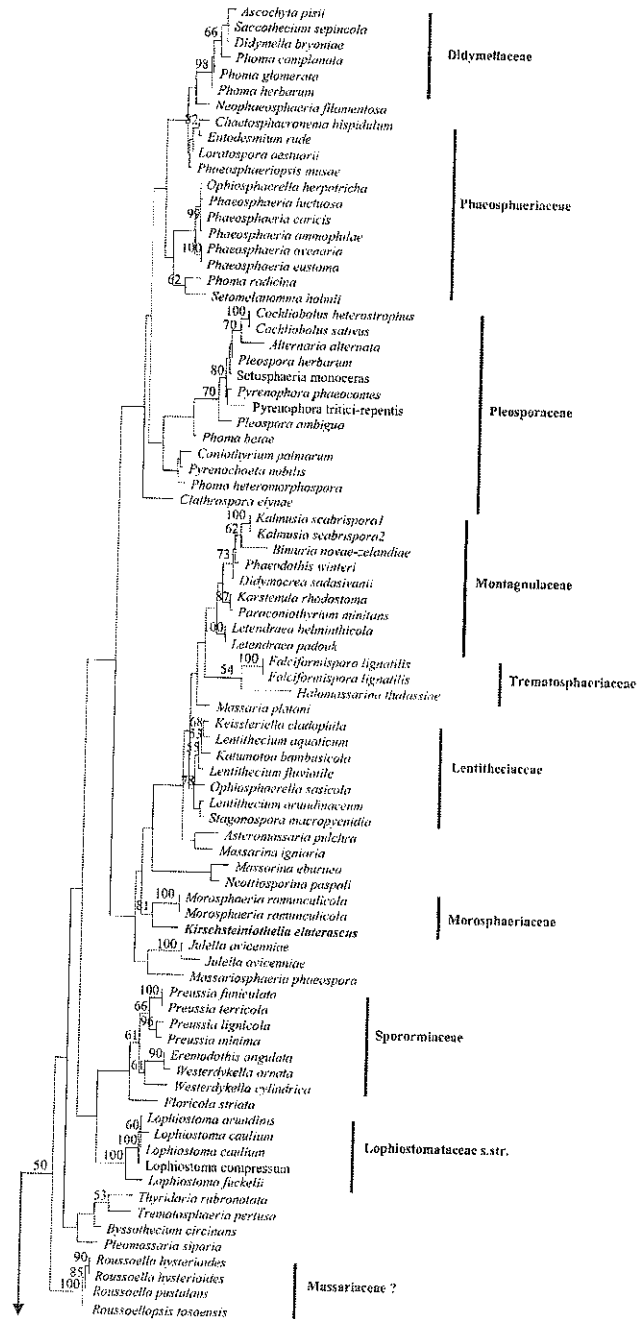


FIG. 1. Maximum parsimony tree constructed with 259 Dothideomycete taxa, mostly from Schoch et al. (2009), plus *Kirschsteiniethelia* species sequenced in this study, based on combined genes (ITS, LSU and SSU). Bootstrap percentage values greater than or equal to 50% are shown on the upper nodes, based on 500 replicates.

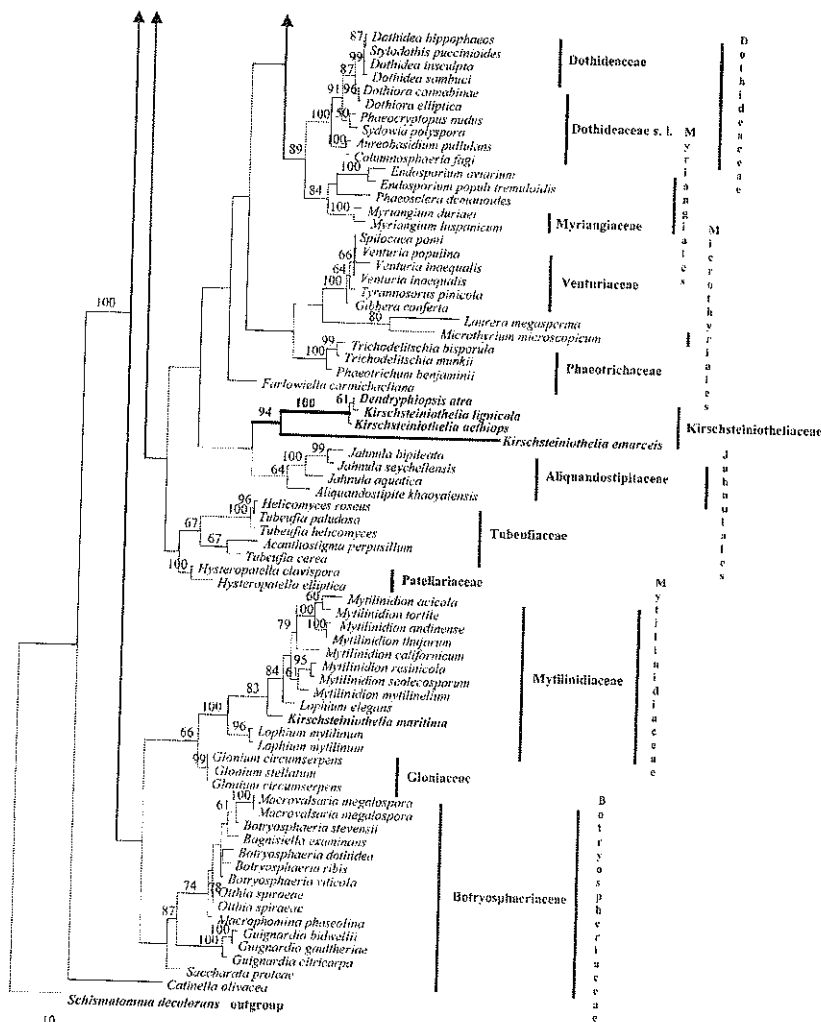


FIG. 1. Continued.

dataset after alignment comprised 1877 characters including gaps. This yielded a maximum parsimony (MP) tree length of 1909 steps (CI = 0.620, RI = 0.672, RC = 0.417, HI = 0.380). Bayesian analysis generated similar tree topology and the Bayesian values are marked below branches (FIG. 2). Twenty-nine fungal strains were grouped in Dothideomycetes within four well defined orders, Mytilinidiales, Pleosporales, Capnodiales and Jahnulales, with the exception of the outgroup *Schismatomma decolorans* (Arthoniomycetes). The phylogenetic analysis revealed an affiliation between the two new species of *Kirschsteiniotelia* from Thailand, described as *K. lignicola* and *K. emarceis*, the generic type *K. aethiops* and the anamorph *D. atra*.

Kirschsteiniotelia maritima and *K. elaterascus* however were placed in different families, with *K. elaterascus* closely related to *Morosphaeria* with strong statistical support (99% BT, 1.00 PP) within Morosphaeriaceae (Pleosporales). *K. elaterascus* is thus transferred to *Morosphaeria*. *K. maritima* clustered with *Mytilinidion* spp. as a sister group in the Mytilinidiaceae clade. Thus it is introduced as a new genus *Halokirschsteiniotelia* in Mytilinidiales.

