



***Wiesneriomyces* a new lineage of Dothideomycetes (Ascomycota) basal to Tubeufiales**

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Abstract

Wiesneriomyces is an asexual genus comprising two species, found growing on submerged leaves and terrestrial leaf litter in tropical habitats. The genus is characterized by conidiomata with setae, macronematous and branched conidiophores, and hyaline conidia in uniseriate chains connected by narrow isthmi. A multigene (SSU & LSU) analysis of 13 strains of *Wiesneriomyces* including *W. conjuntosporus* and *W. laurinus* formed a monophyletic clade in the Dothideomycetes with high support (98 BSMP, 99 BSML & 1.00 BYPP). The *Wiesneriomyces* clade is elevated to a higher taxonomic rank, the family Wiesneriomycetaceae, based on cultural, morphological and multi-gene phylogenetic evidence. The family forms a sister lineage to Tubeufiales with strong support.

Key words: hyphomycetes, LSU rDNA, new lineage, SSU rDNA, Wiesneriomycetaceae

Introduction

Some 60% of hyphomycetes (1,727 species) have no known sexual stages (Hyde *et al.* 2011), although recently molecular techniques have linked a number asexual taxa to families, orders or classes in the ascomycetes and basidiomycetes (Abdel-Wahab *et al.* 2010, Shenoy *et al.* 2007, Dai *et al.* 2012, Diederich *et al.* 2012, Rungjindamai *et al.* 2012, Zhang *et al.* 2012, Hyde *et al.* 2013). However, fewer than 90% of freshwater hyphomycetes have been linked to sexual morphs. In an ongoing investigation of tropical hyphomycetes and coelomycetes (Sivichai & Jones 2003, Plaingam *et al.* 2003, Pinruan *et al.* 2004, 2008, Somrithipol & Jones 2006, Somrithipol *et al.* 2006, 2007, 2008, Pinnoi *et al.* 2007, Rungjindamai *et al.* 2008, Jones *et al.* 2008), we have employed molecular data to determine their phylogenetic relationships. In this study we report on the genus *Wiesneriomyces*, which grows on leaf litter in temperate and tropical forests.

The genus *Wiesneriomyces* was introduced by Koorders (1907) with *W. javanicus* Koord., as the type species. However, this fungus had previously been described 40 years earlier under the name *Volutellaria laurina* Tassi (Tassi 1897). Clements & Shear (1931) proposed *Chaetosira* Clem. for *W. javanica* Koord., but Bisby (1949) pointed out that *Chaetosira* was an invalid change.

Volutellaria laurina Tassi was described from *Laurus nobilis* leaf litter by Tassi (1897). Soon after describing the fungus, Saccardo (in Tassi 1898) introduced the genus *Chaetopeltis* Sacc. and transferred *V. laurina* to the genus as *Chaetopeltis laurina* (Tassi) Sacc. Sydow & Sydow (1919) found that the fungal genus *Chaetopeltis* is the later homonym of the algal genus *Chaetopeltis* Berth. Therefore, they proposed a new genus *Tassia* H. & P. Syd. and made a new combination, *Tassia laurina* (Tassi) H. & P. Syd.

Kirk (1984) examined the holotype of *V. laurina* and opined that it should not be placed in the genus *Volutellaria* because its conidial morphology clearly differs from *Volutellaria acaroids*, the type species. Based on priority the generic name *Wiesneriomyces* is valid. Kirk (1984) proposed a new combination: *Wiesneriomyces laurinus* (Tassi) P.M. Kirk with *V. laurina*, *Wiesneriomyces javanicus*, *Chaetopeltis laurina* (Tassi) Sacc. and *Tassia laurina* (Tassi) H. & P. Syd. as synonyms. Subsequently, Kuthubutheen & Nawawi (1988) described *W. conjunctosporus* on submerged leaf litter from Pasoh Forest Reserve, Malaysia. Both species have been recently collected in Thailand and isolated into axenic culture. These form the basis for this phylogenetic study of the genus *Wiesneriomyces*.

Wiesneriomyces is characterized by conidiomata with thick-walled setae, macronematous and branched conidiophores, and hyaline conidia in uniseriate chains connected by narrow isthmi. Based on the original discussion, *W. conjunctosporus* differs from *W. laurinus* as it has larger setae, conidiomata and conidia and longer conidial chains (Kuthubutheen & Nawawi, 1988). However, a number of specimens collected later reveal the measurements of the two species overlapped. The most distinctive morphology to separate these species is the structure of the conidiomata. The conidioma of *Wiesneriomyces laurinus* is a prominent sporodochium with conidiophores and setae arising from its basal, pseudoparenchymatous stalk. On the other hand, the conidioma of *W. conjunctosporus* is a less prominent sporodochium encircled by a single row of setae which arise from the immersed mycelium.

Genera producing setose, pigmented sporodochia, conidiophores, and isthmospores resembling *Wiesneriomyces* include *Abgliophragma* R.Y. Roy & S. Gujarati and *Gliophragma* Subram. & B.C. Lodha. *Gliophragma* differs from *Wiesneriomyces* in possessing larger setae attached to the synnemata. Pirozynski (1972) and Matsushima (1971) treated *Gliophragma* as a synonym of *Wiesneriomyces*. *Phalangispora* Nawawi & Webster (1982) differs from *Wiesneriomyces* in having branched chains of conidia.

All *Wiesneriomyces* species have been collected from terrestrial leaf litter. *Wiesneriomyces conjunctosporus* was also collected from submerged litter in a stream (Kuthubutheen & Nawawi, 1988). *Wiesneriomyces laurinus* is widely reported from Java (Koorders 1907), India (Subramanian 1956), Panama (Manotis & Strain 1968), Papua New Guinea (Matsushima 1971), UK (Kirk 1983), Taiwan (Matsushima 1980), Australia (Shaw & Sutton 1985) and Malaysia (Kuthubutheen & Nawawi 1988), whereas *W. conjunctosporus* is known only from Malaysia and Thailand.

Materials and methods

Fungal cultures and maintenance

Two species of *Wiesneriomyces* were collected from Thailand. This comprised seven strains of strains of *W. conjunctosporus* and six strains of *W. laurinus*. These *Wiesneriomyces* species were isolated by single spore methodology as outlined by Chomnunti *et al.* (2011) from various locations and on separate occasions in Thailand (Table 1). All cultures are deposited at BIOTEC Culture Collection (BCC), their BCC numbers and accession numbers of two rDNA sequences are shown in Table 1.

