



Article

Doi 10.5943/sif/1/1/5

Copyright © Mushroom Research Foundation 2016

## Saprobic Dothideomycetes in Thailand: *Vaginatispora appendiculata* sp. nov. (*Lophiostomataceae*) introduced based on morphological and molecular data

Wanasinghe DN<sup>1,2</sup>, Jones EBG<sup>3</sup>, Dissanayake AJ<sup>1,2,4</sup> and Hyde KD<sup>1,2,3,4\*</sup>

<sup>1</sup>Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, 57100, Thailand

<sup>2</sup>School of Science, Mae Fah Luang University, Chiang Rai, 57100, Thailand

<sup>3</sup>Department of Botany and Microbiology, King Saudi University, Riyadh, Saudi Arabia

<sup>4</sup>Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, No. 9 of ShuGuangHuaYuanZhongLu, Haidian District, Beijing 100097, P. R. China

Wanasinghe DN, Jones EBG, Dissanayake AJ, Hyde KD 2016 – Saprobian Dothideomycetes in Thailand: *Vaginatispora appendiculata* sp. nov. (*Lophiostomataceae*) introduced based on morphological and molecular data. *Studies in Fungi* 1(1), 56–68, Doi 10.5943/sif/1/1/5

### Abstract

In order to establish the evolutionary relationships and resolve the polyphyletic nature of Dothideomycetes, we are studying their natural classification based on both morphology and multigene phylogeny. In this paper we introduce *Vaginatispora appendiculata*, a novel species on dead twigs from southern Thailand. Morphological character differences and analyses of combined LSU, TEF, SSU and ITS sequence datasets support the validity of the new species and its placement in *Vaginatispora* (*Lophiostomataceae*).

**Keywords** – Appendages – *Massarina* – Phylogeny

### Introduction

We have been studying the diverse members of Dothideomycetes to provide a natural classification based on both morphology and phylogeny (Boonmee et al. 2011, 2012, Liu et al. 2011, 2012, 2015, Hyde et al. 2013, Ariyawansa et al. 2014, 2015a, 2015b, Chomnunti et al. 2014, Phookamsak et al. 2014, 2015, Thambugala et al. 2014, 2015, Wanasinghe et al. 2014a, 2014b, 2015, Wijayawardene et al. 2014, 2014b, 2015, 2016, Tian et al. 2015). This paper reports on a saprobic pleosporalean species which was collected on dead twigs in southern Thailand and identified as a new species of *Vaginatispora* K.D. Hyde. *Vaginatispora* was introduced by Hyde (1995) in *Massarinaceae* Munk to accommodate *Vaginatispora aquatica* K.D. Hyde. The genus and species was characterized by ‘depressed globose ascomata, immersed beneath a blackened neck, with a slot-like ostiole, numerous and filamentous pseudoparaphyses, cylindrical to clavate asci and narrowly ellipsoidal, hyaline, 2-celled ascospores with a mucilaginous collar around its equator and a spreading papilionaceous sheath’ (Hyde 1995, Zhang et al. 2014). *Vaginatispora* was considered as a synonym of *Massarina* for a long time (Hyde et al. 1992, Read et al. 1997). Few studies have been conducted on the family placement of *Vaginatispora* and recently Thambugala et al. (2015) confirmed it as a separate genus in *Lophiostomataceae* Sacc. based on both morphological characteristics and phylogeny.

Combined analyses of LSU, TEF, SSU and ITS sequence data, using maximum-likelihood (ML), maximum-parsimony (MP) and MrBayes (BYPP), clearly show that *Vaginatispora appendiculata* is a well-supported species (100% ML & MP / 1.00 BYPP, Fig. 1) in *Vaginatispora*.

## Materials and methods

### *Sample collection, morphological studies and isolation*

Specimens were collected from Thailand, and processed and examined following the method described in Wanasinghe et al. (2014a). Hand sections of the fruiting structures were mounted in water for microscopic studies and photomicrography. The taxon was examined using a Nikon ECLIPSE 80i compound microscope and photographed with a Canon 450D digital camera fitted to the microscope. Measurements were made with the Tarosoft (R) Image Frame Work program and images used for figures processed with Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems, USA).

Single ascospore isolation was carried out following the method described in Chomnunti et al. (2014). Germinated spores were individually transferred to Potato dextrose agar (PDA) plates and grown at 16°C in the daylight. Colony colour and other characters were observed and measured after three weeks. The specimens are deposited at the Mae Fah Luang University (MFLU) Herbarium, Chiang Rai, Thailand. Living culture is also deposited at the Culture Collection of Mae Fah Luang University (MFLUCC). Faces of Fungi number is provided in Jayasiri et al. (2015) and Index Fungorum numbers as in Index Fungorum (2016).

### *DNA extraction and PCR amplification*

Fungal isolates were grown on potato-dextrose agar (PDA) for 3–4 weeks at 16 °C and total genomic DNA was extracted from 50 to 100 mg of axenic mycelium scraped from the edges of the growing culture (Wu et al. 2001). Mycelium was ground to a fine powder with liquid nitrogen and DNA was extracted using the Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux, P.R. China) following the instructions of the manufacturer.

DNA sequence data was obtained from the partial sequences of four genes, the internal transcribed spacers (5.8S, ITS), small subunit rDNA (18S, SSU), large subunit (28S, LSU) and translation elongation factor 1-alpha gene (TEF). Nuclear ITS was amplified using the primers ITS5 and ITS4 (White et al. 1990). LSU was amplified using the primers LROR and LR5 (Vilgalys & Hester 1990). SSU was amplified using the primers NS1 and NS4 (White et al. 1990), TEF was amplified using primers EF1-983F and EF1-2218R (Rehner 2001).

Polymerase chain reaction (PCR) was carried out following the protocol of Wanasinghe et al. (2014a). PCR amplification was confirmed on 1 % agarose electrophoresis gels stained with ethidium bromide. The amplified PCR fragments were sent to a commercial sequencing provider (BGI, Ltd Shenzhen, P.R. China). The nucleotide sequence data acquired is deposited in GenBank (Table 1).

