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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.

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Kirk (1984) examined the holotype of *V. laurina* and opinioned 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. conjunctosporous* 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 & Nawai 1988), whereas *W. conjunctosporus* is know 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	
Wiesneriomyces				SSU	LSU
W. conjunctosporus	BCC18525	Decaying leaves from hill evergreen forest, Krabi	1 September 2005	KJ425436	KJ425450
W. conjunctosporus	BCC18606	Dead leaf from an urban park, Bangkok	4 October 2005	KJ425437	KJ425451
W. conjunctosporus	BCC18608	Leaf *Lagerstroenia speciosa*, Kasetsart University, Bang Khen, Bangkok	4 October 2005	KJ425438	KJ425452
W. conjunctosporus	BCC20803	Dead leaf from evergreen forest, Khao Yai National Park, Nakhon Ratchasima	23 March 2006	KJ425439	KJ425453
W. conjunctosporus	BCC4027	Leaf, Khao Yai National Park, Nakhon Ratchasima	21 November 1999	KJ425440	KJ425449
W. conjunctosporus	BCC40615	Twig or branch from tropical rain forest, Khao Nan National Park, Nakhon Si Thammarat	25 November 1999	KJ425441	KJ425454
W. conjunctosporus	BCC40633	Leaf from tropical rain forest, Khao Nan National Park, Nakhon Si Thammarat	10 February 2010	KJ425442	KJ435455
W. laurinus	BCC18609	Dead leaf, an urban park, Bangkok	4 October 2005	KJ425443	KJ425459
W. laurinus	BCC2922	Insect, Khao Yai National Park, Nakhon Ratchasima	26 October 2000	KJ425447	KJ425456
W. laurinus	BCC3922	Fruit from evergreen forest, Ton Nga Chang Wildlife Sanctury, Songkhla	21 February 1998	KJ425448	KJ425457
W. laurinus	BCC40614	Leaf from tropical rain forest, Khao Nan National Park, Nakhon Si Thammarat	25 November 2009	KJ425444	KJ425460
W. laurinus	BCC40684	Leaf from tropical rain forest, Khao Nan National Park, Nakhon Si Thammarat	25 November 2009	KJ425445	KJ425461
W. laurinus	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
Acrospermum adeanum	M133	EU940031	EU940104
Acrospermum adeanum	M133	EU940031	EU940104
Acrospermum compressum	M151	EU940012	EU940084
Acrospermum gramineum	M152	EU940013	EU940085
Aigialus grandis	BCC20000	GU479739	GU479775
Aigialus mangrovis	BCC33564	GU479742	GU479777
Aigialus parvus	BCC32558	GU479743	GU479778
Aigialus rhizophorae	BCC33573	GU479746	GU479781
Aliquandostipite khaoyaiensis	SS3321	EF175628	EF175650
Aliquandostipite siamensiae	SS81.02	EF175645	EF175666
Aquaticheirospora lignicola	RK-2006a	AY736377	AY736378
Ascocratera manglicola	JK5262C	GU296136	GU301799
Botryosphaeria ribis	AFTOL1232	DQ678000	DQ678085
Botryosphaeria stevensii	AFTOL1572	DQ678012	DQ678064
Botryosphaeria tsugae	CBS418.64	AF271127	DQ767655
Capnodium salicinum	CBS131.34	DQ677997	DQ678050
Cladosporium cladosporioides	CBS170.54	DQ678057	DQ678004
Cochliobolus heterostrophus	CBS134.39	AY544727	AY544645
Cystocoleus ebeneus	L348	EU048573	EU048580
Davidiella tassiana	AFTOL1591	DQ678022	DQ678074
Decorospora gaudefroyi	CBS322.63	AF394542	N/A
Delitschia winteri	AFTOL1599	DQ678026	DQ678077
Delphinella strobiligena	AFTOL1257	DQ471029	DQ470977
Dendryphiella arenaria	CBS181.85	DQ471022	DQ470971
Dendryphiopsis atra	AFTOL273	DQ677996	DQ678046
Devriesia strelitziae	CBS122379	GU296146	GU301810
Dothidea insculpta	CBS189.58	DQ247810	DQ247802
Dothidea sambuci	AFTOL274	AY544722	AY544681
Dothiora cannabinae	AFTOL1359	DQ479933	DQ470984
Elsinoe centrolobi	AFTOL1854	DQ678041	DQ678094
Flavobathelium epiphyllum	MPN67	JN887382	N/A
Helicoma chiangraiense	MFLUCC-10-0115	JN865176	JN865188
Helicomyces roseus	AFTOL1613	DQ678032	DQ678083
Herpotrichia juniperi	CBS200.31	DDQ678029	DQ678080
Hortaea werneckii	CBS708.76	GU296153	GU301818
Hysteropatella clavispora	CBS247.34	DQ678006	AY541493
Jahnula appendiculata	BCC11445	FJ743445	FJ743439
Jahnula bipileata	AF220.1	EF175634	EF175656
Jahnula sangamonensis	F81-1	EF175641	EF175663
Kirschsteiniothelia aethiops	CBS109.53	AY016344	AY016361
Kirschsteiniothelia lignicola	MFLUCC10-0105	HQ441569	HQ441568
Leptosphaeria doliolum	CBS505.75	U43447	U43474
Lophiostoma compressum	IFRD2014	FJ795480	FJ795437
Lophiostoma crenatum	AFTOL1581	DQ678017	DQ678069
Lophiostoma scabridisporum	BCC22835	GQ925831	GQ925844
Lophiostoma semiliberum	CBS626.86	FJ795484	FJ795441
Lophium mytilinum	AFTOL1609	DQ678030	DQ678081
Macrophomina phaseolina	AFTOL1783	DQ678037	DQ678088
Manglicola guatemalensis	BCC20156	FJ743442	FJ743448
Massaria inquinans	MI19	HQ599444	N/A