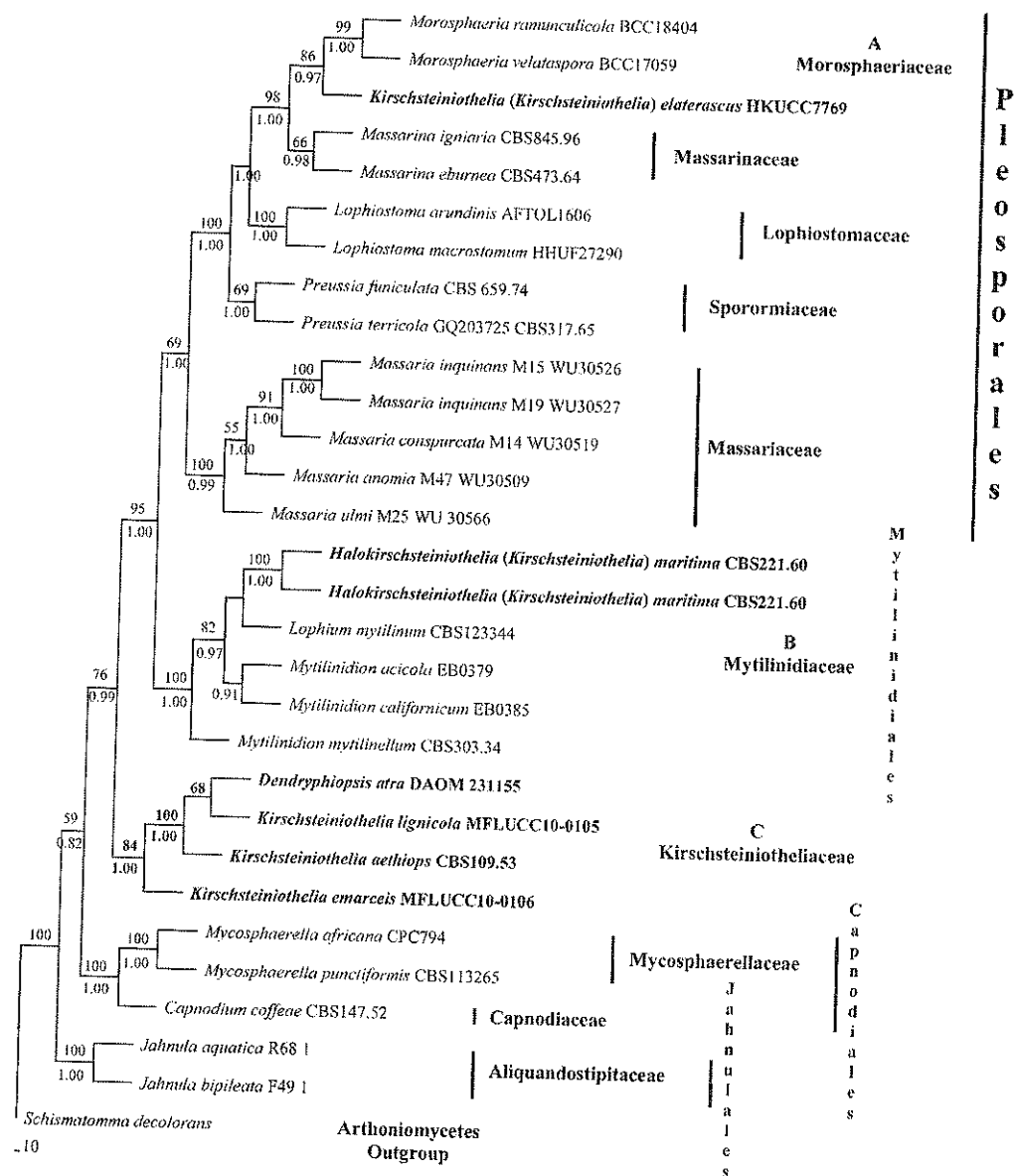


FIG. 2. Phylogram generated from maximum parsimony analysis based on combined LSU, SSU and ITS rDNA, demonstrating the phylogenetic relationships of *K. lignicola* and *K. emarceis* with *K. aethiops* (type species and its *D. atra* anamorph). The *Kirschsteiniethelia* lineage is in bold. *Schmatomma decolorans* is the outgroup. Bootstrap support values higher than 75% are shown at the relevant nodes, based on 1000 replicates. Bayesian posterior probabilities greater than 0.95 are indicated under thickened branches.

TAXONOMY

Clade A: Mytiliniaceae.—*Kirschsteiniiothelia maritima* groups in the Mytiliniaceae clade (Mytiliniales) and as a sister group to *Mytilinidion* species (FIG. 2). *K. maritima* differs from *Mytilinidion* in appearance and in its marine habitat (Hawksworth 1985b, Kohlmeyer and Kohlmeyer 1979, Figueira and Barata 2007). Therefore a new genus, *Halokirschsteiniiothelia* (Mytiliniadales), is introduced to accommodate *K. maritima*. The characters of *Halokirschsteiniiothelia* are unusual for Mytiliniaceae and therefore its inclusion in the family is tentative.

Halokirschsteiniiothelia S. Boonmee & K.D. Hyde gen. nov.

Mycobank MB561017

Etymology. from Greek *hals* = salts, in reference to the marine origin of the fungus.

Ascomata erumpentia ad superficialia, hemisphaerica ad subglobosa, dispersa, leviter papillata. Pseudoparaphyses sparsis, septata, ramosis. Asci bitunicate, octaspori, cylindriclavati, cubiculum ocular, pedicellati. Ascospores ellipsoideae, pallide brunnae, uniseptatae, constrictae ad septum.

Ascomata erumpent to superficial, hemispherical to subglobose, scattered, with a small papillate ostiole. Pseudoparaphyses cellular, persistent, sparse, septate, branched, 2–3 µm thick. Asci 64–85 × 8–12 µm, eight-spored, bitunicate, clavate, pedicellate, and with a small ocular chamber. Ascospores 16–20 × 4.5–6 µm, ellipsoid, pale brown, one-septate, the apices tending to be attenuated, constricted at the septum.

Type species. *Halokirschsteiniiothelia maritima* (Linder), S. Boonmee & K. D. Hyde.

Halokirschsteiniiothelia maritima (Linder) S. Boonmee & K. D. Hyde comb. nov.

Mycobank MB561018

Basionym. *Amphisphaeria maritima* Linder, Farlowia 1:411, 1944.

Synonyms. *Kirschsteiniiothelia maritima* (Linder) D. Hawksw., *J. Linn. Soc., Bot.* 91:193 (1985).

Microthelia maritima (Linder) Kohlm., *Nova Hedwigia* 2:32, 1960.

Microthelia linderi Kohlm., *Trans. Br. Mycol. Soc.* 57:483, 1971.

Illustrations: Wang et al. 2004 (Plate 61, Figs. 1–7). Illustrated as *M. maritima* Linder Kohlm. in *Icones Fungorum Maris*, plate 39, 1964.

This species has been described by Kohlmeyer and Kohlmeyer in *Marine Mycology, the Higher Fungi* p 430–431, 1979, as *Microthelia linderi* Kohlm. with *K. maritima* (Linder) Kohlm. *Trans. Br. Mycol. Soc.* 57:483, 1971. as a synonym. Hawksworth (1985a)

however excluded it from *Microthelia* and placed it in *Kirschsteiniiothelia*.

Specimens examined. UNITED STATES, MASSACHUSETTS, Woods Hole, on test block immersed ca. 7 ft. below low water, 13 Oct 1941, E.S. Barghoorn (FH, HOLOTYPE of *Amphisphaeria maritima*).