### *Sequencing and sequence alignment*

Other sequences used in the analyses (Table 1) were obtained from GenBank based on recently published data (Ariyawansa et al. 2015a, Thambugala et al. 2015). The multiple alignments were automatically done by MAFFT v. 7.036 (<http://mafft.cbrc.jp/alignment/server/index.html>; Katoh & Standley 2013) using the default settings and latter refined where necessary, using BioEdit v. 7.0.5.2 (Hall 1999).

### *Phylogenetic analysis*

Parsimony analysis was carried with the heuristic search option in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002), with the following parameter settings, as described in Wanasinghe et al. (2014a): characters unordered with equal weight, random taxon addition, branch swapping with tree bisection-reconnection (TBR) algorithm, branches collapsing if

**Table 1** Taxa used in the phylogenetic analysis and their corresponding GenBank numbers. The newly generated sequences are indicated in bold

Taxon	Culture Accession No *	GenBank Accession No.			
		LSU	TEF	SSU	ITS
<i>Alpestrisphaeria terricola</i>	SC-12	JX985750	–	JX985749	JN662930
<i>Biappendiculispora japonica</i>	MAFF 239452	AB619005	LC001744	AB618686	LC001728
<i>Biappendiculispora japonica</i>	JCM 17671	AB619007	LC001746	AB618688	LC001730
<i>Biappendiculispora japonica</i>	JCM 17670	AB619006	LC001745	AB618687	LC001729
<i>Capulatispora sagittiformis</i>	JCM 15100	AB369267	LC001756	AB618693	AB369268
<i>Coelodictyosporium muriforme</i>	MFLUCC 13-0351	KP888641	KR075163	KP899127	KP899136
<i>Coelodictyosporium pseudodictyosporium</i>	MFLUCC 13-0451	KR025862	–	–	KR025858
<i>Dimorphiopsis brachystegiae</i>	CPC 22679	KF777213	–	–	KF777160
<i>Floricola striata</i>	JK 5603 K	GU479785	–	GU479751	–
<i>Floricola striata</i>	JK 5678I	GU301813	GU479852	GU296149	–
<i>Guttulispora crataegi</i>	MFLUCC 13-0442	KP888639	KR075161	KP899125	KP899134
<i>Guttulispora crataegi</i>	MFLUCC 14-0993	KP888640	KR075162	KP899126	KP899135
<i>Lophiopoacea paramacrostoma</i>	MFLUCC 11-0463	KP888636	–	KP899122	–
<i>Lophiopoacea winteri</i>	JCM 17648/KT 740	AB619017	LC001763	AB618699	JN942969
<i>Lophiopoacea winteri</i>	MAFF 239454	AB619018	LC001764	AB618700	JN942968
<i>Lophiostoma alpigenum</i>	GKM 1091b	GU385193	GU327758	–	–
<i>Lophiostoma heterosporum</i>	CBS 644.86	AY016369	DQ497609	AY016354	GQ203795
<i>Lophiostoma macrostomoides</i>	CBS 123097	FJ795439	GU456277	FJ795482	–
<i>Lophiostoma macrostomum</i>	JCM 13544	AB619010	LC001751	AB618691	JN942961
<i>Lophiostoma macrostomum</i>	JCM 13546/MAFF 239447	AB433274	LC001753	AB521732	AB433276
<i>Lophiostoma macrostomum</i>	JCM 13545	AB433273	LC001752	AB521731	AB433275
<i>Lophiostoma multiseptatum</i>	JCM 17668	AB619003	LC001742	AB618684	LC001726
<i>Lophiostoma multiseptatum</i>	MAFF 239451	AB619004	LC001743	AB618685	LC001727
<i>Lophiostoma quadrinucleatum</i>	GKM1233	GU385184	GU327760	–	–
<i>Lophiostoma semiliberum</i>	JCM 13548	AB619012	LC001757	AB618694	JN942966
<i>Lophiostoma semiliberum</i>	JCM 13547	AB619013	LC001758	AB618695	JN942967
<i>Lophiostoma semiliberum</i>	JCM 13549/MAFF 239448	AB619014	LC001759	AB618696	JN942970
<i>Lophiostoma triseptatum</i>	SMH 2591	GU385183	–	–	–
<i>Lophiostoma triseptatum</i>	SMH 5287	GU385187	–	–	–
<i>Lophiostoma viridarium</i>	IFRDCC 2090	FJ795443	–	FJ795486	–
<i>Melanomma pulvis-pyrius</i>	CBS 124080	GU456323	GU456265	GU456302	–
<i>Neotrematosphaeria biappendiculata</i>	KTC 1124	GU205227	–	GU205256	–
<i>Paucispora quadrispora</i>	KH 448	LC001722	LC001754	LC001720	LC001733
<i>Paucispora quadrispora</i>	MAFF 239455/KT 843	AB619011	LC001755	AB618692	LC001734
<i>Paucispora versicolor</i>	MAFF 244508	AB918732	LC001760	LC001721	AB918731
<i>Platystomum actinidiae</i>	KT 521	JN941380	LC001747	JN941375	JN942963
<i>Platystomum actinidiae</i>	JCM 13125/MAFF 239635	JN941379	LC001748	JN941376	JN942962
<i>Platystomum actinidiae</i>	IFRD 2014	FJ795437	–	FJ795480	–
<i>Platystomum compressum</i>	MFLUCC 13-0343	KP888643	KR075165	KP899129	–
<i>Platystomum crataegi</i>	MFLUCC 14-0925	KT026109	KT026121	KT026113	KT026117
<i>Platystomum rosae</i>	MFLUCC 15-0633	KT026111	–	KT026115	KT026119
<i>Platystomum salicicola</i>	MFLUCC 15-0632	KT026110	–	KT026114	KT026118