DNA Extraction and PCR amplification

The cultures were grown on potato dextrose agar (PDA) and incubated at room temperature for two weeks. Actively growing mycelia were harvested *en masse* and placed in a 1.5 ml Eppendorf tube. Genomic DNA was extracted using a CTAB method (O'Donnell *et al.* 1997) which was modified and previously described by Rungjindamai *et al.* (2012). The purified genomic DNA was used as a DNA template for PCR amplification. Two regions of rDNA sequences including the small subunit (SSU) and large subunit (LSU) were amplified using primers for NS1, NS3, NS4 and NS6 (for SSU) and JS1 and LR7 (for LSU) (White *et al.* 1990, Bunyard *et al.* 1994) using DyNAzyme II DNA polymerase kit (Fizzymes, Espoo, Finland). PCR amplification was performed using a DNA Engine DYAD ALD 1244 Thermocycler (MJ Research, the US). The PCR conditions were 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, 49°C for 1 min and 72°C for 1 min 30 sec, a final extension at 72°C for 8 min and held at 25°C. 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.

Sequence alignment and phylogenetic analysis

SSU and LSU DNA sequences of 13 *Wiesneriomyces* strains were compared to sequences deposited in GenBank using the BLAST search tool to obtain the closest matched sequences (Altschul *et al.* 1990). Additional

representative taxa from the Dothideomycetes appearing in previously published papers were added into the dataset (Suetrong *et al.* 2011, Jones *et al.* 2012, Hyde *et al.* 2013). The SSU and LSU sequences were multiple aligned using Clustal W 1.6 (Thompson *et al.* 1994) and adjusted manually where necessary using BioEdit 7.5.0.3 (Hall 2006). Manual gap adjustments were made to improve the alignment. Ambiguously aligned regions were excluded. Missing data at the 5'-and 3'-end of partial sequences were coded by a '?'. The final alignment was again optimized by eye and manually corrected using Se-Al v. 2.0a8 (Rambaut 1996). The tree construction procedure was performed in PAUP* 4.0b10 (Swofford 2002). Phylogenetic trees were visualized using the program Treeview (Page 1996). The phylogenetic analyses of different datasets were performed using maximum parsimony, Bayesian and maximum likelihood algorithms.

TABLE 1. Sources, substratum, origin, date of collection and GenBank accession number of the two species of *Wiesneriomyces*.

Taxon	Source	Substratum and origin	Date of collection	GenBank accession number	
				SSU	LSU
<i>Wiesneriomyces</i>					
<i>W. conjunctosporus</i>	BCC18525	Decaying leaves from hill evergreen forest, Krabi	1 September 2005	KJ425436	KJ425450
<i>W. conjunctosporus</i>	BCC18606	Dead leaf from an urban park, Bangkok	4 October 2005	KJ425437	KJ425451
<i>W. conjunctosporus</i>	BCC18608	Leaf <i>Lagerstroenia speciosa</i> , Kasetsart University, Bang Khen, Bangkok	4 October 2005	KJ425438	KJ425452
<i>W. conjunctosporus</i>	BCC20803	Dead leaf from evergreen forest, Khao Yai National Park, Nakhon Ratchasima	23 March 2006	KJ425439	KJ425453
<i>W. conjunctosporus</i>	BCC4027	Leaf, Khao Yai National Park, Nakhon Ratchasima	21 November 1999	KJ425440	KJ425449
<i>W. conjunctosporus</i>	BCC40615	Twig or branch from tropical rain forest, Khao Nan National Park, Nakhon Si Thammarat	25 November 1999	KJ425441	KJ425454
<i>W. conjunctosporus</i>	BCC40633	Leaf from tropical rain forest, Khao Nan National Park, Nakhon Si Thammarat	10 February 2010	KJ425442	KJ435455
<i>W. laurinus</i>	BCC18609	Dead leaf, an urban park, Bangkok	4 October 2005	KJ425443	KJ425459
<i>W. laurinus</i>	BCC2922	Insect, Khao Yai National Park, Nakhon Ratchasima	26 October 2000	KJ425447	KJ425456
<i>W. laurinus</i>	BCC3922	Fruit from evergreen forest, Ton Nga Chang Wildlife Sanctuary, Songkhla	21 February 1998	KJ425448	KJ425457
<i>W. laurinus</i>	BCC40614	Leaf from tropical rain forest, Khao Nan National Park, Nakhon Si Thammarat	25 November 2009	KJ425444	KJ425460
<i>W. laurinus</i>	BCC40684	Leaf from tropical rain forest, Khao Nan National Park, Nakhon Si Thammarat	25 November 2009	KJ425445	KJ425461
<i>W. laurinus</i>	BCC9453	Dicotyledonous leaf from evergreen forest, Khao Yai National Park, Nakhon Ratchasima	3 July 2001	KJ425446	KJ425458

Maximum parsimony analyses were performed using PAUP v. 4.0b10 (Swofford 2002), 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. The consistency indices (CI; Kluge and Farris 1969), retention indices (RI; Farris 1989) and rescaled consistency indices (RC; Farris 1989) were calculated for each tree generated.

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 and 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 5,000,000 generations and sampled every 100 generations. The first 5,000 trees, which represented the burn-in phase of the analysis, were discarded, with 45,000 trees used for calculating posterior probabilities (BYPP) in the consensus tree. Posterior probabilities were obtained for each clade. Confident branch support is defined as Bayesian posterior probabilities equal or more than 0.95.

TABLE 2. Taxa used in the phylogenetic analysis and GenBank accession numbers, culture abbreviations are indicated where available.