This species is widespread in the oceans (Kohlmeyer and Kohlmeyer 1979) and differs from *Kirschsteiniiothelia* in having ostiolate ascomata and small ascospores with acute ends (Wang et al. 2004).

Clade B: Morosphaeriaceae.—The Morosphaeriaceae clade comprises four marine species (*M. ramunculicola*, *M. velatispora*, *Helicascus kamaloanus*, *H. nypae*) and the freshwater species *K. elaterascus*, all commonly found on dead wood (Suetrong et al. 2009). Shearer (1993) distinguished *K. elaterascus* from *K. aethiops* by its ascospores, which are surrounded by a sheath and asci whose pedicel stretch in water. Phylogenetic analyses indicate that *K. elaterascus* groups with *Morosphaeria* (Morosphaeriaceae) with high support (Kodsueb et al. 2006, Schoch et al. 2009, Shearer et al. 2009, Suetrong et al. 2009). In this study *K. elaterascus* grouped with *Morosphaeria* as a sister group with high support (99% BT, 1.00 PP). Based on these results, *K. elaterascus* is transferred to *Morosphaeria*, Morosphaeriaceae.

Morosphaeria elaterascus (Shearer) S. Boonmee & K.D. Hyde comb. nov.

Mycobank MB561019

Basionym. *Kirschsteiniiothelia elaterascus* Shearer, *Mycologia* 85:963, 1993.

The placement of *K. elaterascus* in *Morosphaeria* is supported by both molecular data (FIG. 2) and morphology. *Morosphaeria elaterascus* is similar to other species in the genus in having immersed to superficial ascomata, clavate to cylindrical asci and sheathed ascospores, which are not hyaline as in other *Morosphaeria* species (Suetrong et al. 2009, Lumbsch and Huhndorf 2010). *K. elaterascus* differs from *Morosphaeria* species in the structure and mode of dehiscence of the ascus, its brown ascospores and freshwater habitat (Shearer 1993).

Clade C: Kirschsteiniiotheliaceae.—The clustering of *Kirschsteiniiothelia aethiops*, *K. lignicola*, *K. emarceis* and *Dendryphiopsis atra* in a basal position to *Pleosporales* is shown in the phylogenetic analysis (FIG. 2), and a new family Kirschsteiniiotheliaceae is introduced to accommodate this group.

Kirschsteiniiotheliaceae S. Boonmee & K.D. Hyde fam. nov.

Mycobank MB561021

Familia Plcosporalium. Ascomycetum. Ascomata superficialia, subglobosa ad globosa, atrobrunnea ad nigra.

Peridium texturam angularem. Hamathecium numerosae, pseudoparaphysum. Asci bitunicati, cylindrici vel clavati, apicali rotundati, cum camera ocular. Ascospores ellipsoideae, 1–2-septatae, viridi, brunnea ad atrobrunnea, constrictae. Anamorph. Conidiophora longa stipula, recta vel leniter curvata, atrobrunnea. Conidia producta apice, obovata ad late, fusiformis ad obclavata, ferruginea ad atrobrunnea, griseobrunnea, septatae, constrictae.

Type genus: *Kirschsteiniotelia* D. Hawksw. (1985)

Genus of Pleosporales, ascomata superficial, subglobose to globose, dark brown to black, solitary. Peridium comprising textura angularis. Hamathecium of numerous filiform pseudoparaphyses. Asci cylindrical-clavate, apically rounded with a small ocular chamber. Ascospores biserial, ellipsoidal, 1–2-septate, dull green, olive brown to dark brown, smooth-walled. Anamorph. Conidiophores long-stalked, straight and slightly curved, thickened at the base, transversely septate, apex branched and dark brown. Conidia produced apically, broadly obovoid, fusiform to obclavate, reddish brown to dark brown, grayish brown, septate, constricted at septa, rounded at each end.

Notes. A new family, *Kirschsteinioteliaceae*, is introduced to accommodate the genus *Kirschsteiniotelia* and its anamorph *Dendryphiopsis*. Also the high support indices in the phylogram (FIG. 2) indicate its placement separate from other families of Pleosporales. It differs from other families in the Pleosporales having mostly ellipsoid, septate, colored ascospores, septate and colored and a *Dendryphiopsis* anamorph. Two new species from Thailand are introduced.

Type species. *Kirschsteiniotelia aethiops* (Berk. & M.A. Curtis) D. Hawksw., *J. Linn. Soc., Bot.* 91:185 (1985).

Basionym. *Sphaeria aethiops* Berk. & M.A. Curtis, *Grevilla* 4:143, 1876.

Anamorph. *Dendryphiopsis atra* (Corda) S. Hughes, *Can. J. Bot.* 31:655, 1953.

Kirschsteiniotelia lignicola S. Boonmee & K.D. Hyde, sp. nov. FIGS. 3–31

Mycobank MB561030

Etymology. The species is named in reference to the habitat on wood.

Ascomata superficialia, 152–178 μ m alta $\times$ 156–214 μ m diam. subglobosa ad globosa, atrobrunnea ad nigra, fasciculatae. Pseudoparaphyses numerosae, filiformia, hyalinae. Asci 107–163 $\times$ 19–28.5 μ m, bitunicati, octaspori, fissitunicati, cylindrici-clavati, apice rotundatae. Ascospores 27–30(–33) $\times$ 10–12(–13) μ m, ellipsoideae, viridi, brunnea ad atrobrunnea, constrictae. Conidiophora erecta, septatae, brunnea ad atrobrunnea. Conidia obovata, oblonga ad obclavata, septatae, constrictae, pallide brunneis ad obscure fuscum.

Ascomata 152–178 μ m high $\times$ 156–214 μ m diam, superficial, subglobose to globose, dark brown to black, membranaceous, clustered, solitary, with central papilla 24 μ m wide with 24 μ m high (FIGS. 3–5). Peridium 18–22 μ m thick, composed of one strata, comprising 3–4 layers of cells of textura angularis; inner layer cells pale brown, but outer layer cells dark (FIG. 6). Pseudoparaphyses numerous, filiform, 1–2 μ m wide, hyaline, embedded in a gelatinous matrix (FIG. 7). Asci 107–163 $\times$ 19–28.5 μ m, eight-spored, bitunicate, fissitunicate, cylindrical-clavate, with a long pedicel 14.5–24 μ m, apically rounded, with an ocular chamber 2 μ m wide, (FIGS. 8–11). Ascospores 27–30(–33) $\times$ 10–12(–13) μ m ($\bar{x}$ = 30 $\times$ 11 μ m, n = 20), biserial, ellipsoidal, slightly curved, 1(–2) septate, with median septum or in lower part, some ascospores with secondary septum, dull green, brown to dark brown at maturity, thick-walled, lacking a mucilaginous sheath (FIGS. 12–15).

Ascospores germinating on MEA within 12 h (FIG. 16). Colonies on MEA, reaching up to 2 cm diam in 7 d at 25–28 C, within a month covering the Petri dish, effuse, hairy, mycelium radiating outwards, edged fimbriate, colony dense, dark green to dark brown. Mycelium immersed and superficial; hyphae dark brown, septate, ca. 5 μ m wide (FIGS. 17–19). Conidiophores 39–148 μ m long, 4–7 μ m thick, septate, branched at apex, brown to dark brown, smooth (FIGS. 20–22). Conidia 24.5–35(–41) $\times$ 14–16(–19) μ m, broadly obovoid, reddish brown to dark brown, 1–2 transverse septa, slightly constricted at septa, smooth-walled, rounded at ends (FIGS. 23–27).