Taxon	Culture Accession No *	GenBank Accession No.			
		LSU	TEF	SSU	ITS
<i>Pseudolophiostoma vitigenum</i>	JCM 13534/MAFF 239459	AB619015	LC001761	AB618697	LC001735
<i>Pseudolophiostoma vitigenum</i>	JCM 17676	AB619016	LC001762	AB618698	LC001736
<i>Pseudoplatystomum scabridisporum</i>	BCC 22835	GQ925844	GU479857	GQ925831	–
<i>Pseudoplatystomum scabridisporum</i>	BCC 22836	GQ925845	GU479856	GQ925832	–
<i>Sigarispora arundinis</i>	JCM 13550	AB618998	LC001737	AB618679	JN942964
<i>Sigarispora arundinis</i>	JCM 13551/MAFF 239449	AB618999	LC001738	AB618680	JN942965
<i>Sigarispora caudata</i>	MAFF 239453	AB619000	LC001739	AB618681	LC001723
<i>Sigarispora caulium</i>	MAFF 239450	AB619001	LC001740	AB618682	LC001724
<i>Sigarispora caulium</i>	JCM 17669	AB619002	LC001741	AB618683	LC001725
<i>Sigarispora coronillae</i>	MFLUCC 14-0941	KT026112	–	KT026116	KT026120
<i>Sigarispora ravennica</i>	MFLUCC 14-0005	KP698414	–	KP698415	KP698413
<b><i>Vaginatispora appendiculata</i></b>	<b>MFLUCC 16-0314</b>	<b>KU743218</b>	<b>KU743220</b>	<b>KU743219</b>	<b>KU743217</b>
<i>Vaginatispora aquatica</i>	MFLUCC 11-0083	KJ591576	–	KJ591575	KJ591577
<i>Vaginatispora armatispora</i>	HKLTC 1562	–	–	–	AF383955
<i>Vaginatispora fuckelii</i>	JCM 17672	AB619008	LC001749	AB618689	LC001731
<i>Vaginatispora fuckelii</i>	MAFF 239458	AB619009	LC001750	AB618690	LC001732
<i>Vaginatispora</i> sp.	MFLUCC 11-0577	KJ188101	–	KJ188103	KJ188102

\*ANM: A.N. Miller, ATCC: American Type Culture Collection, Virginia, USA, BCC: BIOTEC Culture Collection, Bangkok, Thailand, BBH: BIOTEC Bangkok Herbarium, Thailand; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, CPC: Working collection of Pedro Crous housed at CBS, DAOM: Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada, GKM: G.K. Mugambi, IFRDCC: Culture Collection, International Fungal Research and Development Centre, Chinese Academy of Forestry, Kunming, China, JCM: the Japan Collection of Microorganisms, Japan, JK: J. Kohlmeyer, KT: K. Tanaka, KH: K. Hirayama, MAFF: Ministry of Agriculture, Forestry and Fisheries, Japan, MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand, NN: NovoNordisk culture collection (now Novozymes, Bagsvaerd, Denmark), SC: Department of Plant Pathology, Sichuan Agricultural University, SMH: S.M. Huhndorf.

the maximum branch length was zero. Alignment gaps were treated as missing characters in the analysis of the combine data set, where they occurred in relatively conserved regions. Parsimony bootstrap analyses were performed using the full heuristic search option, random stepwise addition, and 1000 replicates, with maxtrees set at 1000. Descriptive tree statistics for parsimony (Tree Length [TL], Consistency Index [CI], Retention Index [RI], Relative Consistency Index [RC] and Homoplasy Index [HI] were calculated for trees generated under different optimality criteria. The Kishino-Hasegawa tests (Kishino & Hasegawa 1989) were performed to determine whether the trees inferred under different optimality criteria were meaningfully different. Maximum parsimony bootstrap values (MP) equal or greater than 60 % are given above each node in red (Fig 1).

Evolutionary models for phylogenetic analyses were selected independently for each locus using MrModeltest v. 3.7 (Posada & Crandall 1998) under the Akaike Information Criterion (AIC) implemented in both PAUP v. 4.0b10 and MrBayes v. 3. Phylogenetic reconstructions of combined gene trees were performed using both Bayesian Inference (BI) and Maximum Likelihood (ML) criteria.

Maximum-likelihood (ML) analysis was performed in RAxML (Stamatakis 2008) implemented in raxmlGUI v.0.9b2 (Silvestro & Michalak 2010), employing mixed models of evolution settings of the program and Bootstrap support obtained by running 1000 pseudoreplicates. The online tool Findmodel was used to determine the best nucleotide substitution (<http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html>) model for each partition. Maximum Likelihood bootstrap values (ML) equal or greater than 60 % are given above each node in black (Fig. 1).

A Bayesian analysis was conducted with MrBayes v. 3.1.2 (Huelsenbeck & Ronqvist 2001) to evaluate Posterior probabilities (PP) (Rannala & Yang 1996; Zhaxybayeva & Gogarten 2002) by Markov Chain Monte Carlo sampling (BMCMC). Two parallel runs were conducted, using the default settings, but with the following adjustments:

Six simultaneous Markov chains were run for 2,000,000 generations and trees were sampled every 100th generation and 20,000 trees were obtained. The first 4,000 trees, representing the burn-in phase of the analyses and discarded. The remaining 16000 trees were used for calculating PP in the majority rule consensus tree (Cai et al. 2006, 2008, Ariyawansa et al. 2015). Branches with Bayesian posterior probabilities greater than 0.9 above each node in black (Fig. 1).

Phylograms were visualized with FigTree v1.4.0 program (Rambaut 2012) and reorganized in Microsoft power point (2007) and Adobe Illustrator® CS5 (Version 15.0.0, Adobe®, San Jose, CA).