Taxon	Cultures	SSU	LSU
<i>Acrospermum adeanum</i>	M133	EU940031	EU940104
<i>Acrospermum adeanum</i>	M133	EU940031	EU940104
<i>Acrospermum compressum</i>	M151	EU940012	EU940084
<i>Acrospermum gramineum</i>	M152	EU940013	EU940085
<i>Aigialus grandis</i>	BCC20000	GU479739	GU479775
<i>Aigialus mangrovis</i>	BCC33564	GU479742	GU479777
<i>Aigialus parvus</i>	BCC32558	GU479743	GU479778
<i>Aigialus rhizophorae</i>	BCC33573	GU479746	GU479781
<i>Aliquandostipite khaoyaiensis</i>	SS3321	EF175628	EF175650
<i>Aliquandostipite siamensis</i>	SS81.02	EF175645	EF175666
<i>Aquaticheirosora lignicola</i>	RK-2006a	AY736377	AY736378
<i>Ascocratera manglicola</i>	JK5262C	GU296136	GU301799
<i>Botryosphaeria ribis</i>	AFTOL1232	DQ678000	DQ678085
<i>Botryosphaeria stevensii</i>	AFTOL1572	DQ678012	DQ678064
<i>Botryosphaeria tsugae</i>	CBS418.64	AF271127	DQ767655
<i>Capnodium salicinum</i>	CBS131.34	DQ677997	DQ678050
<i>Cladosporium cladosporioides</i>	CBS170.54	DQ678057	DQ678004
<i>Cochliobolus heterostrophus</i>	CBS134.39	AY544727	AY544645
<i>Cystocoleus ebeneus</i>	L348	EU048573	EU048580
<i>Davidiella tassiana</i>	AFTOL1591	DQ678022	DQ678074
<i>Decorospora gaudefroyi</i>	CBS322.63	AF394542	N/A
<i>Delitschia winteri</i>	AFTOL1599	DQ678026	DQ678077
<i>Delphinella strobiligena</i>	AFTOL1257	DQ471029	DQ470977
<i>Dendryphiella arenaria</i>	CBS181.85	DQ471022	DQ470971
<i>Dendryphiopsis atra</i>	AFTOL273	DQ677996	DQ678046
<i>Devriesia strelitziae</i>	CBS122379	GU296146	GU301810
<i>Dothidea insculpta</i>	CBS189.58	DQ247810	DQ247802
<i>Dothidea sambuci</i>	AFTOL274	AY544722	AY544681
<i>Dothiora cannabinae</i>	AFTOL1359	DQ479933	DQ470984
<i>Elsinoe centrolobi</i>	AFTOL1854	DQ678041	DQ678094
<i>Flavobathelium epiphyllum</i>	MPN67	JN887382	N/A
<i>Helicoma chiangraiense</i>	MFLUCC-10-0115	JN865176	JN865188
<i>Helicomycetes roseus</i>	AFTOL1613	DQ678032	DQ678083
<i>Herpotrichia juniperi</i>	CBS200.31	DDQ678029	DQ678080
<i>Hortaea werneckii</i>	CBS708.76	GU296153	GU301818
<i>Hysteropatella clavisporea</i>	CBS247.34	DQ678006	AY541493
<i>Jahnula appendiculata</i>	BCC11445	FJ743445	FJ743439
<i>Jahnula bipileata</i>	AF220.1	EF175634	EF175656
<i>Jahnula sangamonensis</i>	F81-1	EF175641	EF175663
<i>Kirschsteiniothelia aethiops</i>	CBS109.53	AY016344	AY016361
<i>Kirschsteiniothelia lignicola</i>	MFLUCC10-0105	HQ441569	HQ441568
<i>Leptosphaeria doliolum</i>	CBS505.75	U43447	U43474
<i>Lophiostoma compressum</i>	IFRD2014	FJ795480	FJ795437
<i>Lophiostoma crenatum</i>	AFTOL1581	DQ678017	DQ678069
<i>Lophiostoma scabridisporum</i>	BCC22835	GQ925831	GQ925844
<i>Lophiostoma semiliberum</i>	CBS626.86	FJ795484	FJ795441
<i>Lophium mytilinum</i>	AFTOL1609	DQ678030	DQ678081
<i>Macrophomina phaseolina</i>	AFTOL1783	DQ678037	DQ678088
<i>Manglicola guatemalensis</i>	BCC20156	FJ743442	FJ743448
<i>Massaria inquinans</i>	MI19	HQ599444	N/A

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TABLE 2. (Continued)

Taxon	Cultures	SSU	LSU
<i>Massaria mediterranea</i>	M45	HQ599452	N/A
<i>Massaria platanoidea</i>	M7	HQ599457	N/A
<i>Massarina cisti</i>	CBS266.62	FJ795490	FJ795447
<i>Melanomma pulvis-pyrius</i>	CBS124080	GU456302	GU456323
<i>Mycosphaerella punctiformis</i>	AFTOL942	DQ471017	DQ470968
<i>Myriangium duriaei</i>	CBS260.36	AY016347	DQ678059
<i>Mytilinidion californicum</i>	EB0385	GU323186	GU323208
<i>Neophaeosphaeria filamentosa</i>	CBS102202	GQ387516	GQ387577
<i>Neottiosporina paspali</i>	CBS331.37	EU754073	EU754172
<i>Ochroconis anellii</i>	CBS284.64	KF282670	KF282651
<i>Ochroconis constricta</i>	CBS211.53	KF282671	KF282653
<i>Ochroconis minima</i>	CBS423.64	KF282680	KF282667
<i>Patellaria atrata</i>	BCC28876	GU371836	GU371828
<i>Patellaria atrata</i>	BCC28877	GU371837	GU371829
<i>Phyllobathelium anomalum</i>	MPN242	GU327698	GU327722
<i>Phyllobathelium firmum</i>	MPN545	JN887388	JN887404
<i>Phoma heteromorphospora</i>	CBS115.96	EU754089	EU754188
<i>Pleomassaria siparia</i>	CBS279.74	DQ678027	DQ678078
<i>Pleospora herbarum</i>	CBS191.86	DQ247812	DQ247804
<i>Rocella fuciformis</i>	AFTOL126	AY584678	AY584654
<i>Schismatomma decolorans</i>	DUKE0047570	AY548809	AY548815
<i>Scorias spongiosa</i>	AFTOL1594	DQ678024	DQ678075
<i>Stylothis puccinioides</i>	CBS193.58	AY016353	AY004342
<i>Tubeufia cerea</i>	AFTOL1316	DQ471034	DQ470982
<i>Tubeufia paludosa</i>	CBS120503	GU296203	GU301877
<i>Venturia populina</i>	CBS256.38	GU296206	GU323212
<i>Venturia pyrina</i>	ATCC38995	EF114739	EF114714

Maximum likelihood analyses (ML) were conducted in RAxML v. 7.2.6 (Stamatakis 2006). The dataset was partitioned according to each gene and separated codons (two partitions). The best-scoring ML tree was estimated using a general time reversible (GTR) + I model of sequence evolution with 1,000 pseudoreplicates. Maximum likelihood bootstrap value (BSML) equal or greater than 50% are given above each node. Maximum parsimony (BSMP, left) and likelihood (BSML, right) bootstrap value greater than 50% are given above the node. Bayesian posterior probabilities greater than 0.95 are given below each node (BYPP). The internodes that are highly supported by all bootstrap proportions (100%) and posterior probabilities (1.00) are shown as a thicker line. The rDNA sequences, consisting of SSU and LSU, were submitted into the GenBank database and the new sequences generated for this investigation are listed in Table 1. Species used in this study, sources and GenBank accession numbers are listed in Table 2.

