Cryptogamie, Mycologie, 2017, 38 (2): 259-273

Lauriomyces, a new lineage in the Leotiomycetes with three new species

Sayanh SOMRITHIPOL[a], E.B. Gareth JONES[b], A.H. BAHKALI[b], Satinee SUETRONG[c], Sujinda SOMMAI[a], Chalida CHAMOI[a], Peter R. JOHNSTON[d], Jerry A. COOPER[d] & Nattawut RUNGJINDAMAI[e]*

[a]Microbe Interaction and Ecology Laboratory (BMIE), National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Thailand Science Park, Phahonyothin Road, Khlong Nueng, Khlong Luang, Pathum Thani, Thailand

[b]Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Kingdom of Saudi Arabia, email: torperadgj@gmail.com

[c]Fungal Biodiversity Laboratory (BFBD), National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Thailand Science Park, Phaholyothin Road, Khlong Nueng, Khlong Luang, Pathum Thani 12120, Thailand

[d]Landcare Research, Private Bag 92170, Auckland 1142, New Zealand

[e]Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, 10520, Thailand

Abstract – *Lauriomyces* is an anamorphic genus comprising nine species, found growing on terrestrial leaf litter and wood in tropical habitats. The genus is characterized by solitary or synnematous, pigmented conidiophores bearing acropetal chains of unicellular, hyaline conidia. A multigene (SSU, LSU & 5.8S) analysis of *Lauriomyces* strains reveal three cryptic new species, which are described, illustrated, and published here: *L. acerosus, L. basitruncatus*, and *L. glumateus* spp. nov. *Lauriomyces glumateus* is characterized by narrowly oval conidia while conidia of *L. acerosus* are cylindrical with acute ends and those of *L. basitruncatus* are cylindrical with truncate base. The nine *Lauriomyces* species sampled form a monophyletic clade in the Leotiomycetes, with high molecular support and all with a morphology typical for the genus. The new combination *Dematioscypha catenata* is made for *Haplographium catenatum* in compliance with the one name protocol.

Anamorphic fungi / Leotiomycetes / Saprobic microfungi / Taxonomy

* Corresponding author: sayanh@yahoo.com

doi/10.7872/crym/v38.iss2.2017.259

INTRODUCTION

In an early investigation of tropical hyphomycetes and coelomycetes in Thailand, a large number of fungi were collected and many new species described based on morphological features (Pinruan *et al.*, 2002, 2004; Plaingam *et al.*, 2003, 2005; Somrithipol *et al.*, 2007). We continued their study using molecular techniques to determine their phylogenetic relationships (Somrithipol *et al.*, 2008; Rungjindamai *et al.*, 2008, 2012) with some higher taxa proposed; for example, Wiesneriomycetaceae (Suetrong *et al.*, 2014), Falcocladiaceae (Jones *et al.*, 2014) and Falcocladiales (Maharachchikumbura *et al.*, 2015).

Of the tropical hyphomycetes encountered, *Lauriomyces* is a genus whose species are frequently collected. *Lauriomyces* species are cosmopolitan in distribution and have been reported from different continents from both Northern and Southern Hemispheres; for example, *L. bellulus* from Switzerland (Crous & Wingfield, 1994) and Japan (Ohnuki *et al.*, 2009) and *Lauriomyces heliocephalus* from Brazil (Crous *et al.*, 2009). Some *Lauriomyces* in Thailand have been described morphologically and published as new species (Somrithipol *et al.*, 2006; Somrithipol & Jones, 2007) whereas the others have been identified and recorded as either known or unknown species. Their cultures were deposited in BIOTEC Culture Collection (BCC) without any sequencing information. Recently, Hernández-Restrepo *et al.* (2017) have introduced the family and order Lauriomycetaceae, Lauriomycetales to accommodate *Lauriomyces* species.

In this study, sequence data has been employed on the available *Lauriomyces* strains to determine their phylogenetic relationships. Specimens have been reexamined to determine if there are hidden species among them.

MATERIALS AND METHODS

DNA Extraction and PCR amplification

Lauriomyces cultures were grown on potato dextrose agar (PDA) and incubated at room temperature for two weeks. Actively growing mycelia were harvested and placed in a 1.5-ml Eppendorf tube. Genomic DNA was extracted using a CTAB method previously described by Suetrong *et al.* (2014). The purified genomic DNA was used as a DNA template for PCR amplification. Three regions of rDNA sequences, including the small subunit (SSU), large subunit (LSU) and internal transcribed spacer (ITS), were amplified using primers for NS1, and NS4 (for SSU) and LROR and LR7 (for LSU) and ITS5 and ITS4 (for ITS) (White *et al.*, 1990; Bunyard *et al.*, 1994) using DyNAzyme II DNA polymerase kit (Fizzymes, Espoo, Finland). The amplification cycles for SSU and LSU consisted of initialisation at 95°C (2 min); 35 cycles of denaturation at 95°C (1 min), annealing at 55°C (1 min) and extension at 72°C (2 min); final extension at 72°C (2 min) and final hold at 15°C. The PCR condition for ITS was initialization at 94°C (2 min); 35 cycles of 94°C (1 min), 55°C (1 min), 72°C (2 min); 72°C (10 min) and final hold at 15°C. The PCR amplification was performed using a DNA Engine DYAD ALD 1244 thermocycler (MJ Research, Inc., Waltham, MA). The PCR products were purified with NucleoSpin Extract DNA purification kit (Macherey-Nagel, Germany) and sequenced by Macrogen Inc. (South Korea) using the same primers as for amplification. All sequences newly generated in this study were deposited in the GenBank and the accession numbers are presented in Table 1.

Table 1. GenBank, herbarium and culture collection codes, substrate, and location generated from this study