## Results and Discussion

### Phylogenetic analysis

The combined LSU, TEF, SSU and ITS gene dataset comprised 57 sequences from all genera in *Lophiostomataceae* whose sequences are available in GenBank, plus two strains from *Floricolaceae* (*Floricola striata* JK 5603K and JK 56781) and our new strain of *V. appendiculata*. *Melanomma pulvis-pyrius* (CBS 124080) is the outgroup taxon (Fig. 1). Four different alignments corresponding to each individual gene and a combined alignment of the four genes were analyzed. A best scoring RAxML tree is shown in Fig. 1, with the value of -15629.448302 (ln) and the following model parameters: alpha: 0.609592 and invar:  $\Pi(A)$ : 0.249198,  $\Pi(C)$ : 0.239722,  $\Pi(G)$ : 0.266913 and  $\Pi(T)$ : 0.244167. All trees (ML, MP and BYPP) were similar in topology and did not differ significantly (data not shown) at the generic relationships, which is in agreement with previous studies based on multi-gene analyses (Ariyawansa et al. 2015, Thambugala et al. 2015).

This analysis comprised 3699 characters, of which 2905 were constant, 600 parsimony-informative and 194 parsimony-uninformative. Four equally parsimonious trees were generated and the first was selected (Fig. 1). Bootstrap support (BS) values of ML and MP (equal to or above 60 % based on 1000 replicates) are shown on the upper branches with black (ML) and blue (MP). Branches with Bayesian posterior probabilities (PP) greater than 0.95 from MCMC analyses are given in bold. The Kishino-Hasegawa test shows length = 1994 steps with CI = 0.551, RI = 0.712, RC = 0.392 and HI = 0.449.

Our strain of *V. appendiculata* (MFLUCC 16-0314) grouped in *Lophiostomataceae*, but separated from the other species of *Vaginatispora* with high bootstrap support (100% ML & MP / 1.00 BYPP, Fig. 1).

### Taxonomy

*Vaginatispora* K.D. Hyde, Nova Hedwigia 61(1–2): 234 (1995)

*Facesoffungi* number: FoF00828

*Type species: Vaginatispora aquatica* K.D. Hyde, Nova Hedwigia 61(1–2): 235 (1995)

≡ *Lophiostoma vaginatispora* Zhang, Hyde, Zhao, McKenzie & Zhou, Phytotaxa 176(1): 177 (2014)