Anamorph growing on type material. Conidiophores erect, 287–406 $\times$ 11–13 μ m, branched apically, dark brown, smooth, conidium forming at apex (FIGS. 28–29). Conidia obovoid to broadly, 39–48(–52) $\times$ 21–25(–28) μ m, 1–2 transverse septa, constricted at septa, smooth-walled, dark brown, rounded at ends (FIGS. 30–31).

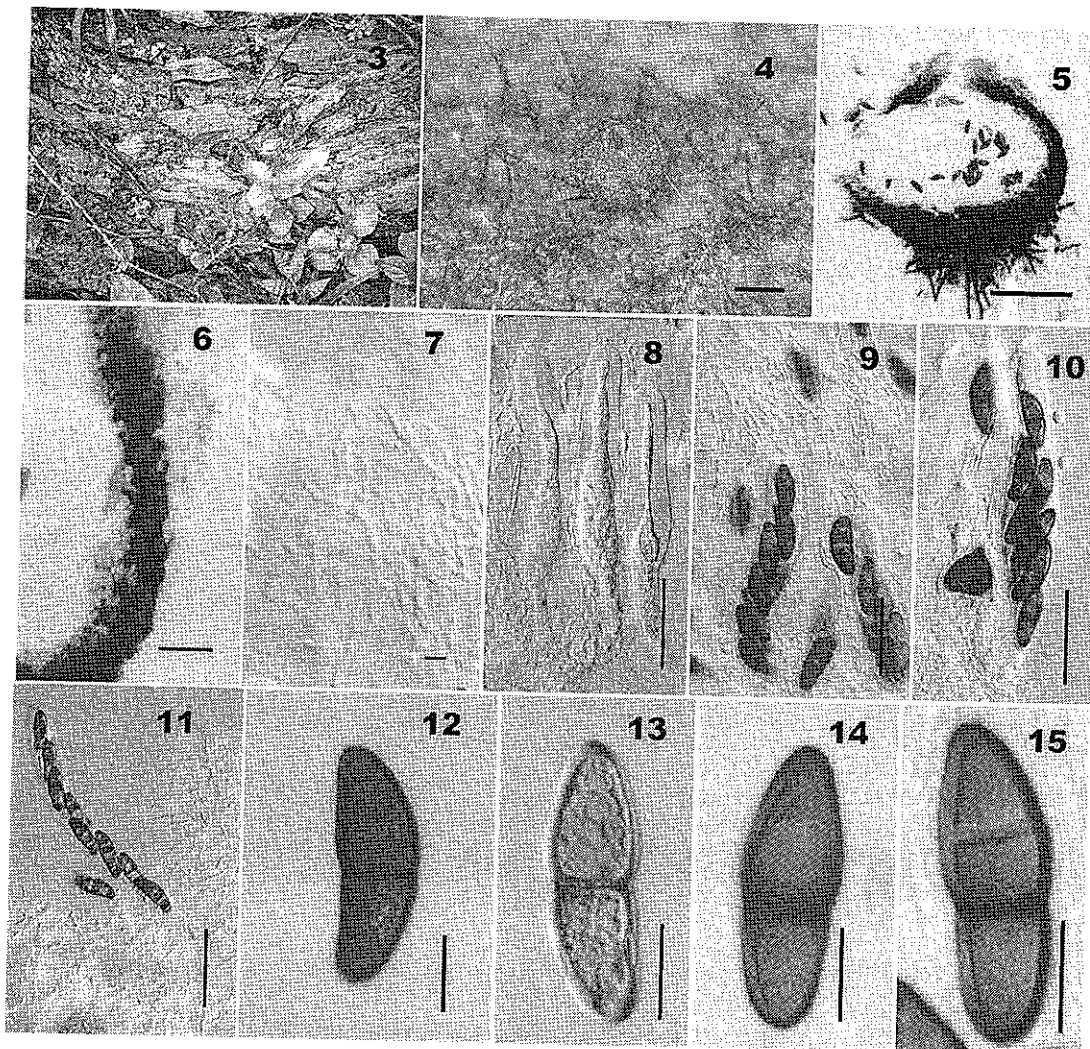
Specimens examined. THAILAND, CHIANG RAI, Muang, Khun Korn Waterfall, as 671 msl. (N19°51'–54' E99°35'–39'), on decaying wood of unidentified tree, 15 Aug 2009, *Savarnyaphat Boonmee* (HOLOTYPE MFLU10-0036), ex-type culture: MFLUCC10-0105 and IFRDC2179.

Habitat. On decaying wood of unidentified tree.

Mode of life. Saprobic.

Distribution. Chiang Rai, Thailand.

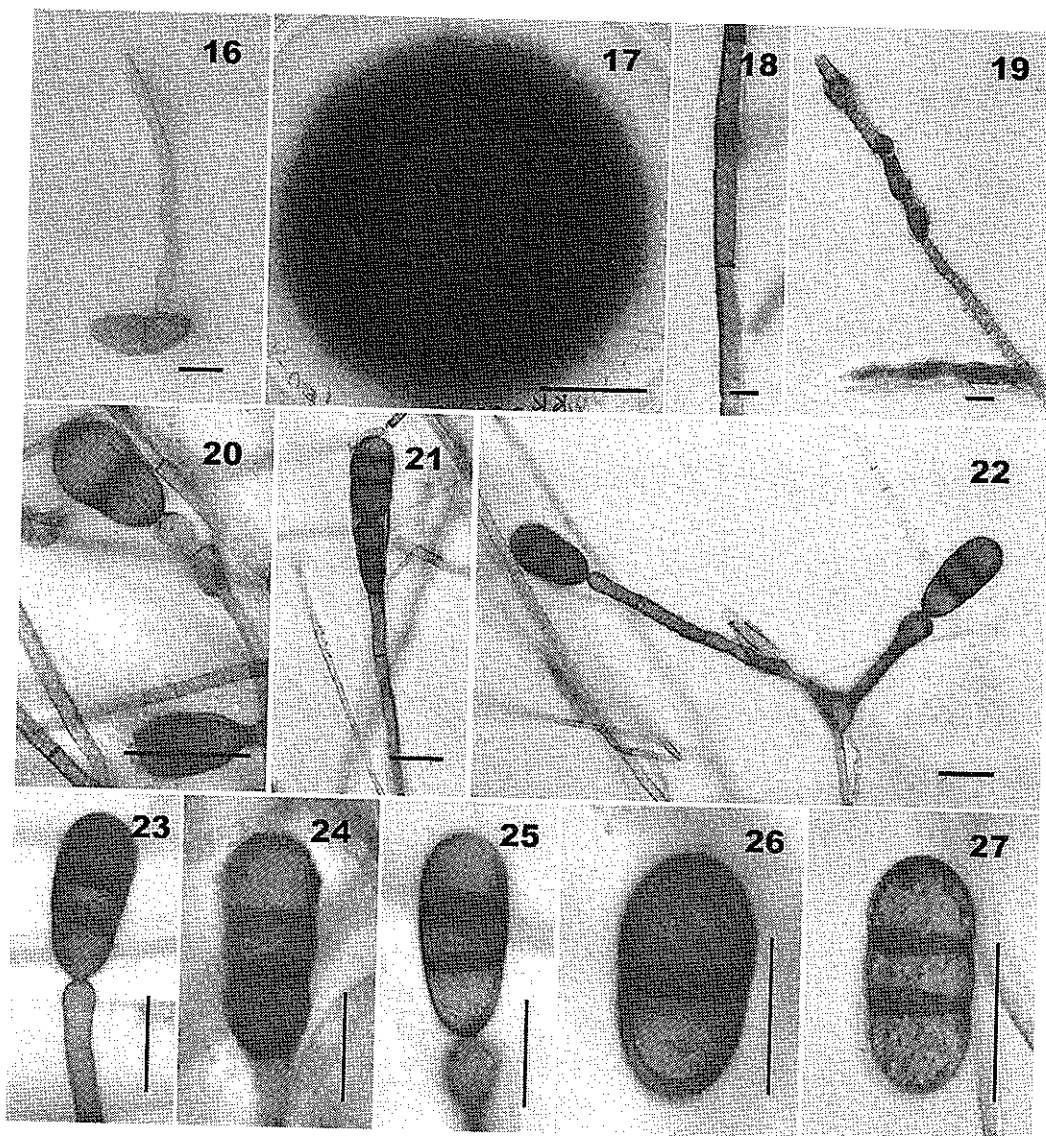
Notes. This species is characterized by superficial ascomata, with mycelia present at the base, a coriaceous wall, short-pedicellate asci, pigmented ascospores, which form secondary septa when mature (FIGS. 5–6, 12–15). *K. lignicola* is similar to *K. populi* (Tracy & Earle) You Z. Wang, Aptroot & K.D. Hyde, *K. recessa* (Cooke & Peck) D. Hawksw., *K. umbrinoidea* (Pass.) You Z. Wang, Aptroot & K.D. Hyde, and *K. xera* (Fairm.) You Z. Wang, Aptroot & K.D. Hyde because