Isolate	*Herbarium Code*	*Culture code*	*Date of collection*	*Substrate*	*Location*	*GenBank Accession number*		
						SSU	*LSU*	*ITS*
Lauriomyces sakaeratensis	SFC01642*	BCC15634**	1 May 2004	Decaying *Dipterocarpus costatus* fruit	Sakaerat Environmental Research Station (Nakhon Ratchasima)	KX649954	KX649965	KX649976
Lauriomyces cylindricus	SFC01649.1*	BCC18576**	15 Jun 2005	Decaying leaf	Nakhon Nayok	KX649955	KX649966	KX649977
Lauriomyces cylindricus	SFC01649.2	BCC18577	15 Jun 2005	Decaying leaf	Nakhon Nayok	KX649956	KX649967	KX649978
Lauriomyces cylindricus	SFC01659	BCC18598	15 Jun 2005	Decaying leaf	Khao Yai National Park (Nakhon Ratchasima)	KX649957	KX649968	KX649979
Lauriomyces cylindricus	SFC01804	BCC40985	1 Dec 2009	Decaying leaf (Unidentified)	Khao Sok National Park (Surat Thani)	KX649958	KX649969	KX649980
Lauriomyces basitruncatus	CC00049*	BCC33398**	13 May 2008	Dead leaf (Unidentified)	Khao Yai National Park (Nakhon Ratchasima)	KX649959	KX649970	KX649981
Lauriomyces ellipticus	SFC00424*	BCC4007**	28 Oct 1998	Decaying seed	Khao Sok National Park (Surat Thani)	KX649960	KX649971	KX649982
Lauriomyces acerosus	CC00030*	BCC33373**	13 May 2008	Dead leaf (Unidentified)	Khao Yai National Park (Nakhon Ratchasima)	KX649961	KX649972	KX649983
Lauriomyces glumaceous	CC00037	BCC33375	13 May 2008	Dead leaf (Unidentified)	Khao Yai National Park (Nakhon Ratchasima)	KX649962	KX649973	KX649984
Lauriomyces glumateus	CC00064	BCC33408	13 May 2008	Dead leaf (Unidentified)	Khao Yai National Park (Nakhon Ratchasima)	KX649963	KX649974	KX649985
Lauriomyces glumateus	SFC02049*	BCC22454**	1 Mar 2006	Dead leaf	Khao Yai National Park (Nakhon Ratchasima)	KX649964	KX649975	KX649986
Lauriomyces bellulus	JAC8612	ICMP15050	9 May 2003	*Weinmannia racemosa*	Bay of Plenty, New Zealand	KT960974	KT960975	EF029218

* Holotype ** Ex-type culture

Sequence alignment and phylogenetic analysis

Initially, three regions (SSU, LSU and 5.8S) of rDNA sequences of *Lauriomyces* strains were compared to sequences deposited in GenBank using the BLAST search tool in order to obtain the closest matched sequences (Altschul *et al.* 1990). The analyses used a concatenated alignment of SSU-LSU-5.8S rDNA with separate partitions created for each gene. The dataset was constructed based on representative taxa from major clades within the Leotiomycetes appearing in previously published papers (Johnston *et al.*, 2014a; Wang *et al.*, 2006a, 2006b; Baschien *et al.*, 2013). The sequences were aligned using MUSCLE (Edgar 2004) and adjusted manually where necessary using BioEdit 7.2.5 (Hall 1999). Manual gap adjustments were made to improve the quality of the sequence alignment. Ambiguously aligned regions were excluded. Missing data at the 5′-and 3′-end of partial sequences were coded by a '?'. The tree construction procedure was performed in three software including PAUP* 4.0b10 (Swofford 2002), MEGA6 (Tamura *et al.,* 2013) and MRBAYES (Huelsenbeck & Ronquist, 2001). The phylogenetic analyses of the combined dataset were performed using maximum parsimony (MP), Maximum Likelihood (ML) and Bayesian algorithms, respectively.

Maximum parsimony analyses were performed using PAUP*, with gaps treated as missing data. Trees were generated using 100 replicates of random stepwise addition of sequence and Tree-Bisection-Reconnection (TBR) branch-swapping algorithm, with all characters given equal weight. Branch support for all parsimony analyses was estimated by performing 1,000 bootstrap replicates (Felsenstein 1985) with a heuristic search of 10 random-addition replicates for each bootstrap replicate. For Maximum Likelihood method, the ML analysis was conducted in MEGA6. The best scoring ML Tree was estimated based on the General Time Reversible (GTR) model with 1,000 replicates. The model of substitution used for Bayesian analyses was chosen using the program Mrmodeltest 2.2 (Nylander 2004).

Independent Bayesian phylogenetic analysis was performed in MrBayes 3.0b4 (Huelsenbeck & Ronquist, 2001) using a uniform [GTR+I+G] model, Isetnst = 6 rates = invgamma; prsetstatefreqpr = dirichlet (1,1,1,1). Four Markov chains were run from random starting tree for 3,000,000 generations and sampled every 100 generations. The first 3,000 trees, which represented the burn-in phase of the analysis, were discarded, with 27,000 trees used for calculating posterior probabilities (BYPP) in the consensus tree. Statistical supports are shown on the node. These include Maximum Parsimony (MPBS) and Maximum Likelihood (MLBS) bootstrap values greater than 50% and Bayesian posterior probabilities (BYPP) greater than 0.95 are indicated on the node.

Specimen examination and morphological description

After the phylogenetic trees had been generated and the cryptic species revealed, their slide specimens were retrieved from BIOTEC Bangkok herbarium (BBH) for morphologically re-examination. Measurement, drawing and photographs were made; and new species are described.

RESULTS

Molecular analyses combined SSU, LSU, 5.8S data

Sequences of *Lauriomyces* species were aligned with other taxa from the Leotiomycetes. The combined SSU, LSU and 5.8S rDNA dataset had 109 taxa with 1,983 characters (base pairs). The dataset was analyzed separately by Maximum Parsimony, Maximum Likelihood and Bayesian inference, and the resulting trees from different methods compared. A maximum parsimony analysis of the dataset resulted in one most parsimonious tree with length = 2,420 (Consistency Index = 0.335160, Retention Index = 0.740371). The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible (GTR) model [1]. The tree with the highest log likelihood (-15761.5829) is shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Evolutionary analyses were conducted in MEGA6. The trees obtained from Maximum Parsimony and Bayesian analyses were topologically similar to the Maximum Likelihood tree. There was only minor swapping in some clades within the Leotiomycetes. Therefore, the tree generated by Maximum Likelihood method is shown (Fig. 1).

From an initial BLAST search, it clearly showed that *Lauriomyces* species belonged in the Leotiomycetes (Ascomycota). The dataset was refined several times and some families and orders related to *Lauriomyces* species were eventually included. Fifteen major clades resolved in Fig. 1 are topologically similar to those reported by Johnston *et al.* (2014a). Statistical supports for nodes indicating these clades within the Leotiomycetes were generally high.

Although *Lauriomyces* shared a node with the *Chaetomella* clade consisting of the three genera *i.e. Chaetomella, Philidium* and *Zoellneria*, there is no statistical support for this relationship. Our molecular phylogeny result confirms that *Lauriomyces* species are monophyletic and emerge as a distinct lineage within the Leotiomycetes. Statistical support of MPBS, MLBS and BYPP for almost every node within the *Lauriomyces* clade are generally high. The *Lauriomyces* clade can be divided into three subclades i.e. (1) Sub-clade I with *L. sakaeratensis*, (2) Sub-clade II with *L. cylindricus* and *L. basitruncatus* and (3) Sub-clade III with *L. ellipticus, L. acerosus, L. glumateus, L. heliocephalus* and *L. bellulus*. *Lauriomyces sakaeratensis* BCC15634 groups with other *Lauriomyces* with high support (99% MPBS, 100 MLBS % and 1.00 BYPP, respectively), but forms long branch length with other *Lauriomyces* spp.