*Vaginatispora appendiculata* Wanasinghe, E.B.G. Jones & K.D. Hyde, **sp. nov.**

Fig. 2

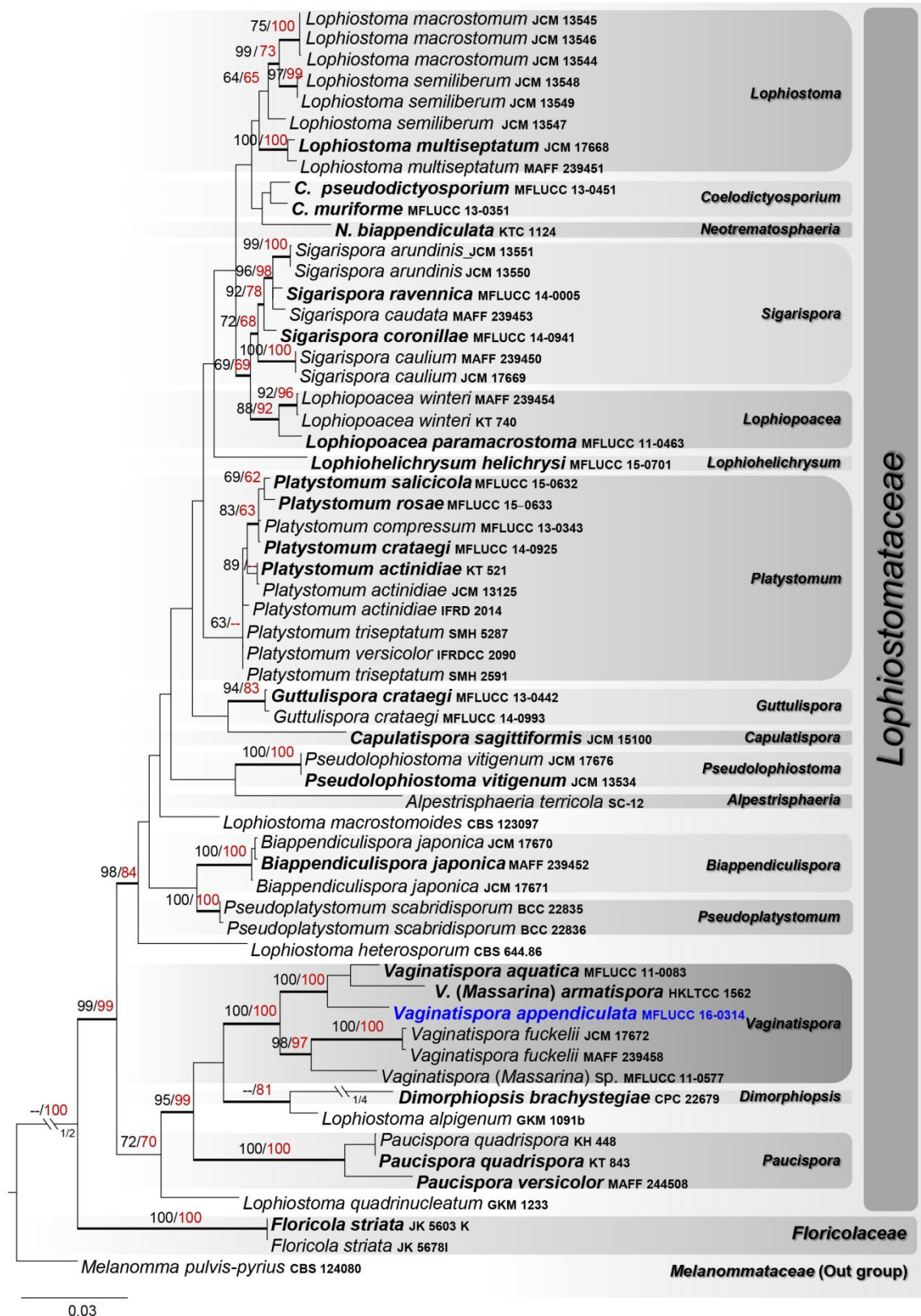
Index Fungorum Number: IF551961

*Facesoffungi* Number: FoF 01926

Etymology – Name reflects the appendages in this species

**Holotype** – MFLU 16-0522

*Saprobic* on dead twigs in terrestrial habitat by waterfall. **Sexual morph:** *Ascomata* 300–400  $\mu\text{m}$  high, 200–400  $\mu\text{m}$  diam. ( $x = 387.3 \times 266.2 \mu\text{m}$ ,  $n = 10$ ), scattered, immersed, coriaceous, black, globose to subglobose, ostiolate. *Ostiole* 100–200  $\mu\text{m}$  high, 50–70  $\mu\text{m}$  ( $x = 165.3 \times 63.9 \mu\text{m}$ ,  $n = 10$ ) diam., slit-like, central, with a crest-like papilla, with an irregular, pore-like opening,



**Fig. 1** – RAxML tree based on a combined dataset of LSU, TEF, SSU and ITS partial sequences. Bootstrap support values for maximum likelihood (ML, black) and maximum parsimony (MP, red) higher than 60 % are defined as above the nodes and branches with Bayesian posterior probabilities (BYPP) greater than 0.90 are given in bold. The ex-type and reference strains are in bold; the new isolates are in blue. The tree is rooted to *Melanomma pulvis-pyrius* (CBS 124080).



plugged by hyaline, filamentous hyphae, and occasionally lighter. *Peridium* 10–15 µm wide at the base, 20–30 µm wide in sides, composed of two layers, outer layer wider, comprising several layers with black, somewhat flattened cells of *textura angularis*, fusing and indistinguishable from the host tissues and inner layer comprising 2–4 layers of lightly pigmented to hyaline cells of *textura angularis*. *Hamathecium* comprising 3–4.5 µm (n = 30), wide septate, cellular pseudoparaphyses, situated between and above the asci, embedded in a gelatinous matrix. *Asci* 130–170 × 20–35 µm (x = 158.9 × 29 µm, n = 35), 8-spored, bitunicate, fissitunicate, cylindrical-clavate, with a developed pedicel (10–30 µm long; x = 21 µm, n = 30), apically rounded with an ocular chamber. *Ascospores* 40–45 × 10–15 µm (x = 43.3 × 12.1 µm, n = 50), uniseriate to partially overlapping 1–2-seriate, hyaline, ellipsoidal, 1-septate, constricted at the septum, with 1–3, distinct large guttules in each cells, smooth-walled, with distinct hyaline appendages (3–6 µm long; x = 4.8 µm, n = 40) at both ends, without a mucilaginous sheath. **Asexual morph:** Undetermined.

Culture characteristics – Colonies on PDA: slow reaching 3 cm diam. after 4 weeks at 25 °C, dirty white at the beginning and dark grey at maturity, convex on the surface, undulate, smooth margins; reverse buff.

Known distribution – Thailand, on dead twigs.

Material examined – Thailand, Prachuap Khiri Khan, Bang Saphan, Ron Thong, Sai Khu Waterfall, on dead stem of undetermined sp., 29 July 2015, D.N. Wanasinghe (MFLU 16-0522, **holotype**) **isotype** in KUN, under the code of HKAS91945, ex-type living culture, MFLUCC 16-0314.

Gene sequence data: ITS – KU743217, LSU – KU743218, SSU – KU743219, TEF – KU743220.

***Vaginatispora armatispora*** (K.D. Hyde, Vrijmoed, Chinnaraj & E.B.G. Jones) Wanasinghe, E.B.G. Jones & K.D. Hyde, **comb. nov.**