......*continued on the next page*

TABLE 2. (Continued)

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

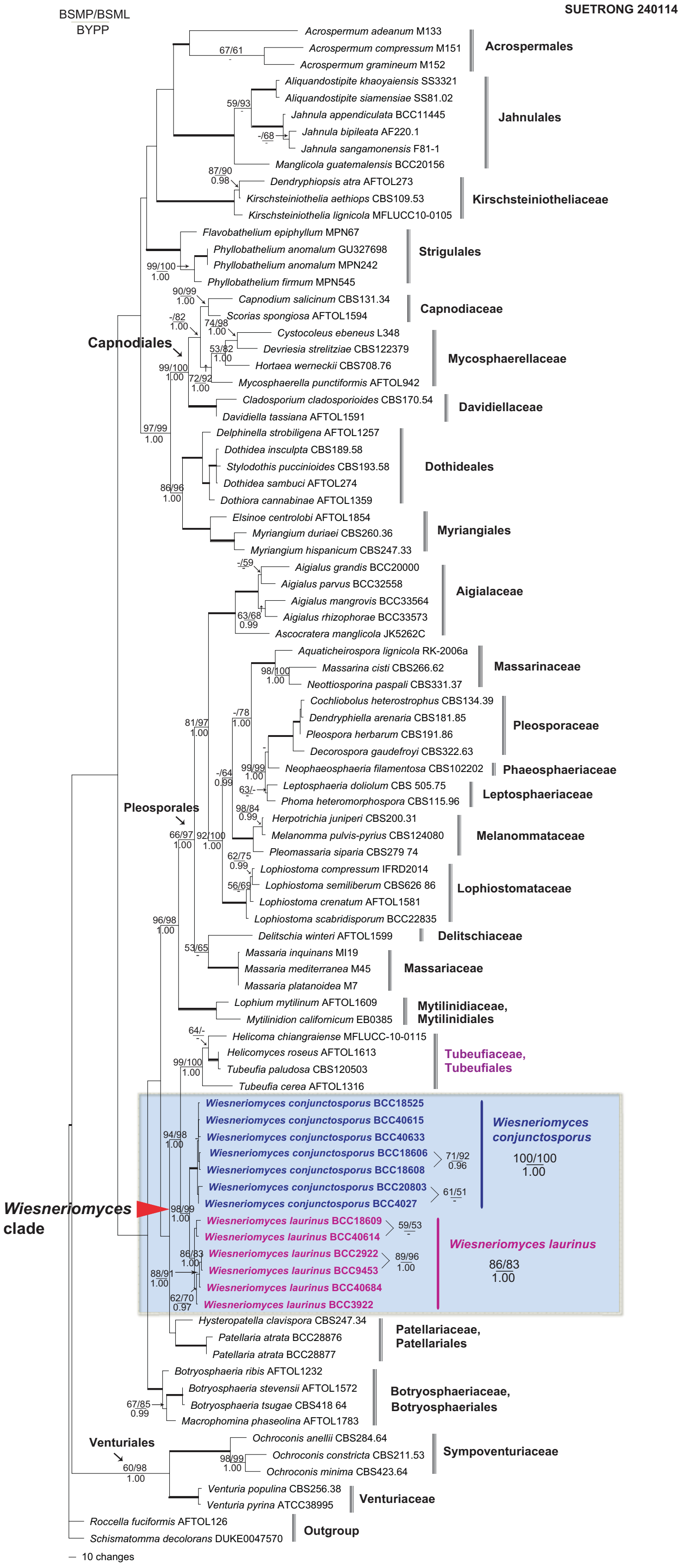

FIGURE 1. One of 34 most parsimonious trees obtained from heuristic searches of an alignment of combined SSU and LSU rDNA sequences (TL = 3502 steps, CI = 0.398, RI = 0.729 & RC = 0.290). *Roccella fuciformis* and *Chismatomma decolorans*are used as outgroups. Maximum parsimony (BSMP, left) and likelihood (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).