FIGS. 3–15. *Kirschsteiniethelia lignicola* (holotype). 3. Habitat. 4. Appearance of ascomata on host surface. Note the light brown ostiole when dry. 5. Section through ascoma. 6. Section of peridium, the latter comprising 3–4 layers of cells of *textura angularis*. 7. Pseudoparaphyses numerous, narrow, embedded in a mucilaginous matrix. 8–11. Asci with eight spores. Note apical inner layer of ascus with small ocular chamber and biserial ascospores. 12–15. Ascospores that are initially hyaline become dull green, then brown to dark brown and 2–3-celled at maturity. Bars: 4–5 = 100 μ m, 6 = 20 μ m, 8–11 = 40 μ m, 7 = 5 μ m, 12–15 = 10 μ m.

ascospores are smooth-walled and lack a sheath (Hawksworth 1985a, Wang et al. 2004) but differs in having thick-walled and larger ascospores with secondary septa. The anamorph was derived from single ascospores, and also found on the substrate (FIGS. 16–27, 28–31). The conidia of the anamorph in culture were smaller than those produced on the natural

substrate. It is not unusual that characters in culture differ from the natural substrate, but because we were unable to isolate the anamorph we cannot unequivocally state these are the same. The characters of conidiophores and conidia are similar to the genus *Dendryphiopsis*, in agreement with the inferred phylogenetic relationships (Hughes 1953, FIG. 2).

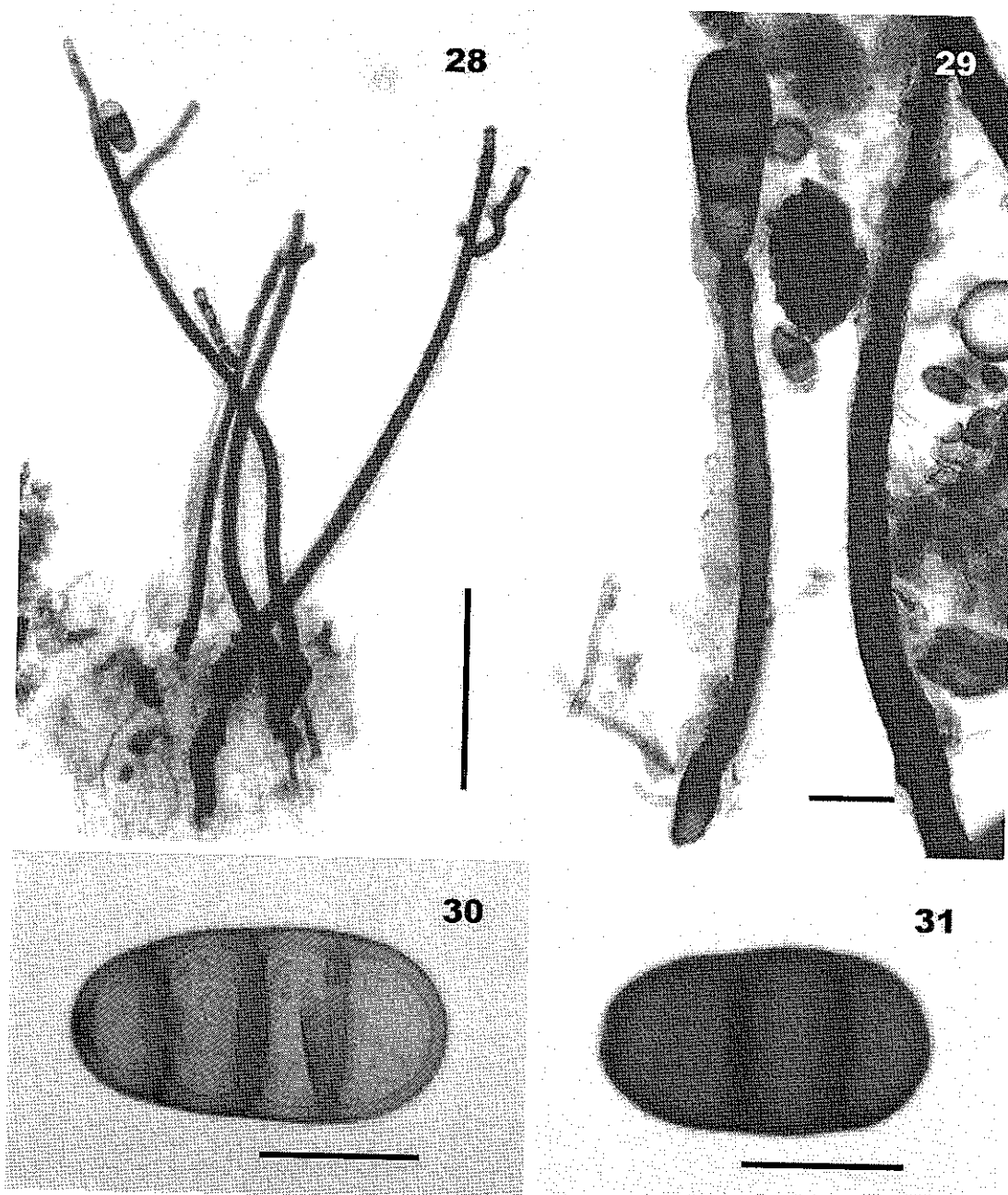


FIGS. 16–27. Anamorph of *Kirschsteiniotelia lignicola* in culture. 16. Germinating ascospore. 17. Colony on MEA. 18. Mycelium on MEA. 19–22. Conidiogenous cells and conidia. 23–27. Conidia. Bars: 16 = 10 μm , 17 = 1 cm, 18–20 = 5 μm , 21–27 = 20 μm .