Lauriomyces was separated from *Haplographium* by Castañeda-Ruiz & Kendrick (1990) chiefly by the persistent chains of conidia. Although a reasonable morphological concept (Somrithipol & Jones, 2007; Somrithipol *et al.*, 2006), it does always hold true. The current phylogenetic analysis reveals that one species placed in *Lauriomyces* by Castañeda-Ruiz & Kendrick (1990) (*L. catenatus*) clusters with *Haplographium delicatum,* the type species of *Haplographium*. *Lauriomyces catenatus* had earlier been transferred to *Haplographium* by Holubova-Jechova (1973). Johnston *et al.* (2014b) recommended that the genus name *Dematioscypha* be protected over the older name *Haplographium*. *H. delicatum* already has a name in *Dematioscypha*, *D. delicata* (Berk. & Broome) Hosoya, here we provide a new combination in *Dematioscypha* for *Haplographium catenatum*.

Molecular analyses of combined ITS, 5.8S, ITS2 data

To determine phylogenetic relationship among *Lauriomyces* species, the complete ITS regions including ITS1, 5.8S and ITS2 of all isolates were used.

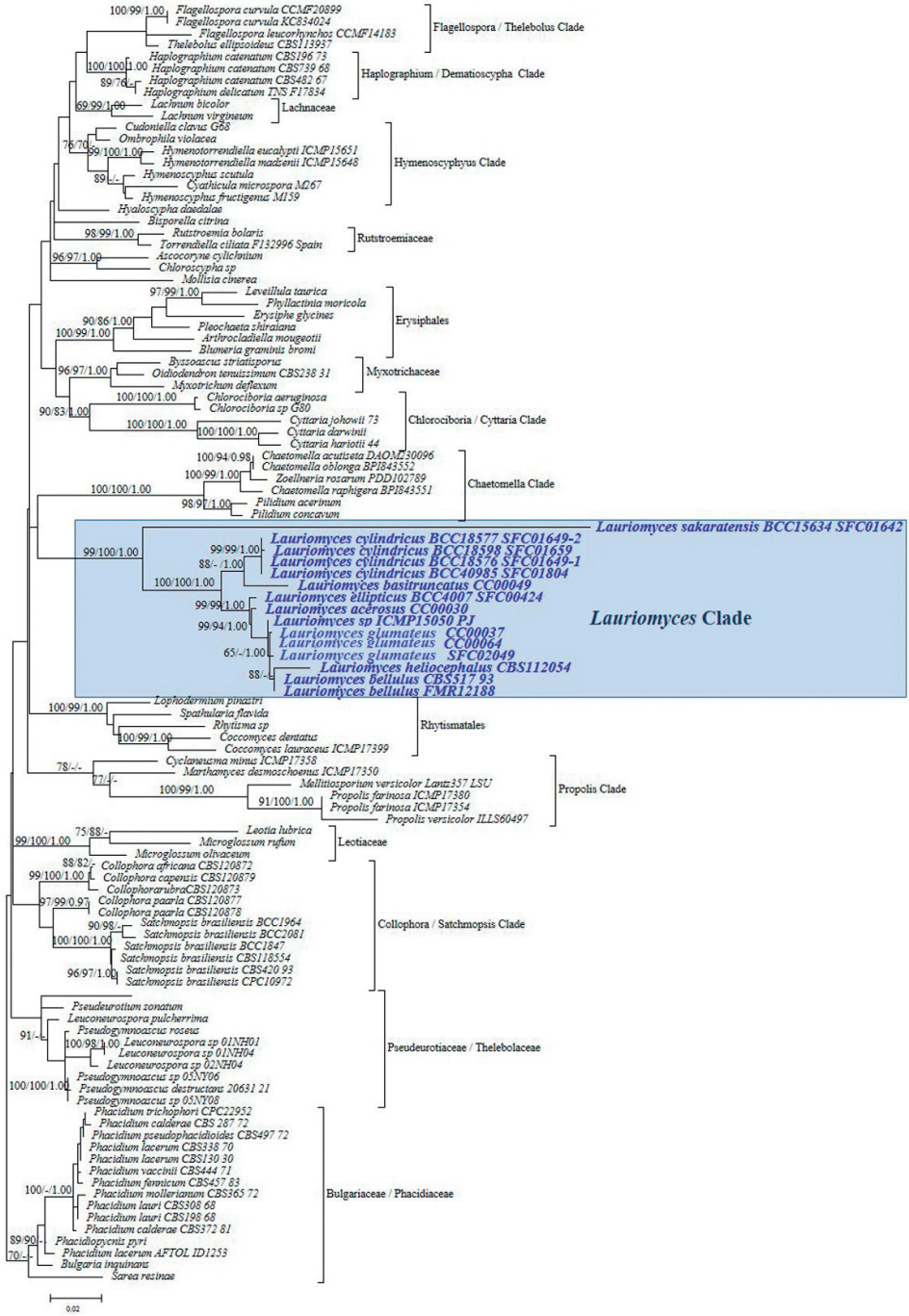


Fig. 1. Phylogenetic relationship of *Lauriomyces* species within the Leotiomycetes. The phylogenetic tree is generated based on a combined dataset of SSU, LSU and 5.8S rDNA sequences and the tree generated by Maximum Likelihood is shown.

The lengths of most ITS sequences were between 500-660 bps. The ITS sequences were automatically multiple aligned using MEGA Software and later manually adjusted to minimize the gaps and ambiguous sequences. Phylogenetic analyses of this dataset were carried out using the same set of programs previously described.

The results for the ITS combined dataset is shown in Figure 12. *Lauriomyces sakaratensis* groups distantly from other *Lauriomyces* species with a long branch length. *Lauriomyces basitruncatus* forms a clade with four isolates *of L. cylindricus* while *L. acerosus*, *L. bellulus, L. ellipticus, L. glumateus* and *L. heliocephalus* were placed in another subclade. The data indicates there is little genetic difference between *L. bellulus* and *L. glumateus* showing that they are closely related. There are slight differences between the three *L. glumateus* strains, but they differ in the longer run of T's near the end of the ITS2 sequence in *L. bellulus.* These two species differ in conidial measurements and morphology (Fig. 11). Moreover, *L. glumateus* lacks setae which are an important character in the delineation of *Lauriomyces* species.

TAXONOMY

Lauriomyces acerosus Somrithipol, Suetrong & E.B.G. Jones**, sp. nov. Figs 2-3, 8**

Mycobank MB 818635

Etymology: referring to the acerose shape of conidia.