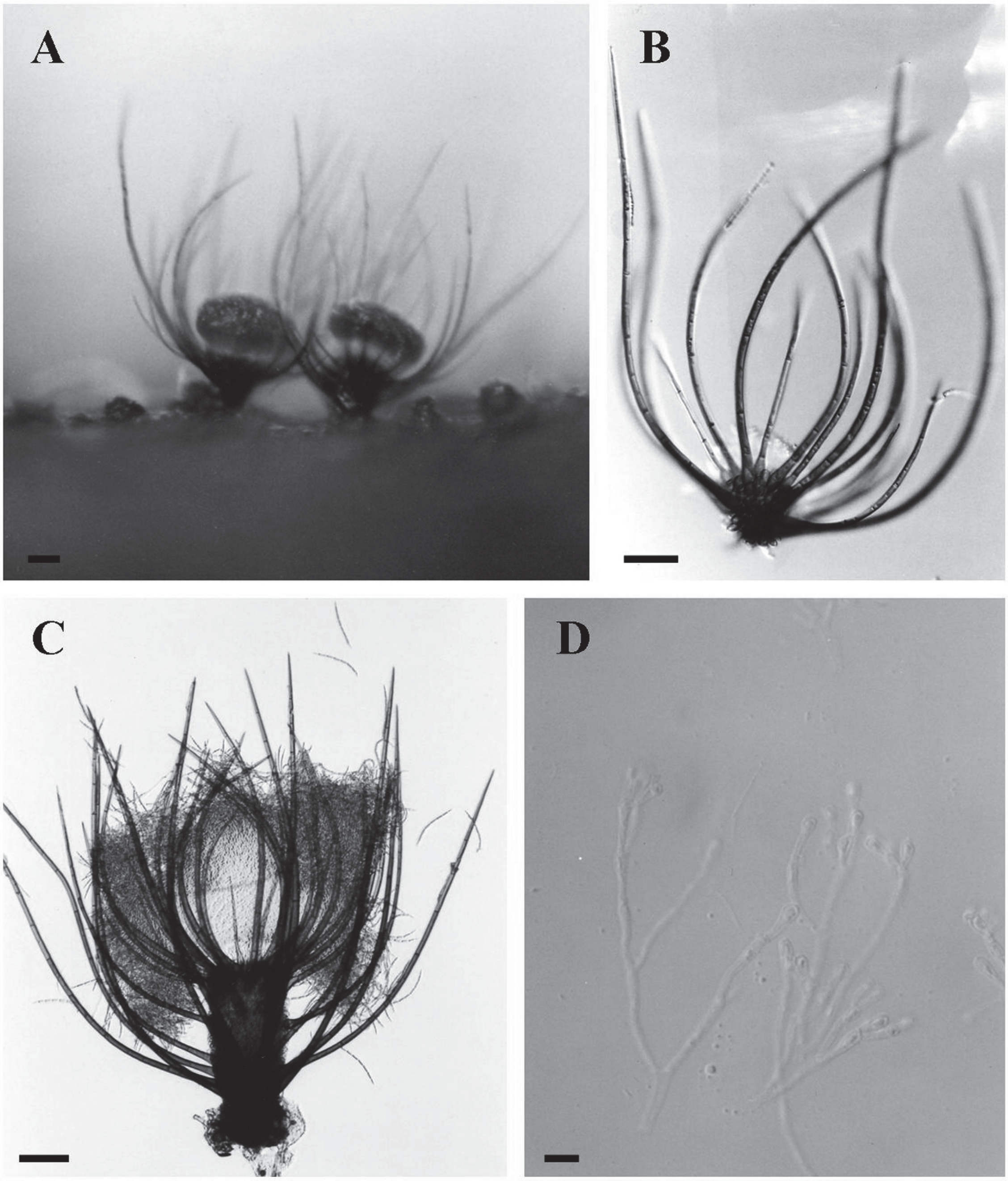

FIGURE 2. *Wiesneriomyces laurinus.* A. Sporodochia on natural substratum. B–C. Sporodochia with incurved setae arising from a prominent sporodochial stalk. D. Branched conidiophores. Scale bars: A–C = 20 µm, D = 10 µm.

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