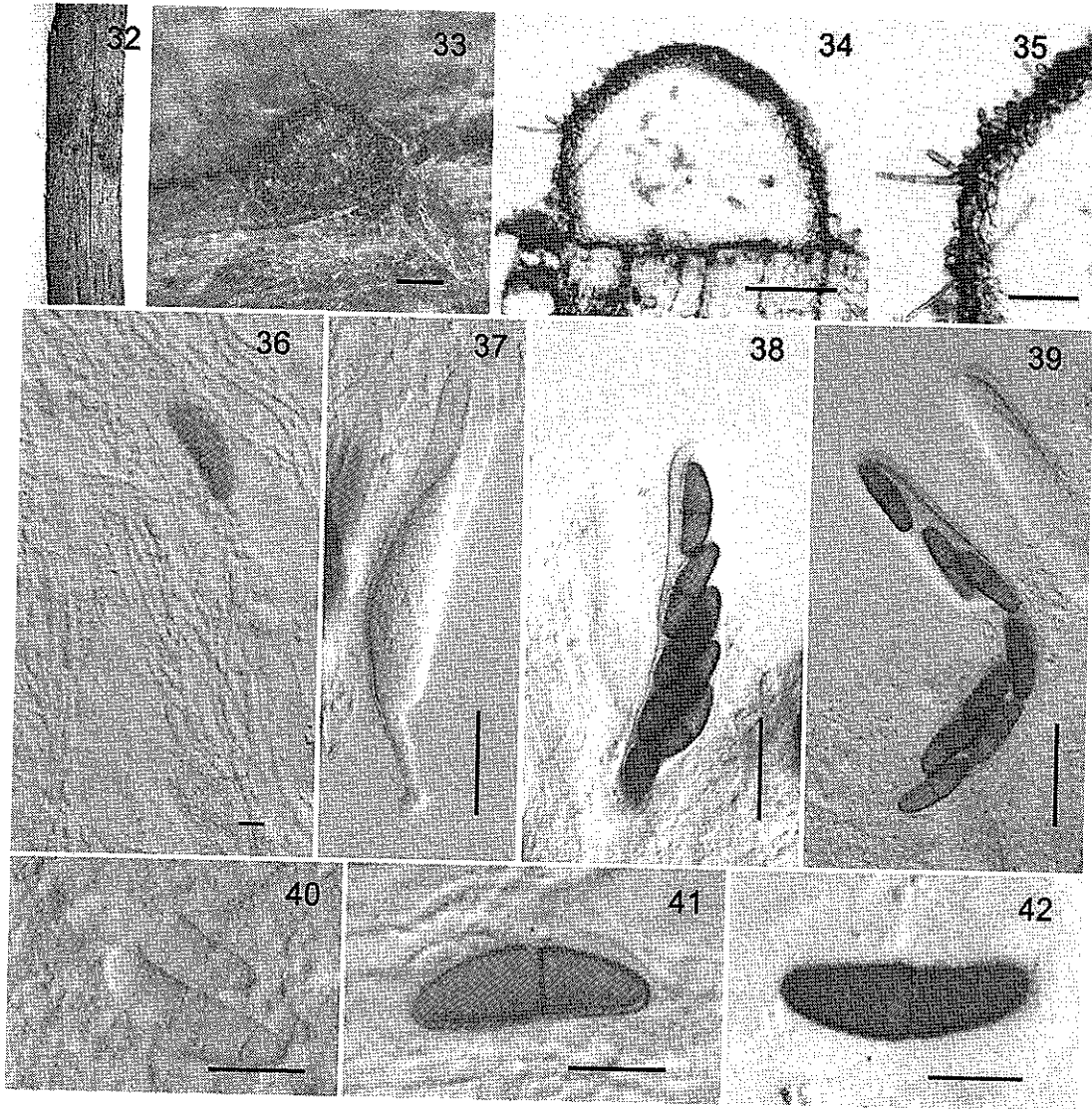
Kirschsteiniotelia emarceis S. Boonmee & K.D Hyde
sp. nov. FIGS. 32–64
Mycobank MB561033

Etymology. The species name refers to MRC (Mushroom Research Centre) where the specimen was collected.

Ascomata superficialia, 109–280 μm alta $\times$ 101–318 μm diam, subglobosa ad globosa, atrobrunnea ad nigra, fasciculatae, solitariae vel dispersae, paucis sparsis capillis-like. Hamothecium numerosae, filiformibus pseudoparaphysum, hyalinae. Asci 88–140 $\times$ 18–24 μm , bitunicati, octaspori, cylindrici-clavati, fissitunicati, pedicellis parvis, cubiculum



FIGS. 28–31. Anamorph of *Kirschsteiniotelia lignicola* on natural substrate. 28. Conidiophores. 29–31. Conidia. Bars: 28 = 100 μ m, 29–31 = 20 μ m.