Asexual morph: ***Colonies*** scattered, with white sporulation. Mycelium mostly immersed. ***Stroma*** and ***hyphopodia*** absent. ***Setae*** not observed. ***Conidiophores*** macronematous, mononematous. ***Stipe*** straight or flexuous, bulbous, thick and smooth-walled, brown to dark brown, paler toward the rounded and thin-walled apex, up to 110 µm long, 4-5 µm wide at the lower part, and 8 µm wide at the swelling base. ***Primary branches*** cylindrical to doliiform, thin- and smooth-walled, hyaline to subhyaline, in clusters at the apical stipe. ***Ramoconidia*** and ***conidia*** holoblastic, schizolytic secession, unicellular, hyaline to subhyaline, thin- and smooth-walled, in acropetal chains. ***Ramoconidia*** cylindrical to obclavate, 4-6 µm µm long and 1.0-1.5 µm wide. ***Conidia*** cylindrical with acute ends, 4-6 µm long ($\bar{x}$ = 4.7 ± 0.5 µm, n = 50), 0.8-1.2 µm wide at the broadest part ($\bar{x}$ = 1.1 ± 0.2 µm, n = 50). ***Sexual morph***: not observed.

Holotype: THAILAND, Nakhon Rashasima Province, on dead leaf in a microscopic slide, 13 May 2008, C. Chamoi, CC0030 in BBH; ex-type living culture (BCC33373).

Known distribution: Thailand.

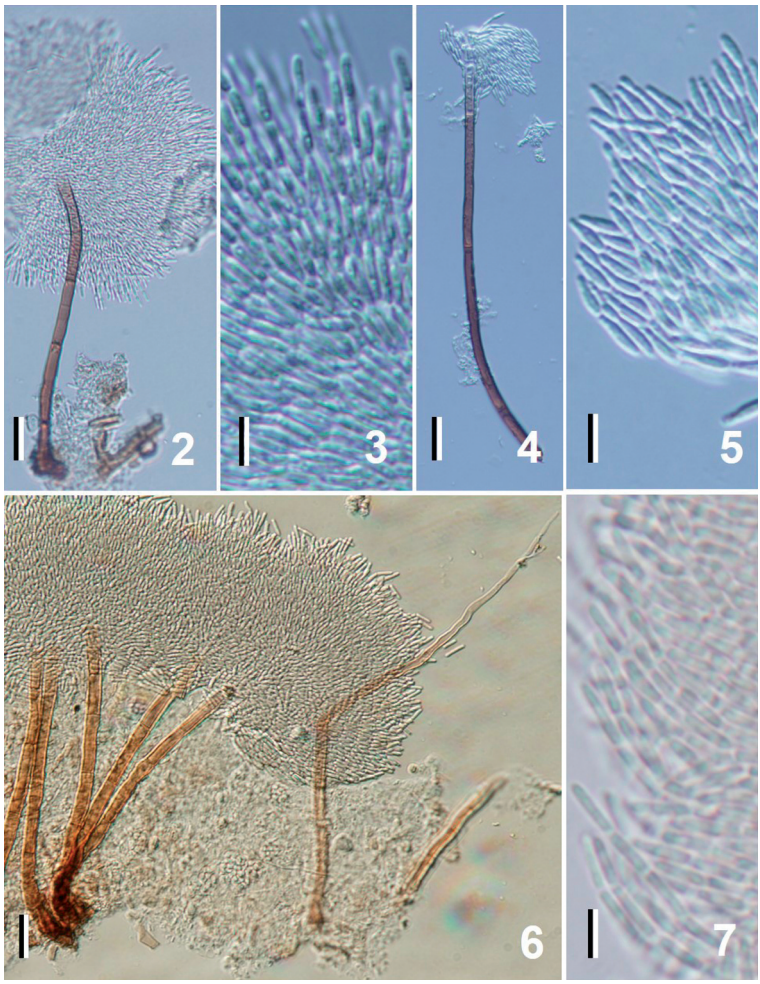
Notes: *Lauriomyces acerosus* morphologically differs from other species of *Lauriomyces* in having cylindrical conidia with acute ends and supported by sequence data.

Lauriomyces glumateus Somrithipol, Suetrong, & E.B.G. Jones, **sp. nov. Figs 4-5, 10**

Mycobank MB 818637

Etymology: referring to the glume shape of conidia.

Asexual morph: colonies scattered, with white sporulation. Mycelium mostly immersed. ***Stroma*** and ***hyphopodia*** absent. ***Setae*** not observed. ***Conidiophores*** macronematous, mononematous. ***Stipe*** straight or flexuous, bulbous, thick and



Figs 2-7. Morphological features of novel species under a light microscope (from holotypes). **2-3.** *Lauriomyces acerosus*. **4-5.** *Lauriomyces glumateus*. **6-7.** *Lauriomyces basitruncatus*. Scale bars: 2,4,6 = 10 µm; 3,5,7 = 20 µm.

smooth-walled, brown to dark brown, paler toward the rounded and thin-walled apex, up to 175 µm long, 3-5 µm wide at the lower part, and 7-10 µm wide at the swelling base. ***Primary branches*** cylindrical to doliiform, thin- and smooth-walled, hyaline to subhyaline, in clusters of 3-5 at the apical stipe. 6-10 µm long and 2-4 µm wide. ***Ramoconidia*** and ***conidia*** holoblastic, schizolytic secession, unicellular, hyaline to subhyaline, thin- and smooth- walled, in acropetal chains. ***Ramoconidia*** cylindrical to obclavate, 4-5 µm long and 1.0-1.2 µm wide. ***Conidia*** narrowly oval, 3-7 µm long ($\bar{x}$ = 4.1 ± 0.5 µm, n = 50), 0.8-1.5 µm wide ($\bar{x}$ = 1.1 ± 0.2 µm, n = 50). ***Sexual morph***: not observed.

Holotype: THAILAND, Nakhon Rashasima Province, on dead leaf in a microscopic slide, 1 Mar 2006, N. Sudhom, SFC2049 in BBH; ex-type living culture (BCC22454).

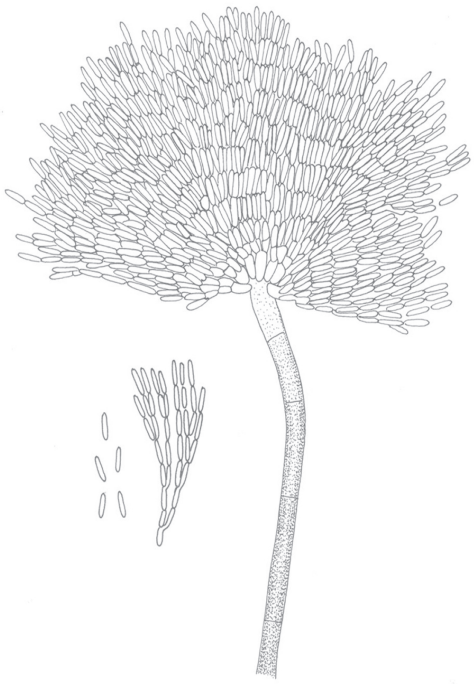


Fig. 8. Line drawing of *Lauriomyces acerosus* from the holotype. Scale bar = 10 µm.