Morphological structures	***W. conjunctosporus***	***W. laurinus***
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

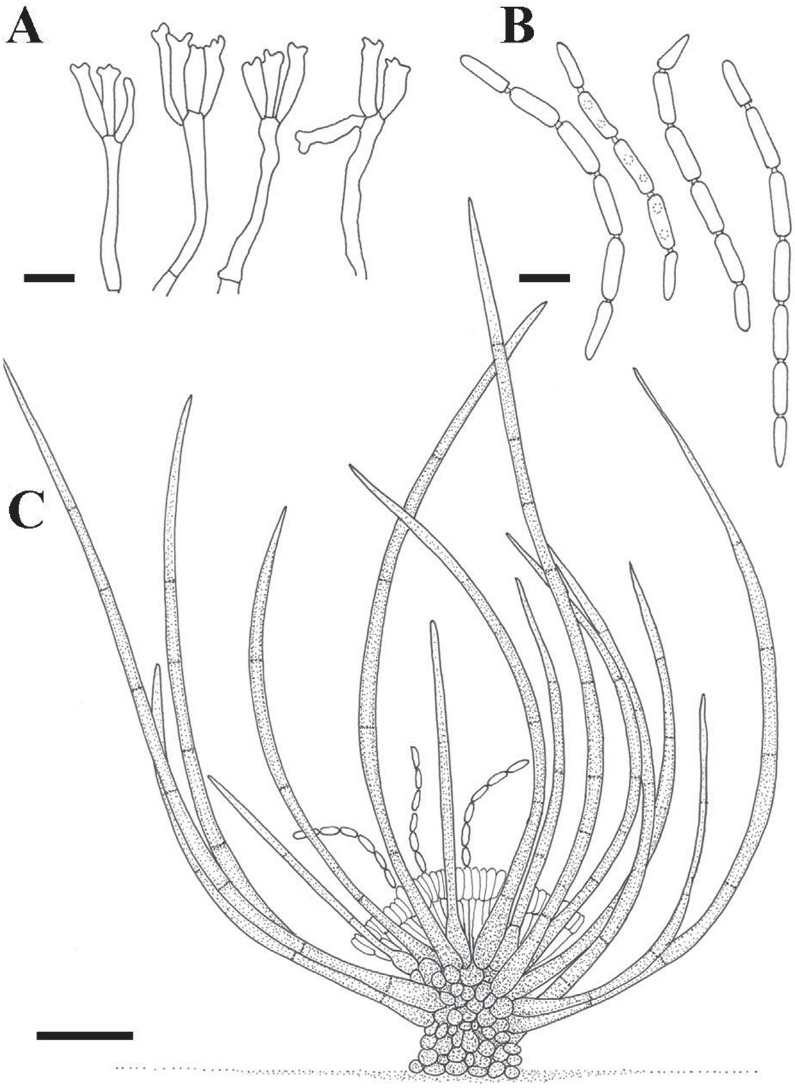

FIGURE 3 *Wiesneriomyces laurinus.* A. Branched conidiophores. B. Conidia. C. Sporodochia with incurved setae. Scale bars: A–B = 10 µm, C = 20 µm.