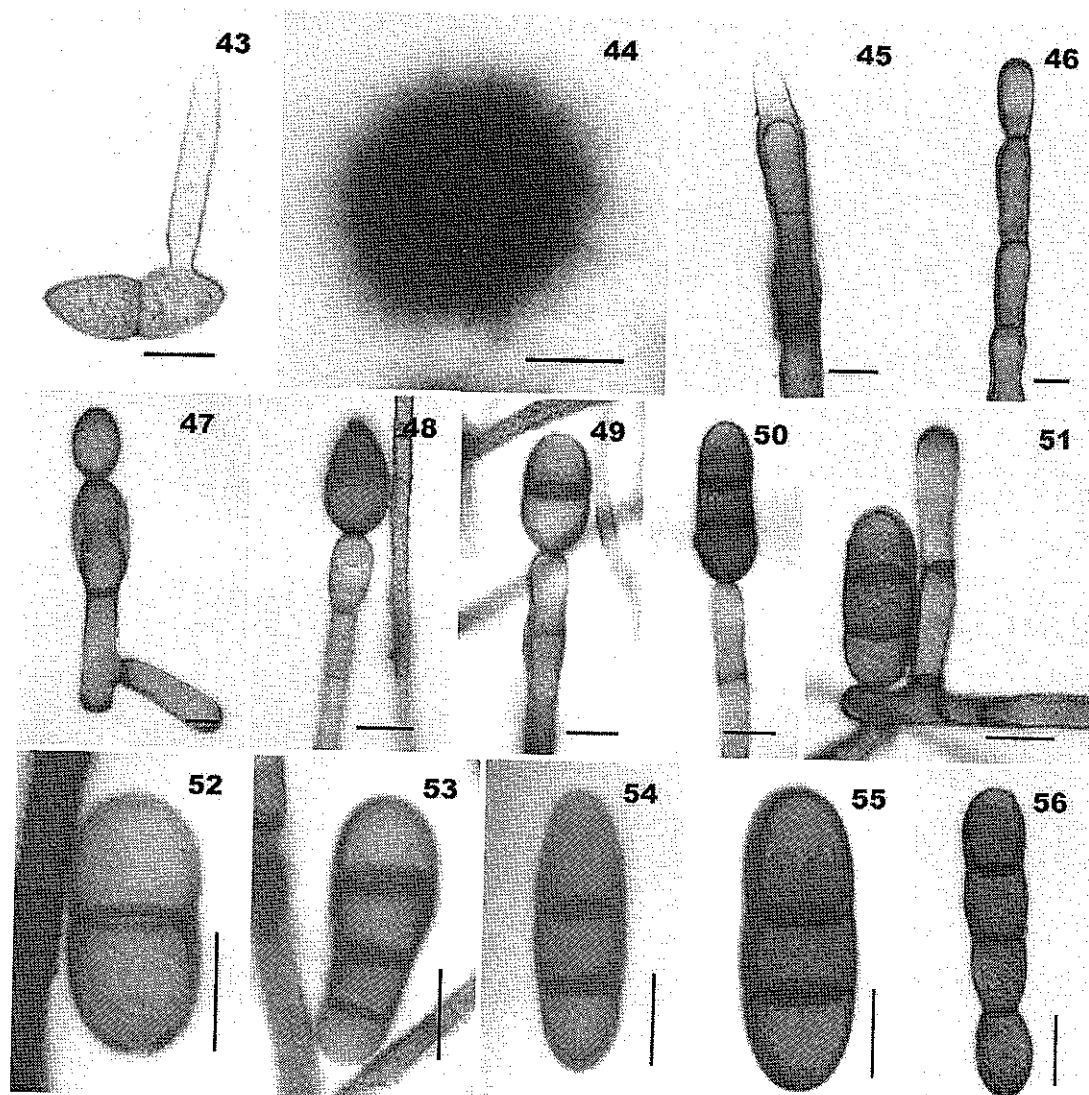


FIGS. 32–42. *Kirschsteiniotelia emarceis* (holotype). 32. Habitat. 33. Ascomata on host surface. Note the dome-shaped ascomata with a flattened base. 34. Section through ascoma covered in sparse hairs. 35. Peridium comprising *textura angularis*. 36. Pseudoparaphyses numerous and embedded in mucilaginous matrix. 37–39. Asci eight-spored, biseriate to uniseriate. 40–42. Ascospores one-septate. Smooth-walled. Bars: 33–34 = 100 μ m, 35 = 40 μ m, 36 = 5 μ m, 37–39 = 20 μ m, 40–42 = 10 μ m.

ocular. Ascospores (23.5–)25–28 $\times$ 8–9(–10) μ m, ellipsoideae, leniter curvatae, viridi, brunnea ad atrobrunnea, uniseptatae, constrictae. Conidiophora apice ramosis, brunneae ad atrobrunnea, laeves. Conidia fusiformia-obclavati, ferruginea ad atrobrunnea, septatae, constrictae.

Ascomata 109–280 μ m high $\times$ 101–318 μ m diam, dark brown to black, clustered, solitary or scattered,

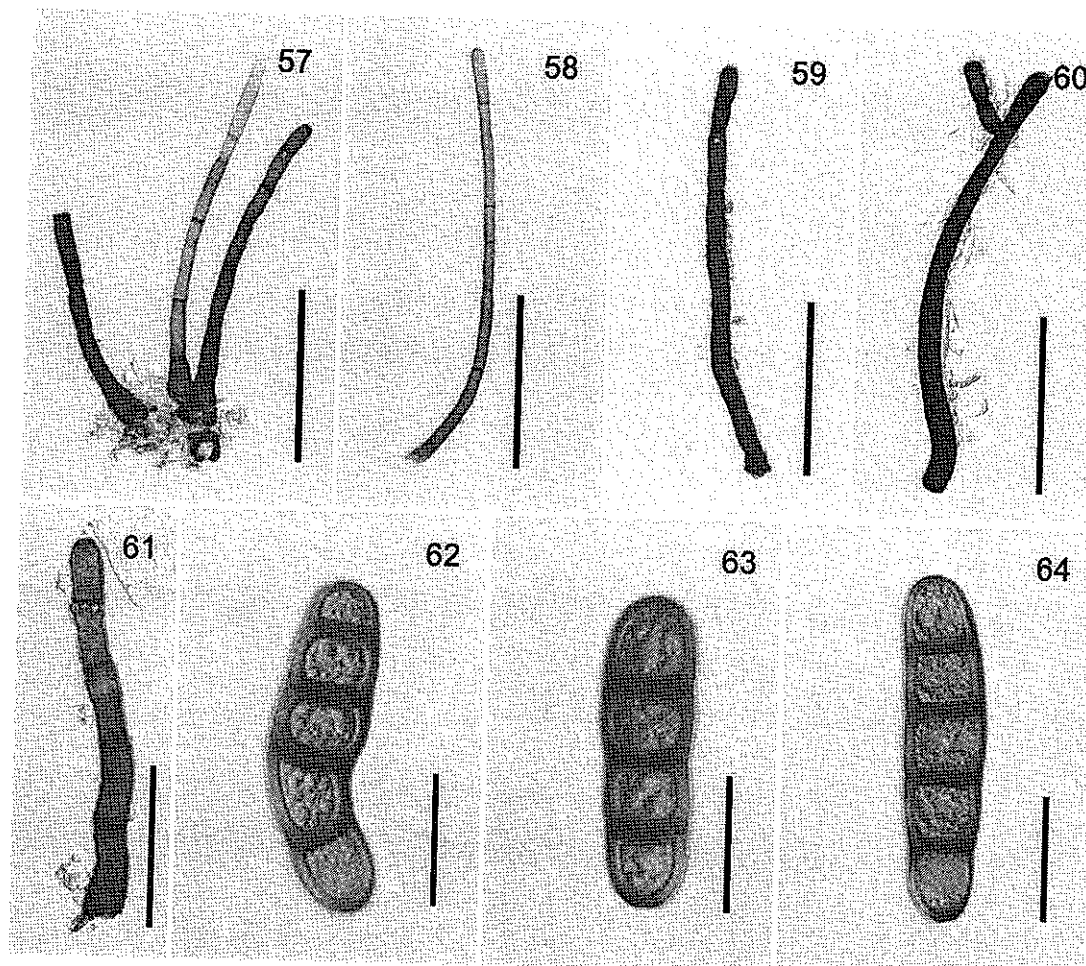
superficial, subglobose to globose, slightly immersed, base flattened, with central ostiole, but unclear, with a few sparse hair-like setae (FIGS. 32–34). Peridium 12–40 μ m thick, composed of 3–4 layers, comprising cells of *textura angularis*; inner layer cells pale brown, but cells of outer layer black (FIG. 35). Hamathecium comprising numerous narrow filiform pseudoparaphyses,



FIGS. 43–56. Anamorph of *Kürsteiniiothelia emarceis* in culture. 43. Germinating ascospore. 44. Colony on MEA. 45–47. Conidiophores and conidiogenous cells. 48–56. 2–3-septate conidia. Bars: 43, 48–56 = 10 μ m; 44 = 1 cm; 45–47 = 5 μ m.

1–2 μ m wide, hyaline, embedded in a gelatinous matrix (FIG. 36). Asci 88–140 $\times$ 18–24 μ m, eight-spored, bitunicate, fissitunicate, cylindrical to clavate, apically rounded, pedicel ca. 3–13 μ m long and with small ocular chamber ca. 2 μ m wide (FIGS. 37–39). Ascospores (23.5–)25–28 $\times$ 8–9(–10) μ m ($\bar{x}$ = 27 $\times$ 9 μ m, n = 20), biserial, ellipsoidal, slightly curved, septum median to supra-median, dull green, becoming brown to dark brown at maturity, one-septate, two-celled, smooth-walled, lacking a mucilaginous sheath (FIGS. 40–42).