Results

Molecular analysis

Sequences were aligned and analyzed separately by maximum parsimony, maximum likelihood and Bayesian inference, and the resulting trees compared. The combined SSU and LSU rDNA dataset had 91 taxa with 2317 characters, of which 1376 were constant, 224 variable and 717 parsimony informative. A maximum parsimony analysis of the dataset resulted in 34 most parsimonious trees (MPTs) with a length of 3502 steps (CI = 0.398, RI = 0.729 and RC = 0.290). *Rocella fuciformis* and *Schismatomma decolorans* were used as outgroups. One hundred successive searches using a rapid hill-climbing algorithm from distinct randomised starting trees in RAxML yielded a best scoring likely tree (data not shown) with log likelihood -21305.765992, alpha: 0.468586, invar: 0.367755, tree-Length: 4.032663, rate A <-> C: 0.920048, rate A <-> G: 2.698746, rate A <-> T: 0.929304, rate C

<-> G: 0.892172, rate C <-> T: 6.029737, rate G <-> T: 1.000000, freq pi(A): 0.256937, freq pi(C): 0.209368, freq pi(G): 0.280418, freq pi(T): 0.253277. Heuristic searches run for 100 replicates of random stepwise addition of sequence that treated gaps as missing data. Independent Bayesian phylogenetic analysis was performed using a uniform GTR+I+G model, as selected by hLRT in Mrmodeltest 2.2: [GTR+I+G] Prsetstatefreqpr = dirichlet (1,1,1,1), Lsetnst = 6 rates = invgamma. The trees obtained from maximum likelihood and Bayesian analyses were topologically similar to the maximum parsimony tree. One of the 34 most parsimonious trees is shown in Fig. 1.

Molecular phylogeny of Wiesneriomyces species

From an initial BLAST search, it was shown that *Wiesneriomyces* species belonged in the Dothideomycetes. Therefore, a dataset was constructed based on various major families and orders from the Dothideomycetes (Hyde *et al.* 2013, Boehm *et al.* 2009, Shearer *et al.* 2009). The dataset was refined several times and some orders which were related with the *Wiesneriomyces* species were eventually included, with *Roccella fuciformis* and *Schismatomma decolorans* as the out group taxa. Twelve orders within the Dothideomycetes were included: Acrospermales, Botryosphaerales, Capnodiales, Dothideales, Myrangiiales, Mytilinidiales, Jahnulales, Patellariales, Pleosporales, Strigulales, Tubeufiales and Venturiales. Statistical supports for nodes indicating the orders and families within the Dothideomycetes were generally high.

The *Wiesneriomyces* species form a separate clade with the Botryosphaerales, Mytilinidiales, Patellariales, Pleosporales and Tubeufiales in the Dothideomycetes (Fig. 1); there is, however, no statistical support for this clade. Among these five orders, *Wiesneriomyces* fell between the Patellariales and Tubeufiales. The closest sister group was the order Tubeufiales with high statistical support (94 BSMP, 98 BSML and 1.00 BYPP). *Wiesneriomyces conjunctosporus* and *W. laurinus* strains are monophyletic with high statistical support (98 BSMP, 99 BSML and 1.00 BYPP), but with a clear separation.

Taxonomy

Wiesneriomycetaceae Suetrong, Rungjindamai, Somrithipol. & E.B.G. Jones, *fam. nov.* MycoBank: MB809097

Sexual state: Unknown. Asexual state: *Colonies* effuse, consisting of scattered conidiomata. *Mycelium* immersed. *Conidiomata* sporodochial, solitary to gregarious, setose. Setae subulate, septate, pigmented, thick-walled, arising from basal pseudoparenchymatous stalk or mycelium immersed in the substrata. *Conidiophores* macronematous, septate, branched. *Conidiogenous cells* holoblastic, discrete, determinate, terminate, clavate to cylindrical. *Conidia* hyaline but greenish, yellowish, or whitish in mass, non-septate; the conidium at each end of a chain tapered, intermediate ones more or less cylindrical, formed in uniseriate, acropetal chains and connected by narrow isthmi.

Taxonomic rank: Fungi, Ascomycota, Dothideomycetes, Order *incertae sedis*, Wiesneriomycetaceae

Type:—*Wiesneriomyces* Koord., Verh. K. Akad. Wet., tweede sect. 13(4): 246 (1907) MB 10438

≡ *Chaetosira* Clem., in Clements & Shear, Genera Fungi: 223, 403 (1931) [MB 7589]

= *Gamospora* Sacc., Atti dell'Istituto Veneto Scienze 3: 719 (1885) [MB 22161]

= *Gamonaemella* Fairm., Proc. Rochester Acad. Sci. 6: 123 (1922) [MB 8304]

= *Setodoichium* Bat. & Cif., Atti dell'Istituto Botanico della Università e Laboratorio Crittogamico di Pavia 15: 59 (1957) [MB 9908]

= *Aschizotrichum* Rieuf, Cahiers de la Recherche Agronomique 15: 61 (1962) [MB 7238]

= *Abgliophragma* R.Y. Roy & Gujarati, Trans. Br. mycol. Soc. 49(3): 363 (1966) [MB 7001]

= *Chaetopeltis* Sacc., in Tassi, Bulletin Labor. Orto Bot. de R. Univ. Siena 1: 14 (1898) [MB 7580]

= *Tassia* Syd. & P. Syd., Anns mycol. 17(1): 44 (1919) [MB 10178]

Type:—*Wiesneriomyces laurinus* (Tassi) P.M. Kirk

Key to species of *Wiesneriomyces*

1. Conidiomata with incurved setae arising from a sporodochial stalk *W. laurinus*
1. Conidiomata with setae arising from immersed mycelium around a sporodochial stalk *W. conjunctosporus*