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 µm, 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 Sanctury, 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: KhaoYai National Park, dicotyledonous leaf from evergreen forest, 3 July 2001, *S. Somrithipol* (BCC18609).

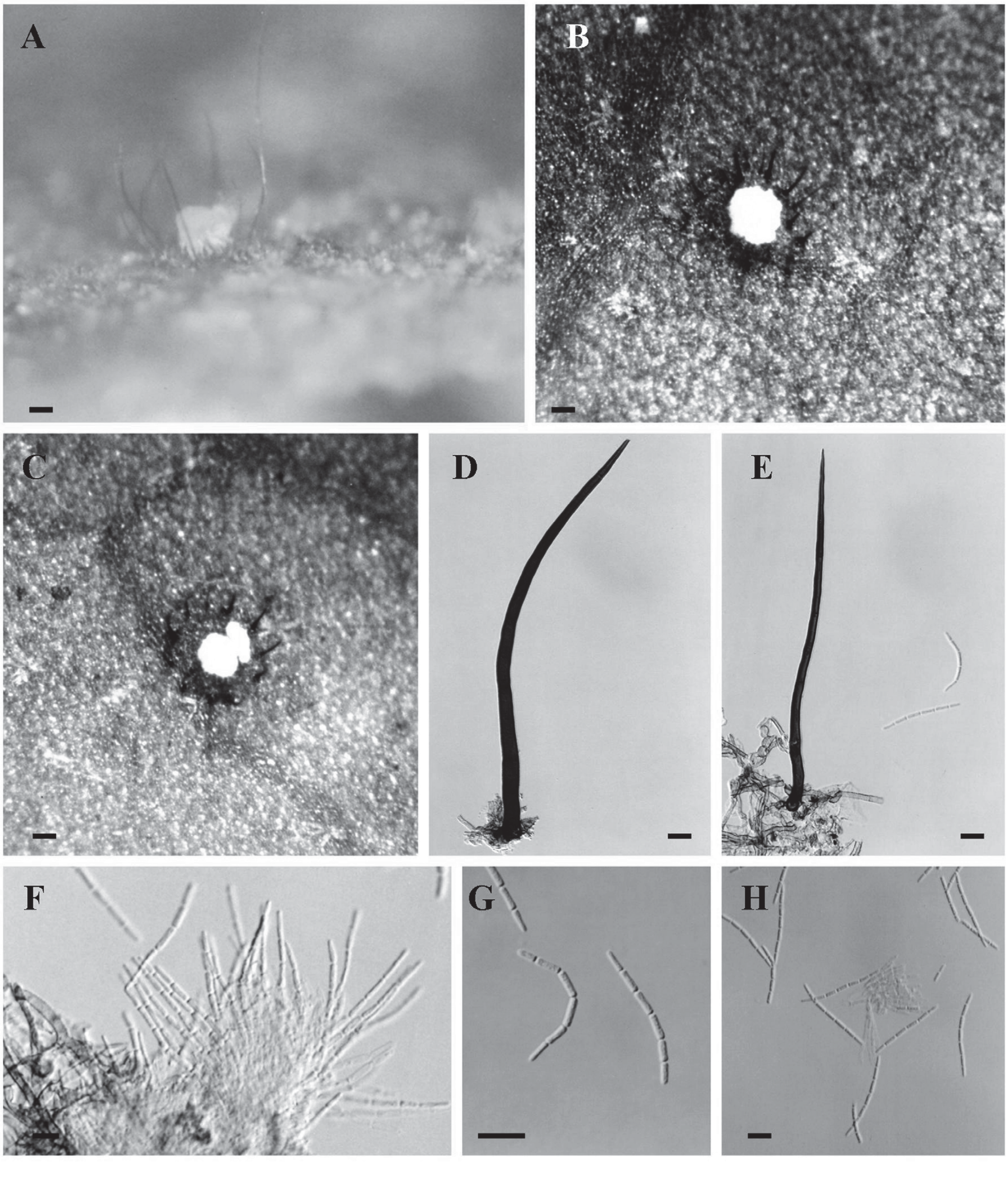

FIGURE 4 *Wiesneriomyces conjunctosporus.* A–C. Sporodochia on natural substratum. D–E. Setae. F. Conidiophores. G–H. Conidia. Scale bars: A–C = 200 µm, D–E, G–H = 20 µm, F = 10 µm.