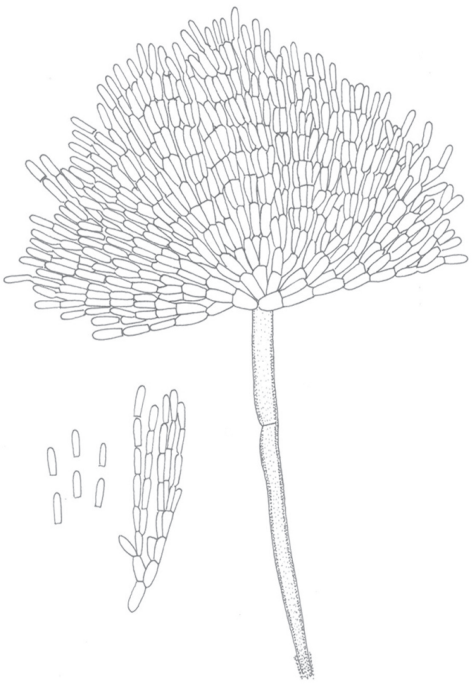


Fig. 9. Line drawing of *Lauriomyces basitruncatus* from the holotype. Scale bar = 10 µm.

Other material examined: THAILAND, Nakhon Rashasima Province, on dead leaf in a microscopic slide, 13 May 2008, C. Chamoi, CC0037 in BBH; ex-type living culture (BCC33375); THAILAND, Nakhon Rashasima Province, on dead leaf in a microscopic slide, 13 May 2008, C. Chamoi, CC0064 in BBH; ex-type living culture (BCC33408).

Known distribution: Thailand.

Notes: *Lauriomyces glumateus* groups in Sub-clade III along with *L. ellipticus, L. acerosus, L. heliocephalus* and *L. bellulus*, the various species differing in conidial measurements and other morphological detail. *L. glumateus* and *L. bellulus* are genetically almost identical, but *L. glumateus* lacks setae and has narrowly oval conidia while *L. bellulus* has cylindrical to ellipsoidal conidia rounding towards flattened, subtruncate ends. We report *L. bellulus* from New Zealand for the first time, the New Zealand specimen possessing the setae characteristic of *L. bellulus* (https://scd.landcareresearch.co.nz/specimen/ICMP%2015050). Morphologically *L. glumateus* and *L. sakaeratensis* both possess narrowly oval conidia with overlapping dimensions, but differ in that the new species lacks setae and sequence data places them in separate subclades. The presence of setae *in L. sakaeratensis* is confirmed by recent collections by Barbosa & Gusmão (2011) from Brazil.

Lauriomyces basitruncatus Somrithipol, Suetrong & E.B.G. Jones, **sp. nov. Figs 6-7, 9**

Mycobank MB 818636

Etymology: referring to the truncate base of conidia.

Fig. 10. Line drawing of *Lauriomyces glumateus* from the holotype. Scale bar = 10 µm.

Asexual morph: colonies scattered, with white sporulation. Mycelium mostly immersed. ***Stroma*** and ***hyphopodia*** absent. ***Setae*** simple, subulate, bulbous, smooth-walled, thick-walled and brown at the base, becoming thin-walled and paler toward the apex, up to 175 µm long, 4-5 µm wide at the lower part, and 7-10 µm wide at the swelling base. ***Conidiophores*** macronematous, mononematous. ***Stipe*** straight or flexuous, bulbous, thick and smooth-walled, brown to dark brown, paler toward the rounded and thin-walled apex, 80-100 µm long, 4-5 µm wide at the lower part, and 7-10 µm wide at the swelling base. ***Primary branches*** cylindrical to doliiform, thin- and smooth-walled, hyaline to subhyaline, in clusters of 3-5 at the apical stipe. 10-15 µm long and 3-5 µm wide. ***Ramoconidia*** and ***conidia*** holoblastic, schizolytic secession, unicellular, hyaline to subhyaline, thin- and smooth- walled, in acropetal chains. ***Ramoconidia*** cylindrical to obclavate, 5-6 µm long and 1.2-2.0 µm wide. ***Conidia*** cylindrical with truncate base and rounded apex, 4-6 µm long ($\bar{x}$ = 4.7 ± 0.6 µm, n = 50), 1.0-1.5 µm wide ($\bar{x}$ = 1.1 ± 0.1 µm, n = 50). ***Sexual morph***: not observed.

Holotype: THAILAND, Nakhon Rashasima Province, on dead leaf in a microscopic slide, 10 June 2008, C. Chamoi, CC0049 in BBH; ex-type living culture (BCC33398).

Known distribution: Thailand.

Notes: *Lauriomyces basitruncatus* morphologically differs from other species of *Lauriomyces* in having cylindrical conidia with a truncate base and rounded apex. *Lauriomyces basitruncatus* and *L. heliocephalus* show little difference in conidial morphology and both possess setae, however they group in different