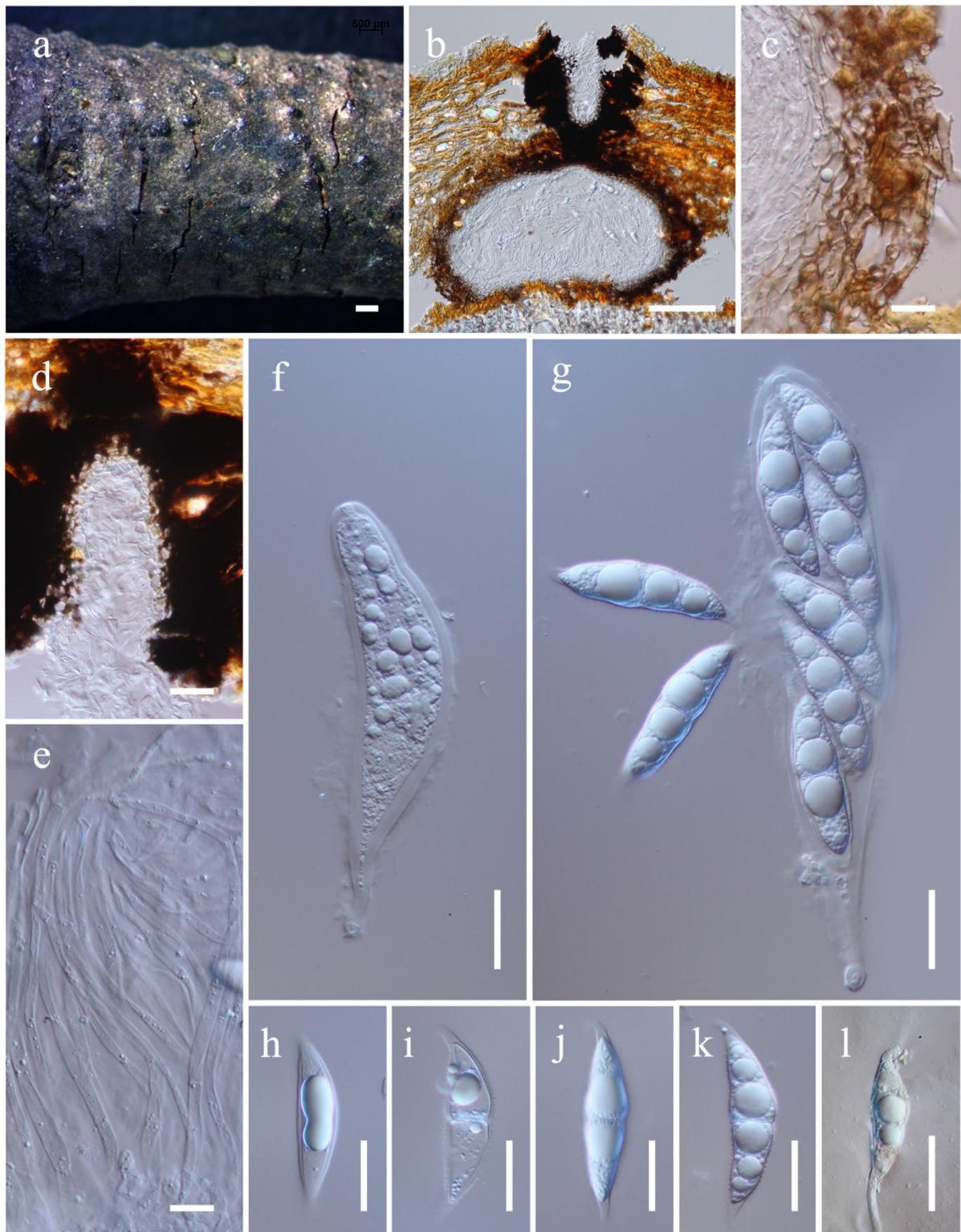
≡ *Massarina armatispora* K.D. Hyde, Vrijmoed, Chinnaraj & E.B.G. Jones, Bot. Mar. 35(4): 325 (1992)

= *Lophiostoma armatisporum* (K.D. Hyde, Vrijmoed, Chinnaraj & E.B.G. Jones) E.C.Y. Liew, Aptroot & K.D. Hyde, Mycologia 94(5): 812 (2002)

Notes – *Massarina armatispora* was introduced by Hyde et al. (1992) in *Massarina* to accommodate an intertidal mangrove taxon from the coast of Southern China and India; which differed from other *Massarina* species at that time. Later, Liew et al. (2002) synonymised *M. armatispora* under *Lophiostoma armatisporum* based on both molecular (ITS) and morphological evidence. In this study *M. armatispora* grouped in *Vaginatispora* with high statistical support (100% ML, 100% MP and 1.00 BYPP, Fig. 1). This fungus is morphologically more similar to *Parapaucispora pseudoarmatispora* Hay. Takah., K. Hiray. & Kaz. Tanaka in its ascospores (Li et al. 2016). However, *P. pseudoarmatispora* groups separately from *Vaginatispora* in multi-gene phylogenetic analyses and has a close relationship with *Lophiostoma alpigenum* (Li et al. 2016).

### Key to species of *Vaginatispora*

1. Semi-immersed ascomata with a diameter less than 250 µm, asci shorter than 100 µm and ascospores smaller than 20 µm..... ***V. fuckelii***
1. Immersed ascomata with a diameter more than 250 µm, asci longer than 100 µm and ascospores larger than 20 µm..... 2
2. Thick peridium (up to 100 µm), pseudoparaphyses less than 2.5 µm wide and ascospores without appendages, with a papilionaceous sheath..... ***V. aquatica***
2. Thin peridium (up to 30 µm), pseudoparaphyses more than 2.5 µm wide and ascospores with appendages, without a sheath..... 3
3. Pseudoparaphyses width less than 3 µm wide, ascospores shorter than 40 µm and lacking guttules..... ***V. armatispora***
3. Pseudoparaphyses width more than 3 µm wide, ascospores longer than 40 µm and with distinct large guttules in each cell ***V. appendiculata***



**Fig. 2 – *Vaginatispora appendiculata* (holotype).** **a** Appearance of ascomata on host substrate. **b** Section of ascoma. **c** Peridium. **d** Close up of ostiole. **e** Pseudoparaphyses. **f, g** Asci. **h-k** Ascospores. **l** Germinated spore. Scale bars: a = 500 µm, b = 100 µm, c, e = 10 µm, d, f-l = 20 µm.

### Discussion

In our combined gene analyses of *Lophiostomataceae* (Fig. 1), taxa from the genus *Vaginatispora* formed a distinct clade with high bootstrap (100% in ML and MP) and a high PP value (1.00 in Bayesian analysis). The type species *Vaginatispora aquatica* (MFLUCC 11-0083) *V. (Massarina) armatispora* (HKLTCC 1562), *V. fuckelii* (JCM 17672 & MAFF 239458) and



*Vaginatispora* (*Massarina*) sp. (MFLUCC 11-0577) clustered together in a well-supported clade within the family *Lophiostomataceae*. Thus, we confirm their generic placement in *Lophiostomataceae*. *Vaginatispora* species are similar to *Lophiostoma* (*L. macrostomum*) and *Lophiopoacea* (*L. winteri*) in having a slot-like papillate ostiole, cylindro-clavate asci with a long pedicel, hyaline, 1-septate, euseptate ascospores. However, phylogenetically these taxa are not closely related with *Vaginatispora* strains (Fig. 1).