Wiesneriomyces conjunctosporus Kuthub. & Nawawi 1988 MycoBank: MB10438 (Figs 4, 5)

Sexual state: Unknown. Asexual state: *Colonies* effuse, olivaceous-brown to dark brown. *Conidiomata* sporodochial 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.

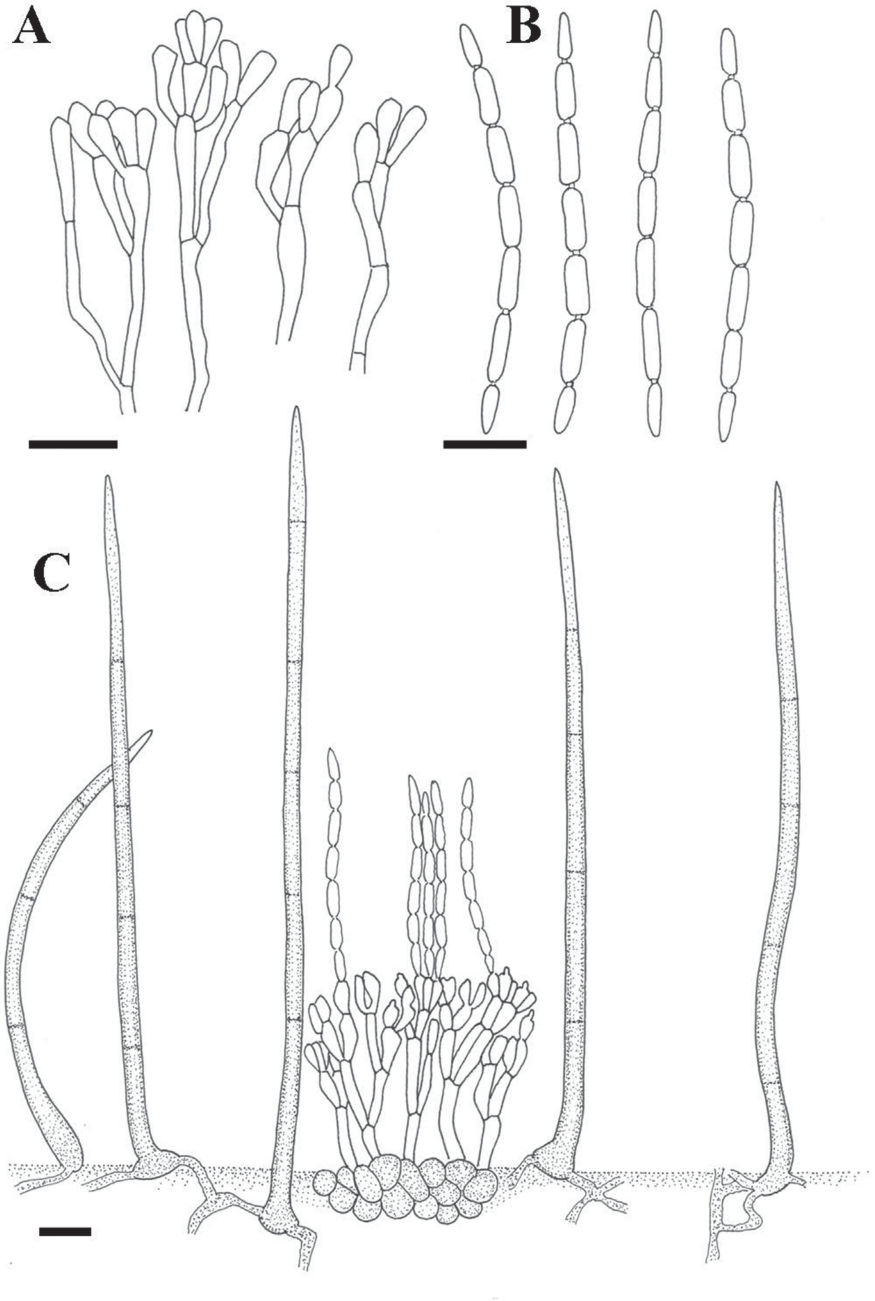

FIGURE 5 *Wiesneriomyces conjunctosporus.* A. Conidiophores. B. Conidia. C. Sporodochia and setae. Scale bars: A–B = 20 µm, C = 50 µm.

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 *Lagerstroenia 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, Botryosphaeriales, 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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