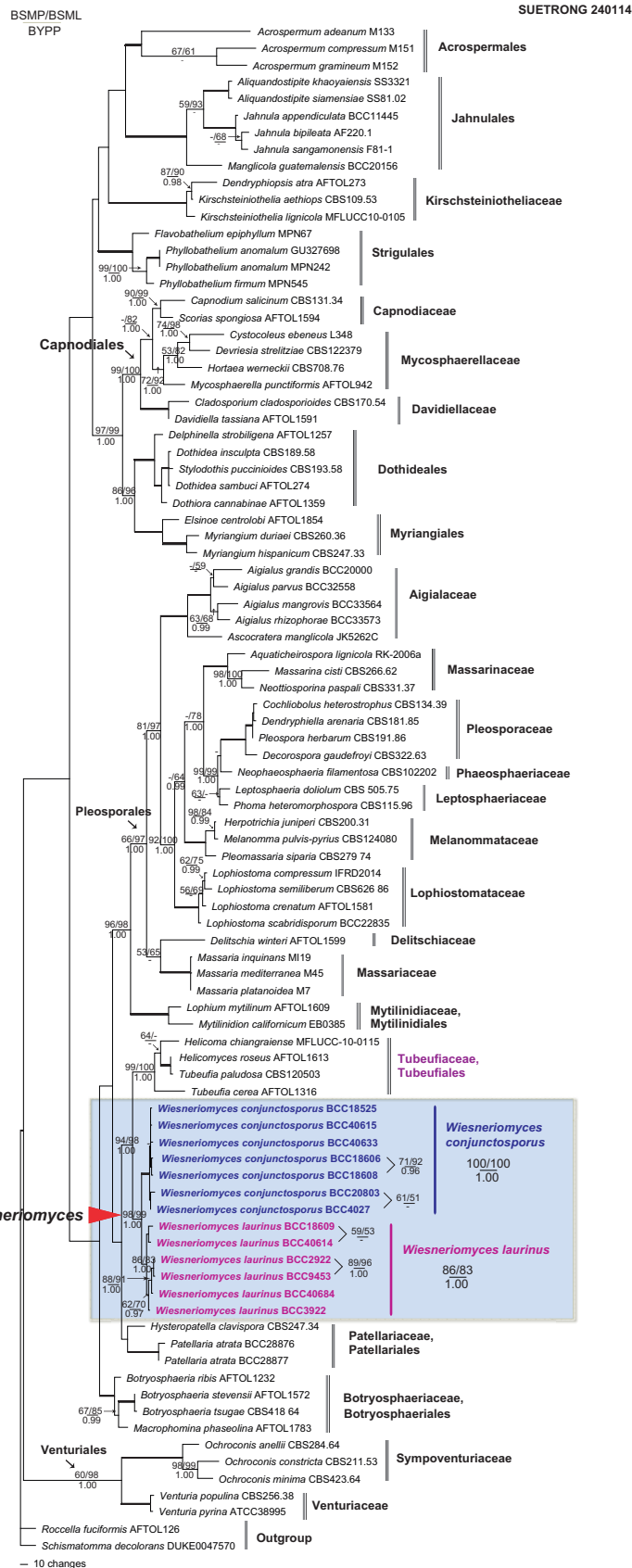


FIGURE 1. One of 34 most parsimonious trees obtained from heuristic searches of an alignment of combined SSU and LSU rDNA sequences (TL = 3502 steps, CI = 0.398, RI = 0.729 & RC = 0.290). *Roccella fuciformis* and *Chismatomma decolorans* are used as outgroups. Maximum parsimony (BSMP, left) and likelihood (BSML, right) bootstrap values greater than 50% are given above the nodes. Bayesian posterior probabilities greater than 0.95 are given below each node (BYPP). Scale bar indicates 10 character state changes. The internodes that are highly supported by all bootstrap (100%) and posterior probabilities (1.00) are shown as a thicker line.

Notes:—There is no known sexual stage and induction of cultures to form ascomata has been unsuccessful. Further collections are required, perhaps on more woody substrata, to yield the sexual stage. The family is well placed in the Dothideomycetes with strong bootstrap support and grouping with the order Tubeufiales, an order with many asexual genera (Boonmee *et al.* 2014).

Morphological characterization and phylogenetic reconstruction have shown that members of the *Wiesneriomyces* clade form a monophyletic clade in the Dothideomycetes, and are a sister lineage to Tubeufiales (Tsui & Berbee 2006, Schoch *et al.* 2006, 2009, Boonmee *et al.* 2011, Zhang *et al.* 2011, 2012, Hyde *et al.* 2013). The *Wiesneriomyces* clade is elevated to a higher taxonomic rank, the family Wiesneriomycetaceae, based on cultural, morphological and multi-gene phylogenetic evidence (Fig. 1). The Wiesneriomycetaceae is a monophyletic clade as revealed by analysis of combined molecular data and differs from other families Wiesneriomycetaceae in the Dothideomycetes with strong support (Fig. 1).