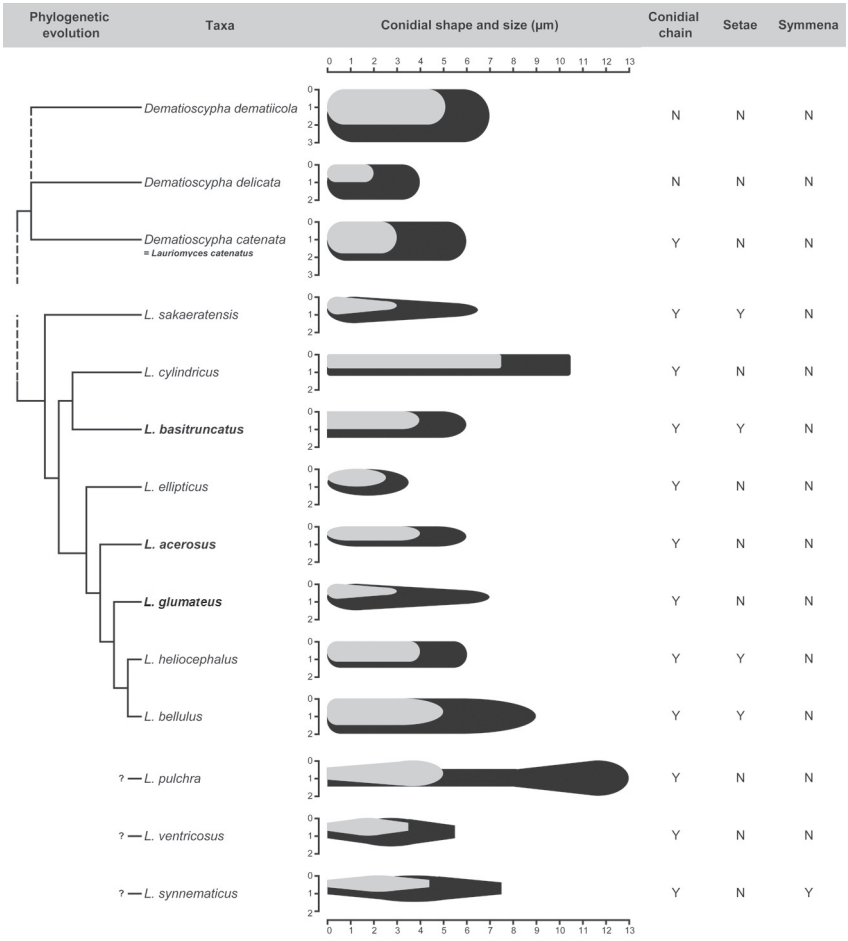


Fig. 11. Diagrammatic key of *Lauriomyces* species and the related taxa, showing phylogenetic relationship, conidial shape, maximum (black) and minimum (grey) sizes, and occurrence of conidial chain, seta, and synnema (Y: presence, N: absence).

subclades. *Lauriomyces basitruncatus* forms a sister group to *L. cylindricus*, but they differ in conidial morphology and the absence of setae in the latter species.

Dematioscypha catenata (Preuss) P.R. Johnst., **comb. nov.**

Registration identifier: MB821471

Basionym: *Stilbum catenatum* Preuss, *Linnaea* 24: 132 (1851) [MB157089]

Synonyms: *Stysanus catenatus* (Preuss) Sacc., *Sylloge Fungorum* 4: 622 (1886) [MB143602]

Haplographium catenatum (Preuss) Hol.-Jech., *Proceedings van de Koninklijke Nederlandse Akademie van Wetenschappen Section C* 76 (3): 301 (1973), [MB314894]

Lauriomyces catenatus (Preuss) R.F. Castañeda & W.B. Kendr., *University of Waterloo Biology Series* 32: 26 (1990) [MB267909]

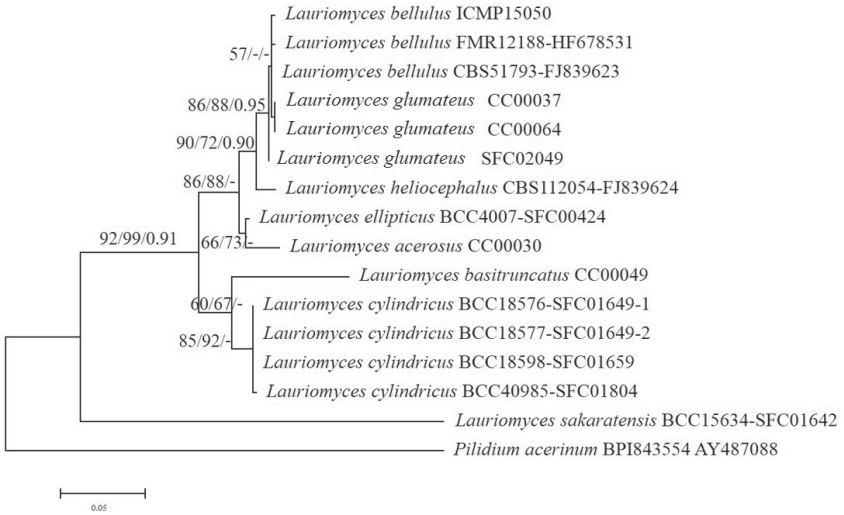


Fig. 12. ITS Phylogeny of 15 isolates of *Lauriomyces* spp. Three regions of ITS consisting of ITS1, 5.8S and ITS2, were used.

DISCUSSION

The Leotiomycetes have been morphologically and phylogenetically studied for decades (Wang *et al.*, 2006a, 2006b). The classification at higher taxonomic levels (familial and ordinal levels) is still not fully resolved and phylogenetic relationships among taxa within this taxonomic group are poorly understood. Selection of genes for sequencing is problematical and not resolved at this time. Considering phylogenetic studies of the Leotiomycetes based on a single gene, the SSU region is too highly conserved to differentiate the distinct lineages within the Leotiomycetes (Gernandt *et al.*, 2001), while ITS is too variable for determining phylogenetic relationships above species level (Wang *et al.*, 2006a). In this study, all three regions were combined into one dataset and ITS1 and ITS2 regions were excluded from our analysis due to alignment problem of their hypervariable sequences and a conserved part of 5.8S was aligned along with SSU and LSU. With the combined analysis, *Lauriomyces* emerges as a single and robust monophyletic group within the Leotiomycetes.

It is clear that molecular analysis based on combined datasets are essential for the taxonomic study of the Leotiomycetes. Recent studies have shown that the incorporation of protein coding genes in phylogenetic analyses may better resolve relationships of these fungi (e.g. Chen *et al.* 2015; Ge *et al.* 2014; Tanney *et al.* 2016) but the number of sequences of those genes currently available for the leotiomycetous fungi in GenBank is scant.

Species within *Lauriomyces* are mainly distinguished by conidial shape and size as previously mentioned (Somrithipol & Jones, 2007; Somrithipol *et al.*, 2006), and this is summarized in Fig. 11. Some species with little variation in conidial morphology can only be distinguished using molecular data. Apart from conidial morphology, setose and synnematous characteristics are specifically considered for

some species. All *Lauriomyces* have catenate conidia and do not have any known sexual morph. Based on the present phylogenetic study, species in Sub-clade III, although they have diverse conidial shapes ranging from oval to cylindrical forms, share the common characteristic in the rounded base of conidia. Two species in Sub-clade II are similar in having cylindrical conidia with slightly truncate base. There are no obvious morphological characters for distinguishing Sub-clade I from other Sub-clades although sequence data clearly separates them. The single species in this sub-clade (*L. sakaeratensis*) possesses the same conidial morphology as *L. glumateus* in the Sub-clade II, but differs in having setae. However, setae are characteristically present in some species in all Sub-clades.