Our collection of *V. appendiculata*, grouped in a well-supported clade (100% ML, 100% MP and 1.00 PP) with *V. aquatica* and *V. armatispora*. However, *V. appendiculata* is different from *V. aquatica* in having ascospores without a sheath but with terminal appendages (Fig. 2), while *V. aquatica* has ascospores without appendages, but with a large mucilaginous sheath constricted in the central septum. *Vaginatispora armatispora* differs from *V. appendiculata* in having ascospores lacking guttules, while *V. appendiculata* has distinct large guttules in each cell, *Vaginatispora armatispora* differs from *V. appendiculata* in having ascospores lacking guttules, while *V. appendiculata* has distinct large guttules in each cell, ascospore measurements are also different with *V. appendiculata* larger ( $40\text{--}45 \times 10\text{--}15 \mu\text{m}$ ) than those of *V. armatispora* ( $28\text{--}38 \times 7\text{--}9.8 \mu\text{m}$ ). Read et al. (1997) have shown that the appendage in *V. armatispora* is quite complex: external to the bipartite mesosporium there is the episporium surround by a mucilaginous sheath which is extended apically to form 3-7  $\mu\text{m}$  tapering, curved polar appendage. An electron-dense fibrillar layer is located external to the sheath. This ultra-structural observation may become a mandatory additional criterion for a complete description of micro fungi, especially taxa with a sheath. The phylogenetic significance of the ultrastructural variation should be further investigated. Consequently we introduce a new species *V. appendiculata* to accommodate the fungus collected from dead twigs from southern Thailand. Most of the members of *Vaginatispora* were collected from submerged wood in freshwater and mangroves in marine environments. Our species was collected from a terrestrial habitat, but it was also a well moistened environment near to a waterfall. Thus, *Vaginatispora* is most likely to be a freshwater genus.

## Acknowledgments

This work was supported by the International Research Group Program (IRG-14-27), Deanship of Scientific Research, King Saud University, Saudi Arabia. Also we would like to thank Humidtropics, a CGIAR Research Program that aims to develop new opportunities for improved livelihoods in a sustainable environment, for partially funding this work.

## References

- Ariyawansa HA, Hyde KD, Jayasiri SC, Buyck B, Kandawatte WTC, Cui YY, Dai DQ, Dai YC, Daranagama DA, Jayawardena RS, Lücking R, Ghobad-Nejhad M, Niskanen T, Thambugala KM, Voigt K, Zhao RL, Boonmee S, Bahkali AH, Chen J, Cui BK, Dayarathne MC, Dissanayake AJ, Ekanayaka AH, Hashimoto A, Hongsanan S, Jones EBG, Larsson E, Lewis D, Li WJ, Li QR, Liu JK, Luo ZL, Maharachchikumbura SSN, Mapook A, McKenzie EHC, Norphanphoun C, Pang KL, Perera RH, Phookamsak R, Phukhamsakda C, Randrianjohany E, Senanayake IC, Singtripop C, Shang Q, Tanaka K, Tian Q, Tian CM, Tibpromma S, Verbeken A, Abdel-Wahab MA, Wanasinghe DN, Wijayawardene NN, Zhang JF, Zhang H, Abdel-Aziz FA, Adamčík S, Ammirati JF, Bulgakov T, Cabral AL, Callaghan TM, Callac P, Chang CH, Coca LF, Dal-Forno M, Dollhofer V, Fliegerová K, Greiner K, Griffith GW, Ho HM, Hofstetter V, Jeewon R, Kang JC, Kirk PM, Kytövuori I, Lawrey JD, Li JXH, Liu ZY, Zhong X, Li, Liimatainen K, Lumbsch HT, Matumura M, Moncada B, Nuankaew S, Parnmen S, Santiago MDA, Sato G, Sommai S, Song Y, De Souza CAF, De Souza-Motta CM, Su HY, Suetrong S, Wang Y, Wei SF, Wen TC, Shen H, Yuan HS, Zhou LW, Reblova M, Fournier J, Camporesi E. 2015a – Fungal Diversity Notes 111–252—Taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity* 75, 27–274. (DOI 10.1007/s13225-015-0346-5).