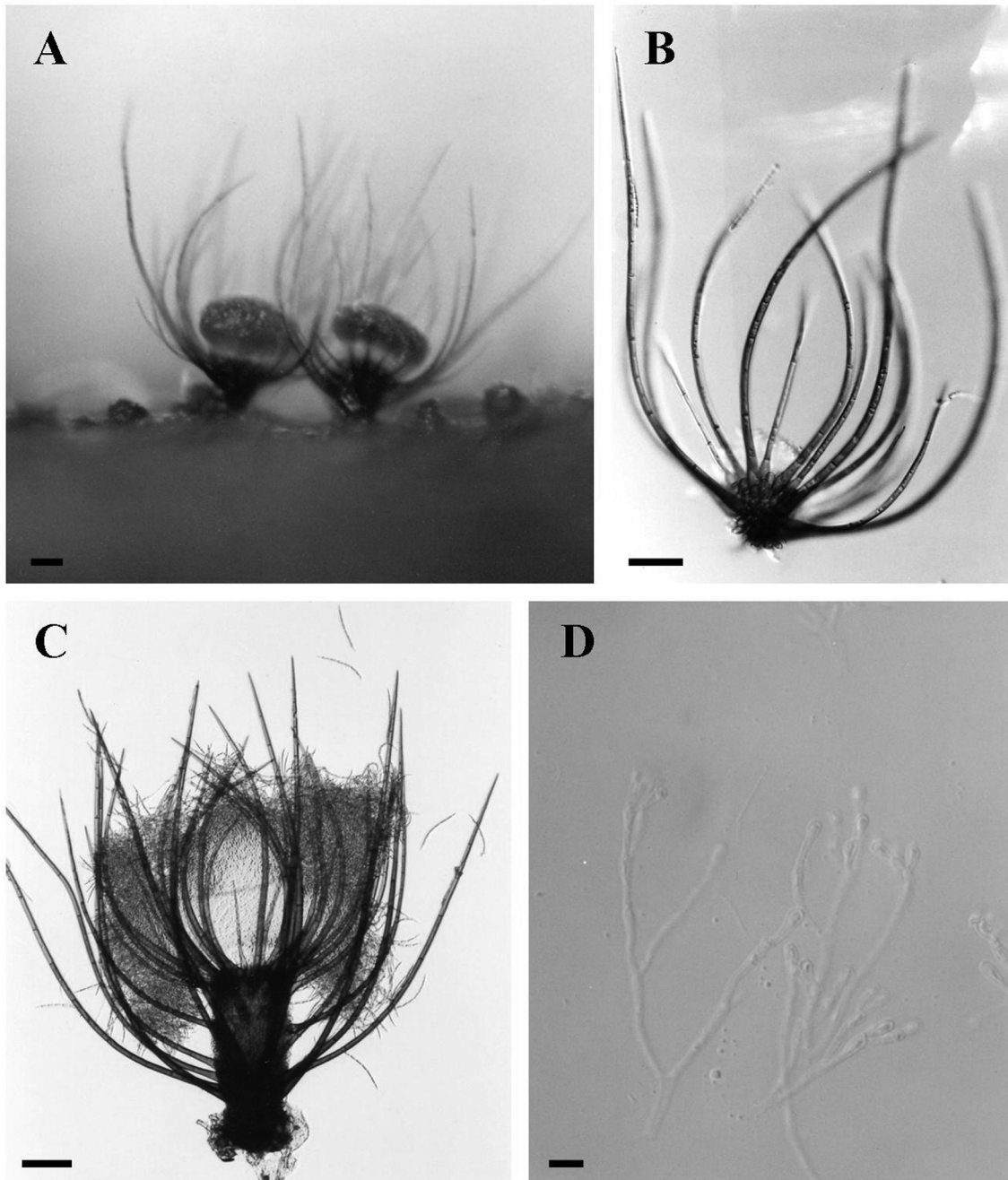


FIGURE 2. *Wiesneriomyces laurinus*. A. Sporodochia on natural substratum. B–C. Sporodochia with incurved setae arising from a prominent sporodochial stalk. D. Branched conidiophores. Scale bars: A–C = 20 μm , D = 10 μm .

TABLE 3. Morphological comparison between *W. conjunctosporus* and *W. laurinus*.

Morphological structures	<i>W. conjunctosporus</i>	<i>W. laurinus</i>
Sporodochia	Usually with a prominent stalk	Usually without a prominent stalk
Setae	Incurved, arising from basal stalk of the sporodochium	Slightly curved or flexuous, arising from mycelium immersed in the substratum, and usually encircling the sporodochium
Conidiophores	Irregularly branched, usually in the alternate step	Regularly branched, usually in a penicillate cluster
Conidiogenous cells	Longer, cylindrical	Shorter, clavate

Accepted species of *Wiesneriomyces*

Wiesneriomyces laurinus (Tassi) P.M. Kirk 1984 (Figs 2, 3)

= *Volutellaria laurina* Tassi 1897 MycoBank MB 107371

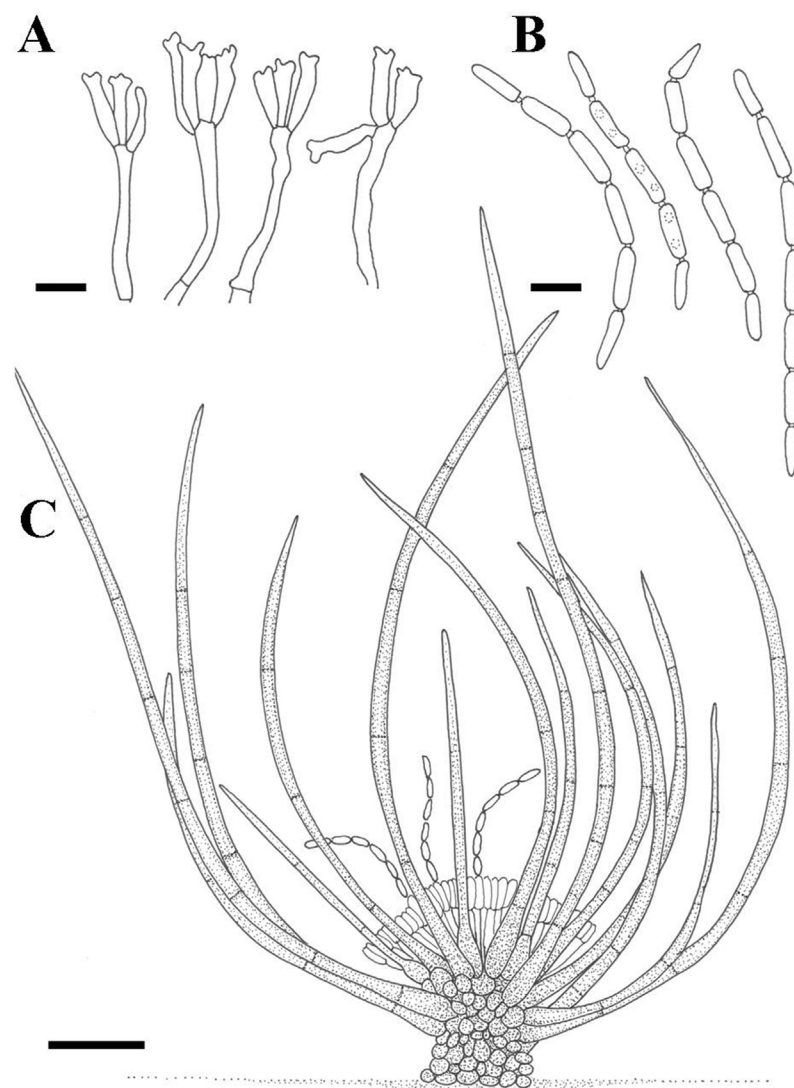


FIGURE 3 *Wiesneriomyces laurinus*. A. Branched conidiophores. B. Conidia. C. Sporodochia with incurved setae. Scale bars: A–B = 10 μ m, C = 20 μ m.

Saprobic on leaf litter. Sexual state: Unknown. Asexual state: Colonies effuse, olivaceous-brown to dark brown. *Conidiomata* sporodochial, dispersed, on a dark pseudoparenchymatous stalk, consisting of 2–3 μ m, dark, inflated, thick-walled cells. *Setae* subulate, apex acute, septate, thick-walled, pale brown, arising at the margins of the stalk

base, up to 600 μm tall, 9–14 μm at the base. *Conidiophores* erect, pale brown to subhyaline at the base, hyaline towards the apex, septate, up to 50 μm tall, 3–4 μm wide, 1–3 times branched, with primary, secondary and tertiary branches. *Conidiogenous cells* terminal, clavate, hyaline, 8–12 μm long, 3–4 μm . *Conidia* hyaline, aseptate, cylindrical, 10–12 μm long, 3–4.5 μm wide, with a short isthmi separating the conidia. Conidia in chains (up to 15) arising from a single conidiogenous cell.