Currently some 2,873 asexual genera of Ascomycota and Basidiomycota are known, while for 1,728 (60.15%) of these no sexual morph link has been established (Hyde *et al.*, 2011). For some ecological groups this is even greater, for example, 90% of freshwater aquatic hyphomycetes are not yet connected with sexual states. Seifert *et al.* (2011) noted that "although thousands of anamorph-teleomorph connections are known, the majority of hyphomycetes remain orphaned" Traditionally such connections have been established by culture techniques, but in recent years' sequence data has highlighted putative sexual stages of a variety of hyphomycetes. As part of a continuing study of Thai coelomycetes we have examined a selected number of genera at the molecular level resulting in their higher order placement, e.g. *Giulia* (Corticiaceae, Agariomycetes, Rungjindamai *et al.*, 2008), *Wiesneariomyces* (Wiesneriomycetaceae, Dothideomycetes, Suetrong *et al.*, 2014), *Falcocladium* (Falcocladiaceae, Sordariomycetes, Jones *et al.*, 2014), and *Canalisporium* (Savoyellales, Sordariomycetes, Boonyuen *et al.*, 2011). Seifert *et al.* (2011) list 59 hyphomycetous genera of Leotiomycetes, and a further 28 genera *incertae sedis*, with putative sexual morphs. They also stress that numbers of hyphomycetous genera associated with some higher taxa of the Dothideomycetes and Leotiomycetes are increasing when evidence drawn from phylogenetic studies are used in the classification of hyphomycetes. Thus, the current study confirms the placement of the asexual genus *Lauriomyces* as a member of the Leotiomycetes and contributes to the resolution of taxa within the class. Key to understanding relationships within the Leotiomycetes is combining data from fungi known only from the sexual state with those known only from the asexual state. Traditionally these different groups of species have been studied independently, although the current study and other recent studies have started to break down these barriers, e.g. Baschien *et al.* (2013) and Tanney *et al.* (2016).

Acknowledgments. We acknowledge the International Research Group Program (IRG-14-27), Deanship of Scientific Research, King Saud University, Saudi Arabia. S. Somrithipol acknowledges the Biodiversity Research and Training Program in Thailand for a grant BRT R_150007 supporting surveys, material collection, and the morphological study; BIOTEC-Novartis collaboration project for a grant P-14-51395 supporting the molecular study; and N. Sudhom for isolation of *L. glumateus* during her internship.

REFERENCES

ALTSCHUL S.F., GISH W., MILLER W., MYERS E.W. & LIPMAN D.J., 1990 — Basic local alignment search tool. *Journal of Molecular Biology* 215: 403-10.

BARBOSA F.R. & GUSMÃO L.F.P., 2011 — Conidial fungi from semi-arid Caatinga Biome of Brazil. Rare freshwater hyphomycetes and other new records. *Mycosphere* 2: 475-485.

BASCHIEN C., TSUI C.K., GULIS V., SZEWZYK U. & MARVANOVA L., 2013 — The molecular phylogeny of aquatic hyphomycetes with affinity to the Leotiomycetes. *Fungal Biology* 117: 660-72.

BOONYUEN N., CHUASEEHARONNACHAI C., SUETRONG S., SRI-INDRASUTDHI V., SIVICHAI S., JONES E.B.G. & PANG K.L., 2011 — *Savoryellales* (Hypocreomycetideae, Sordariomycetes): a novel lineage of aquatic ascomycetes inferred from multiple-gene phylogenies of the genera *Ascotaiwania*, *Ascothailandia* and *Savoryella*. *Mycologia* 103: 1351-1350.

BUNYARD B.A., NICHOLSON M.S. & ROYSE D.J., 1994 — A systematic assessment of *Morchella* using RFLP analysis of the 28S ribosomal RNA gene. *Mycologia* 86: 762-722.

CASTAÑEDA-RUÍZ R.F. & KENDRICK B., 1990 — Conidial Fungi from Cuba: II. *Univ. Waterloo Biol. Ser.* 33: 1-61.

CHEN C., VERKLEY G.C.M., SUN G., GROENEWALD J.Z. & CROUS P.W., 2015 — Redefining common endophytes and plant pathogens in *Neofabraea, Pezicula*, and related genera. Fungal Biology 120: 1291-1322.

CROUS P.W., BRAUN U., WINGFIELD M.J., WOOD A.R., SHIN H.D., SUMMERELL B.A., ALFENAS A.C., CUMAGUN C.J.R. & GROENEWALD J.Z., 2009 — Phylogeny and taxonomy of obscure genera of microfungi. *Persoonia* 22: 139-161.

CROUS P. W. & WINGFIELD M. J., 1994 — *Sporendocladia fumosa* and *Lauriomyces bellulus* sp. nov. from *Castanea cupules* in Switzerland. *Sydowia* 46: 193-203.

DELGADO G., MILLER A.N. & FERNÁNDEZ F.A., 2015 — *Curviclavula*, a new genus of anamorphic Helotiales (Leotiomycetes) isolated from air. *Mycological Progress* 14: 1-7.

EDGAR R.C., 2004 — MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792-1797.

FELSENSTEIN J., 1985 — Phylogenies and the comparative method. *The American Naturalist* 125: 1-15.

GE Z.-W., YANG Z.L., PFISTER D.H., CARBONE M., BAU T. & SMITH M.E., 2014 — Multigene Molecular phylogeny and biogeographic diversification of the earth tongue fungi in the genera *Cudonia* and *Spathularia* (Rhytismatales, Ascomycota). *PLoS ONE* 9(8): e103457.

GERNANDT D.S., PLATT J.L., STONE J.K., SPATAFORA J.W., HOLST-JENSEN A., HAMELIN R.C. & KOHN L.M., 2001 — Phylogenetics of Helotiales and Rhytismatales based on partial small subunit nuclear ribosomal DNA sequences. *Mycologia* 93: 915-933.

HALL T. A., 1999 — BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95-98.

HERNÁNDEZ-RESTREPO M., GENÉ J., CASTAÑEDA-RUIZ R.F., MENA-PORTALES J., CROUS P.W., & GUARRO J., 2017 — Phylogeny of saprobic microfungi from Southern Europe. Studies in Mycology DOI: 10.1016/j.simyco.2017.05.002.

HUELSENBECK J.P. & RONQUIST F., 2001 — MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754-755.

HYDE K.D., MCKENZIE E.H.C. & KOKO T.W., 2011 — Towards incorporating anamorphic fungi in a natural classification - checklist and notes for, 2010. *Mycosphere* 2: 1-88.

JOHNSTON P., PARK D., BARAL H.-O., GALÁN R., PLATAS G. & TENA R., 2014a — The phylogenetic relationships of *Torrendiella* and *Hymenotorrendiella* gen. nov. within the Leotiomycetes. *Phytotaxa* 177: 1-25.

JOHNSTON P.R., SEIFERT K.A., STONE J.K., ROSSMAN A.Y. & MARVANOVA L., 2014b — Recommendations on generic names competing for use in Leotiomycetes (Ascomycota). *IMA Fungus* 5: 91-120.