Ascospores germinating on MEA within 12 h (FIG. 43). Colonies on MEA, reaching up to 2 cm diam in 7 d at 25–28 C and within a month covering Petri dish. Aerial mycelium, radiating outward, effuse, hairy, edge fimbriate, colony dense, dull green to dark brown. Mycelium immersed and superficial, dark brown; hyphae septate, ca. 5 μ m wide (FIG. 44). Conidiophores produced on MEA 32–92 μ m long, 5–7 μ m thick, branched at apex, brown to dark brown, smooth (FIGS. 45–50). Conidia (21–)27–28(–36) $\times$ 9–



FIGS. 57–64. Anamorph of *Kirschsteiniotelia emarceis* on natural substrate. 57–61. Conidiophores. 62–64. Conidia that are almost oblong and slightly curved. Bars: 57–61 = 100 μ m, 62–64 = 20 μ m.

13(–15) μ m, reddish brown to dark brown, 1–2(–3) transverse septate, constricted at septa, smooth-walled, rounded at the ends, fusiform to obclavate (FIGS. 51–56).

Anamorph also growing on type material. Conidiophores erect, 162–271 $\times$ 7–14 μ m, branched apically, brown to dark brown, smooth, conidia forming at apex (FIGS. 57–61). Conidia (40–)45–56(–67) $\times$ (10–)14–15(–17) μ m, oblong to obclavate, grayish brown to dark brown, 3–4(–5) septate, constricted at septa, smooth-walled (FIGS. 62–64).

Specimens examined. THAILAND, CHIANG MAI: Mae Taeng, Ban Pha Pae, Mushroom Research Center, ca. 900 msl. (N19°17.123' E 98°44.009'), on dead wood of unidentified tree, 6 Sep 2009, *Saranyaphat Boonmee* (HO-

LOTYPE MFLU10-0037), ex-type culture: MFLUCC10-0106 and IFRDC2181.

Habitat. On dead wood of unidentified tree.

Mode of life. Saprobic.

Distribution. Chiang Mai, Thailand.

Notes. *Kirschsteiniotelia emarceis* ascospores are similar to that of *K. populi* (Tracy & Earle) You Z. Wang, Aptroot & K.D. Hyde, *K. recessa* (Cooke & Peck) D. Hawksw., *K. umbrinoidea* (Pass.) You Z. Wang, Aptroot & K.D. Hyde, and *K. xera* (Fairm.) You Z. Wang, Aptroot & K.D. Hyde, being brown to dark brown, one-septate and have asymmetrical ends (Hawksworth 1985a, Wang et al. 2004). It differs from all other *Kirschsteiniotelia* species by having sparse ascomatal hairs (FIGS. 33–35). Its anamorph in culture

and on the natural substrate produce oblong to obclavate, dark brown conidia with several septa, typical of *Dendryphiopsis* (Hughes 1953, Figs. 43–64). The conidia of the anamorph in culture were smaller and lighter than on the substrate as with *K. lignicola*. It is not unusual that characters in culture differ from those of the substrate, but because we were unable to obtain an isolate from the anamorph we cannot unequivocally state these are the same species. The phylogenetic analysis shows *K. emarceis* to form a sister relationship with *K. aethiops* and *K. lignicola*, with a long branch length (Fig. 2).

DISCUSSION

Morphological characters of two new species of Kirschsteinioteliaceae.—An additional two new species (*K. lignicola* and *K. emarceis*) are described herein from Thailand and included in this family. Molecular phylogenetic analyses have demonstrated that the genus, as currently understood, is polyphyletic (Fig. 2) with *M. elaterascus* and *K. maritima* grouping in the Morosphaeriaceae and Mytiliniaceae respectively. The two new species from Thailand (*K. lignicola* and *K. emarceis*) grouped with the type species *K. aethiops* in a clade that is distinct from other families in the Dothideomycetes with good bootstrap support. Therefore a new family is introduced to accommodate these species. Morphologically they share common features as well as having anamorphs similar to the genus *Dendryphiopsis* (Hughes 1953). The anamorphs share straight to slightly curved, darkened conidiophores and oval-oblong, 2–3-septate, dark brown conidia (Figs. 16–31, 43–64).

Phylogenetic evidence.—Species currently classified in *Kirschsteiniotelia* are scattered in Capnodiales, Jahnuales, Mytilinidiales and Pleosporales (Fig. 2). Studies on morphology and phylogeny of *Kirschsteiniotelia* highlighted its uncertain status (Hawksworth 1985a, b; Barr 1987; Lumbsch and Lindemuth 2001; Wang et al. 2004; Nelsen et al. 2009; Ruibal et al. 2009; Schoch et al. 2006; Shearer et al. 2009; Lumbsch and Huhndorf 2010). Phylogenetic studies have indicated its polyphyly (Suetrong et al. 2009). *K. elaterascus* thus is transferred to the genus *Morosphaeria* (Morosphaeriaceae) based on morphological and molecular evidence. This is in agreement with studies by Shearer et al. (2009), Schoch et al. (2009) and Suetrong et al. (2009). *K. maritima* has been shown to be affiliated with the Mytiliniaceae in a well supported monophyletic clade (Schoch et al. 2009, Suetrong et al. 2009) and a new genus (*Halokirschsteiniotelia*) is erected to accommodate

it. However *H. maritima* has few morphological characters in common with the Mytiliniaceae, except they are all saprobic fungi found on non-living plants, especially on deadwood (Hawksworth 1985a; Boehm et al. 2009a, b). Finally *Kirschsteiniotelia aethiops* is the type of the new family *Kirschsteinioteliaceae* in a well supported clade along with the two new species described in this paper with a *Dendryphiopsis* anamorph. The taxonomic position of four species has been confirmed, while the affiliation of other taxa in the genus remains to be resolved. This study has advanced our understanding of the higher order taxonomy of a genus previously regarded as polyphyletic.

ACKNOWLEDGMENTS

This work was supported by the Thailand Research Fund BRG528002, the Global Research Network for Fungal Biology and King Saud University.

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Mycologia

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Published online before print January 2012, doi: 10.3852/11-089
Mycologia May/June 2012 vol. 104 no. 3 698-714

**Two new *Kirschsteiniothelia* species with
Dendryphiopsis anamorphs cluster in
Kirschsteiniotheliaceae fam. nov.**

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Abstract

Two new *Kirschsteiniothelia* species are proposed in this study; both were collected on decaying wood from Chiang Mai and Chiang Rai provinces in northern Thailand. The taxa were isolated and the morphological characters are described and illustrated. ITS, LSU and SSU combined sequence analysis showed taxa of *Kirschsteiniothelia* separating into three lineages: (i) *K. elaterascus* grouped within *Morosphaeriaceae* (Pleosporales); (ii) *K. maritima* clustered with *Mytilinidion* spp. as a sister group in the *Mytiliniaceae* clade; and (iii) the two new *Kirschsteiniothelia* species, which produce *Dendryphiopsis* anamorphs in culture, clustered with *K. aethiops* (the generic type) and the anamorph *D. atra*. The new family *Kirschsteiniotheliaceae* is introduced to accommodate taxa grouping with *K. aethiops*. *K. elaterascus* is transferred to *Morosphaeria* (*Morosphaeriaceae*) and a new genus *Halokirschsteiniothelia* is introduced to accommodate *K. maritima* (*Mytiliniaceae*).

Dothideomycetes *Kirschsteiniotheliaceae* new species phylogeny



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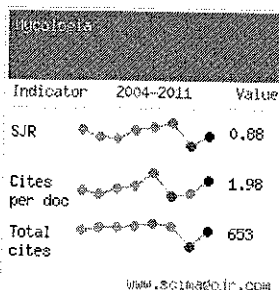
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