Material examined:—THAILAND. Bangkok Province: dead leaf, an urban park, 4 October 2005, *S. Somrithipol* (BCC18609, SFC01930); Nakhon Ratchasima Province: Khao Yai National Park, insect, 26 October 2000, *S. Somrithipol* (SFC00755); Songkhla Province: Ton Nga Chang Wildlife Sanctuary, fruit from evergreen forest, 21 February 1998, *S. Somrithipol* (SFC00151); Nakhon Si Thammarat Province: Khao Nan National Park, leaf from tropical rain forest, 25 November 2009, *S. Somrithipol* (SFC01773); Nakhon Si Thammarat Province: Khao Nan National Park, leaf from tropical rain forest, 25 November 2009, *S. Somrithipol* (SFC01783); Nakhon Ratchasima Province: Khao Yai National Park, dicotyledonous leaf from evergreen forest, 3 July 2001, *S. Somrithipol* (BCC18609).

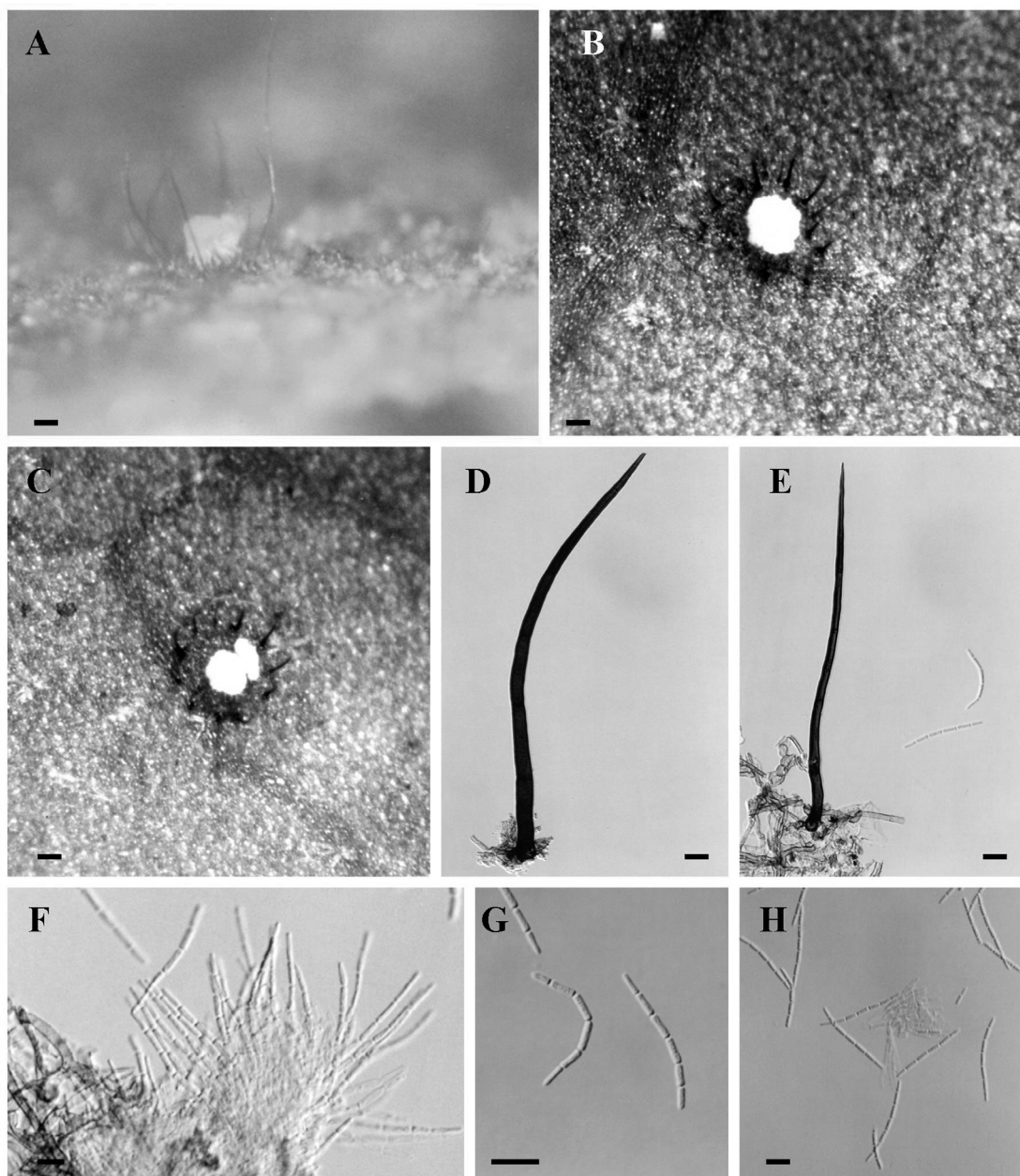


FIGURE 4 *Wiesneriomyces conjunctosporus*. A–C. Sporodochia on natural substratum. D–E. Setae. F. Conidiophores. G–H. Conidia. Scale bars: A–C = 200 μm , D–E, G–H = 20 μm , F = 10 μm .

Sexual state: Unknown. Asexual state: Colonies effuse, olivaceous-brown to dark brown. *Conidiomata* sporochial solitary to gregarious, on a dark pseudoparenchymatous stalks, consisting of 2–3 μm , dark, inflated, thick-walled cells, with 2–10 setae arising at the margins of the stalk base. Setae subulate with acute apex, septate, thick-walled 2–3 μm diam., dark brown up to 650 μm tall, 9–11 μm wide at the base. *Conidiophores* semi-mucronematous, arising close to one another, pale brown to subhyaline at the base, hyaline towards the apex, septate, 1–3 times branched, 37–60 μm tall, 1.5–3.0 μm wide, with primary, secondary and tertiary branches. *Conidiogenous cells* terminal, cylindrical, hyaline, integrated. *Conidia* hyaline, aseptate, cylindrical, 15–21 μm cells connected by narrow short isthmi separating the conidia. Conidia in chains arising (280–360 μm long) from a single conidiogenous cell.