JONES E.B.G., SUETRONG S., CHENG W.-H., RUNGJINDAMAI N., SAKAYAROJ J., BOONYUEN N., SOMRITHIPOL S., ABDEL-WAHAB M.A. & PANG K.-L., 2014 — An additional fungal lineage in the Hypocreomycetidae (*Falcocladium* Species) and the taxonomic re-evaluation of *Chaetosphaeria chaetosa* and *Swampomyces* species, based on morphology, ecology and phylogeny. *Cryptogamie Mycologie* 35: 119-138.

MAHARACHCHIKUMBURA S.S.N., HYDE K.D., JONES E.B.G., MCKENZIE E.H.C., HUANG S.-K., ABDEL-WAHAB M.A., DARANAGAMA D.A., DAYARATHNE M., D'SOUZA M.J., GOONASEKARA I.D., HONGSANAN S., JAYAWARDENA R.S., KIRK P.M., KONTA S., LIU J.-K., LIU Z.-Y., NORPHANPHOUN C., PANG K.-L., PERERA R.H., SENANAYAKE I.C., SHANG Q., XIAO Y., BAHKALI A.H., KANG J., SOMRITHIPOL S., SUETRONG S., WEN T. & XU J., 2015 — Towards a natural classification and backbone tree for Sordariomycetes. *Fungal Diversity* 72: 199-301.

NYLANDER J.A.A., 2004 — Bayesian phylogenetics and the evolution of gall wasps. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology, Uppsala, 937: pp. 43.

OHNUKI T., YANO T., ONO Y., KOZUMA S., SUZUKI T., OGAWA Y. & TAKATSU T., 2009 — Haplofungins, novel inositol phosphorylceramide synthase inhibitors, from *Lauriomyces bellulus* SANK 26899 I. Taxonomy, fermentation, isolation and biological activities. *Journal of Antibiotics* 62: 545-549.

PINRUAN U., JONES E.B.G. & HYDE K.D., 2002 — Aquatic fungi from peat swamp palm: *Jahnula appendiculata* sp. nov. *Sydowia* 54: 242-247.

PINRUAN U., MCKENZIE E.H.C., JONES E.B.G. & HYDE K.D., 2004 — Two new species of *Stachybotrys*, and a key to the genus. *Fungal Diversity* 17: 147-157.

PLAINGAM N., SOMRITHIPOL S. & JONES E.B.G., 2003 — *Infundibulomyces*: a new genus of coelomycetes from Thailand. *Canadian Journal of Botany* 81: 732-737.

PLAINGAM N., SOMRITHIPOL S. & JONES E.B.G., 2005 — *Pseudorobillarda siamensis* sp. nov. and notes on *P. sojae* and *P. texana* from Thailand. *Nova Hedwigia* 80: 335-348.

HOLUBOVÁ-JECHOVÁ V., 1973 — Lignicolous Hyphomycetes from the Netherlands. *Proceedings van de Koninklijke Nederlandse Akademie van Wetenschappen Section C*. 76: 297-302.

HUELSENBECK J.P. & RONQUIST F.R., 2001 — MRBAYES: Bayesian inference of 380 phylogenetic trees. *Biometrics* 17:754-755.

RUNGJINDAMAI N., SAKAYAROJ J., PLAINGAM N., SOMRITHIPOL S. & JONES E.B.G., 2008 — Putative teleomorphs of the coelomycete genera *Chaetospermum, Guilia* and *Mycotribulus* based on nuclear large and small subunit rDNA. *Mycological Research* 112: 802-810.

RUNGJINDAMAI N., SAKAYAROJ J., SOMRITHIPOL S., PLAINGAM N. & JONES E.B.G., 2012 — Phylogeny of the appendaged coelomycete genera: *Pseudorobillarda*, *Robillarda*, and *Xepiculopsis* based on nuclear ribosomal DNA sequences. *Cryptogamie Mycologie* 33: 319-332.

SEIFERT K., MORGAN-JONES G., GAMS W. & KENDRICK B., 2011 — The genera of hyphomycetes. *CBS Biodiversity Series* 9: 1-997.

SOMRITHIPOL S. & JONES E.B.G., 2007 — *Lauriomyces cylindricus* and *Lauriomyces ellipticus* spp. nov., two new hyphomycetes from tropical forest of Thailand. *Nova Hedwigia* 84: 479-486.

SOMRITHIPOL S., KOSOL S. & JONES E.B.G., 2006 — *Lauriomyces sakaeratensis* sp. nov., a new hyphomycete on decaying *Dipterocarpus costatus* fruits from Sakaerat Biosphere Reserve, Thailand. *Nova Hedwigia* 82: 209-215.

SOMRITHIPOL S., SUDHOM N., TIPPAWAN S. & JONES E.B.G., 2007 — A new species of *Falcocladium* (Hyphomycetes) with turbinate vesicles from Thailand. *Sydowia* 58: 133-140.

SOMRITHIPOL S., SAKAYAROJ J., RUNGJIDAMAI N., PLAINGAM N. & JONES E.B.G., 2008 — Phylogenetic relationship of the coelomycete genus *Infundibulomyces* based on nuclear rDNA data. *Mycologia* 100: 735-741.

SUETRONG S., RUNGJINDAMAI N., SOMMAI S., RUNG-AREERATE P., SOMMRITHIPOL S. & JONES E.B.G., 2014 — *Wiesneriomyces* a new lineage of Dothideomycetes (Ascomycota) basal to Tubeufiales. *Phytotaxa* 176: 283-297.

SWOFFORD D.L., 2002 — PAUP*: phylogenetic analysis using parsimony, version 4.0b10. Sinauer Associates, Inc. Publishers, Sunderland.

TAMURA K., STECHER G., PETERSON D., FILIPSKI A. & KUMAR S., 2013 — MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30: 2725-2729.

TANNEY J.B., DOUGLAS B. & SEIFERT K.A., 2016 — Sexual and asexual states of some endophytic *Phialocephala* species of Picea. *Mycologia* 108: 255-280.

WANG Z., BINDER M., SCHOCH C.L., JOHNSTON P.R., SPATAFORA J.W. & HIBBETT D.S., 2006a — Evolution of helotialean fungi (Leotiomycetes, Pezizomycotina): A nuclear rDNA phylogeny. *Molecular Phylogenetics and Evolution* 41: 295-312.

WANG Z., JOHNSTON P. R., TAKAMATSU S., SPATAFORA J. W. & HIBBETT D. S., 2006b — Toward a phylogenetic classification of the Leotiomycetes based on rDNA data. *Mycologia* 98: 1065-1075.

WHITE T.F., BRUNS T., LEE S. & TAYLOR J., 1990 — Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: a guide to methods and applications (Innis M.A., Gelfand D.H., Sninsky F.S. & White T.J. eds). Academic Press, San Diego, U.S.A. pp. 315-322.