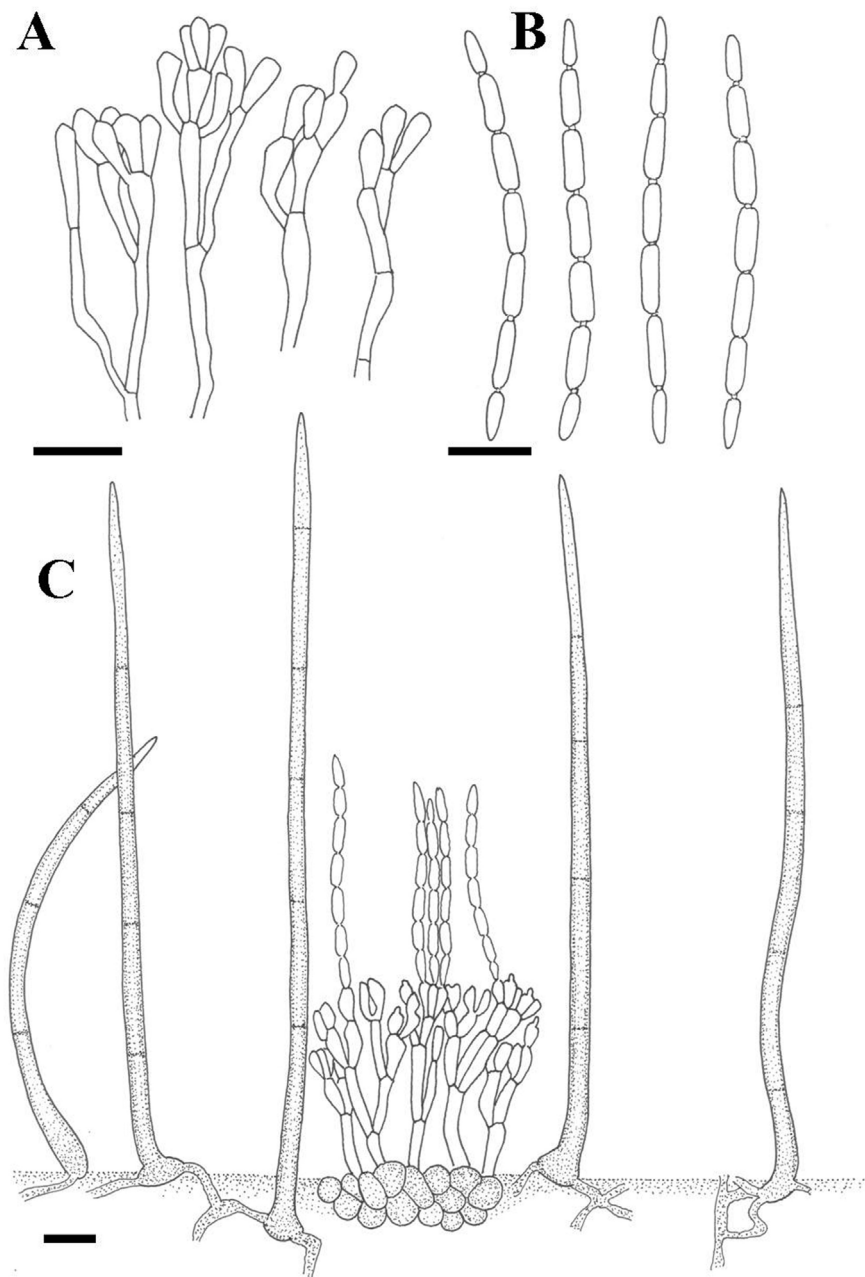


FIGURE 5 *Wiesneriomyces conjunctosporus*. A. Conidiophores. B. Conidia. C. Sporochia and setae. Scale bars: A–B = 20 μm , C = 50 μm .

Habitat and host range:—Terrestrial leaf litter and submerged litter in a stream.

Geographical distribution:—India, Java, Malaysia, Papua New Guinea, Taiwan, Thailand.

Material examined:—THAILAND. Krabi Province: decaying leaves from evergreen forest, 1 September 2005, *S. Somrithipol* (SFC01911); Bangkok Province: dead leaf from an urban park, 4 October 2005, *S. Somrithipol* (SFC01927); Bangkok Province: Kasetsart University, Bang Khen, leaf *Lagerstroemia speciosa*, 4 October 2005, *S. Somrithipol* (SFC01929); Nakhon Ratchasima Province: Khao Yai National Park, dead leaf from evergreen forest, 23 March 2006, *S. Somrithipol* (SFC01688); Nakhon Ratchasima Province: Khao Yai National Park, leaf, 21 November 1999, *S. Somrithipol* (SFC00425); Nakhon Si Thammarat Province: Khao Nan National Park, twig or branch from tropical rain forest, 25 November 1999, *S. Somrithipol* (SFC01776); Nakhon Si Thammarat Province: Khao Nan National Park, leaf from tropical rain forest, 10 February 2010, *S. Somrithipol* (SFC01828).

Discussion

Wiesneriomyces has been referred to the Ascomycota (Mycobank) and, Pezizomycotina genera *incertae sedis* (Index Fungorum 2013, Hyde *et al.* 2011) as no molecular studies have been undertaken for this genus. The 13 *Wiesneriomyces* strains form a well-supported monophyletic clade with members of the Tubeufiaceae, Tubeufiales as a sister clade (Fig. 1). Sequence data and morphological observations strongly support the delineation of *W. conjunctosporus* from *W. laurinus*. *Wiesneriomyces* also has an affinity with three sequences of the Patellariales (*Patellaria atrata* and *Hysteropetella clavispoa*), but bootstrap support between these two taxa are lacking.

The data set comprises the Dothideales, Botryosphaerales, Jahnulales, Mytilinidiales, Pleosporales and Venturiales in the class Dothideomycetes, with the *Wiesneriomyces* clade not grouping with any of these orders. In a separate analysis we included sequences of three *Pseudorobillarda* species (*P. phragmitis*, *P. texana*, *P. siamensis*) which form a well-supported clade with the Mytilinidiales as a sister clade (data not shown, Suetrong *et al.* 2009, Rungjindamai *et al.* 2012). The two *Wiesneriomyces* species form a unique new lineage in the Dothideomycetes which warrants the introduction of a new family, Wiesneriomycetaceae.

Tsui & Barbee (2006), in a study of many asexual and sexual genera of the *Tubeufia sensu lato*, pointed out that traditional generic characters of this family such as conidia, conidiophores, thickness of the conidial filament were more useful for species delimitation than for clarifying higher level relationships. Although these genera possess similar barrel-shaped spore forms, they were polyphyletic and had evolved in different ascomycete orders. They speculated that the convergent of spore forms may represent adaptation for dispersal to aquatic environments. This shows that molecular systematics is vital in classification of fungi related to the Tubeufiaceae.

Boonmee *et al.* (2014) will introduce the order Tubeufiales which is strongly supported by molecular data as well as ecological and morphological data; this includes a distinct habitat, and helicosporous asexual morphs. Although *Wiesneriomyces* species are related to the Tubeufiales and Patellariales, it is a new lineage within the Dothideomycetes rather than a subgroup of those two orders. This stresses the importance of molecular data to clarify the complexity of dothideomycetous taxa.

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