- Ariyawansa HA, Tanaka K, Thambugala KM, Phookamsak R, Tian Q, Camporesi E, Hongsanan S, Monkai J, Wanasinghe DN, Chukeatirote E, Kang JC, Xu JC, McKenzie EHC, Jones EBG, Hyde KD. 2014 – A molecular phylogenetic reappraisal of the *Didymosphaeriaceae* (= *Montagnulaceae*). *Fungal Diversity* 68, 69–104. (DOI 10.1007/s13225-014-0305-6)
- Ariyawansa HA, Thambugala KM, Manamgoda DS, Jayawardena R, Camporesi E, Boonmee S, Wanasinghe DN, Phookamsak R, Hongsanan S, Singtripop C, Chukeatirote E, Kang JC, Jones EBG, Hyde KD. 2015b — Towards a natural classification and backbone tree for *Pleosporaceae*. *Fungal Diversity* 71, 85–139. (DOI 10.1007/s13225-015-0323-z)
- Boonmee S, KoKo TW, Chukeatirote E, Hyde KD, Chen H, Cai L, McKenzie EHC, Jones EBG, Kodsueb R, Hassan BA. 2012 – Two new *Kirschsteiniothelia* species with *Dendryphiopsis* anamorphs cluster in *Kirschsteiniotheliaceae* fam. nov. *Mycologia* 104, 698–714. (DOI: 10.3852/11-089)
- Boonmee S, Zhang Y, Chomnunti P, Chukeatirote E, Tsui CKM, Bahkali AH, Hyde KD. 2011 – Revision of lignicolous Tubeufiaceae based on morphological reexamination and phylogenetic analysis. *Fungal Diversity* 51, 63–102. (DOI 10.1007/s13225-011-0147-4)
- Cai L, Guo XY, Hyde KD. 2008 – Morphological and molecular characterization of a new anamorphic genus *Cheirosporium*, from freshwater in China. *Persoonia* 20, 53–58. (DOI 10.3767/003158508X314732)
- Cai L, Jeewon R, Hyde KD. 2006 – Phylogenetic investigations of *Sordariaceae* based on multiple gene sequences and morphology. *Mycological Research* 110, 137–150. (DOI 10.1016/j.mycres.2005.09.014)
- Chomnunti P, Hongsanan S, Hudson BA, Tian Q, Peršoh D, Dhamsi MK, Alias AS, Xu J, Liu X, Stadler M, Hyde KD. 2014 – The Sooty Moulds. *Fungal Diversity* 66, 1–36. (DOI 10.1007/s13225-014-0278-5)
- Hall TA. 1999 — BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: *Nucleic Acids Symposium Series* 41, 95–98
- Huelsenbeck JP, Ronquist F. 2001 – MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17, 754–755. (DOI 10.1093/bioinformatics/17.8.754)
- Hyde KD, Jones EBG, Liu JK, Ariyawansa H, Boehm E, Boonmee S, Braun U, Chomnunti P, Crous PW, Dai DQ, Diederich P, Dissanayake A, Doilom M, Doveri F, Hongsanan S, Jayawardena R, Lawrey JD, Li YM, Liu YX, Lücking R, Monkai J, Muggia L, Nelsen MP, Pang KL, Phookamsak R, Senanayake IC, Shearer CA, Suetrong S, Tanaka K, Thambugala KM, Wijayawardene NN, Wikee S, Wu HX, Zhang Y, Aguirre-Hudson B, Alias SA, Aptroot A, Bahkali A, Bezerra JL, Bhat DJ, Camporesi E, Chukeatirote E, Gueidan C, Hawksworth DL, Hirayama K, Hoog SD, Kang JC, Knudsen K, Li WJ, Li XH, Liu ZY, Mapook A, McKenzie EHC, Miller AN, Mortimer PE, Phillips AJL, Raja HA, Scheuer C, Schumm F, Taylor JE, Tian Q, Tibpromma S, Wanasinghe DN, Wang Y, Xu JC, Yacharoen S, Yan JY, Zhang M. 2013 – Families of Dothideomycetes. *Fungal Diversity* 63, 1–313. (DOI 10.1007/s13225-013-0263-4)
- Hyde KD, Vrijmoed LLP, Chinnaraj S, Jones EBG. 1992 – *Massarina armatispora* sp. nov., a new intertidal ascomycete from mangroves. *Botanica Marina* 35, 325–328. (DOI: 10.1515/botm.1992.35.4.325)
- Hyde KD. 1995 – Tropical Australasian fungi. IX. *Vaginatispora aquatica* gen. et sp. nov. *Nova Hedwigia* 61, 233–241.
- Jayasiri SC, Hyde KD, Ariyawansa HA, Bhat J, Buyck B, Cai L, Dai YC, Abdelsalam KA, Ertz D, Hidayat I, Jeewon R, Jones EBG, Bahkali AH, Karunarathna SC, Liu JK, Luangsa-Ard JJ, Lumbsch HT, Maharachchikumbura SSN, McKenzie EHC, Moncalvo JM, Ghobadnejhad M, Nilsson H, Pang KA, Pereira OL, Phillips AJL, Raspé O, Rollins AW, Romero AI, Etayo J, Selçuk F, Stephenson SL, Suetrong S, Taylor JE, Tsui CKM, Vizzini A, Abdel-Wahab MA, Wen TC, Boonmee S, Dai DQ, Daranagama DA, Dissanayake AJ, Ekanayaka AH, Fryar SC, Hongsanan S, Jayawardena R, Li WJ, Perera RH, Phookamsak R, De Silva NI, Thambugala KM, Tian Q, Wijayawardene NN, Zhao RL, Zhao Q, Kang JC, Promputtha I. 2015 — The

- Faces of Fungi database: fungal names linked with morphology, phylogeny and human impacts. *Fungal Diversity* 74, 3–18. (DOI 10.1007/s13225-015-0351-8)
- Katoh K, Standley K. 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology & Evolution* 30, 772–780. (DOI 10.1093/molbev/mst010)
- Kishino H, Hasegawa M. 1989 – Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in hominoidea. *Journal of Molecular Evolution* 29, 170–179. (DOI 10.1007/BF02100115)
- Li GJ, Hyde KD, Zhao RL, Sinang H, Abdel-Aziz FA, Abdel-Wahab MA, Silva GA, Ammirati J, Ariyawansa HA, Baghela A, Bahkali AH, Beug M, Bojantchev D, Boonpratuang T, Bulgakov T, Camporesi E, Castilho BM, Ceska O, Chakraborty D, Chen JJ, Chethana KWT, Consiglio G, Cui BK, Dai YC, Daranagama DA, Das K, Dayarathna MC, Crop ED, De Oliveira RJV, De Souza CAF, Dentinger BTM, Dissanayake AJ, Doilom M, Drechsler-Santos ER, Ghobad-Nejhad M, Gilmore SP, Góes-Neto A, Gorczak M, Haitjema CH, Hapuarachchi KK, Hashimoto A, He MQ, Henrique JG, Henske JK, Hirayama K, Ivanildo DSJ, Jayasiri SC, Jayawardena RS, Jeon SJ, Jesus AL, Jones EBG, Josefina IM, Karunarathna SC, Kirk PM, Konta S, Kuhnert E, Langer E, Lee HS, Lee HB, Li WJ, Li XH, Liimatainen K, Lima DX, Lin CG, Luangsa-ard JJ, Lücking R, Lumbsch HT, Lumyong S, Maharachchikumbura SSN, Malibiran LE, Marano AV, Matsumura M, McKenzie EHC, Nguyen TTT, Niskanen T, Norphanphoun C, O'Malley MA, Pablo A, Parnmen S, Pawłowska J, Perera RH, Phookamsak R, Phukhamsakda C, Pires-Zottarelli CLA, Raspé O, Reck MA, Santiago ALCMDA, Setti L, Shang QJ, Singh SK, Sir EB, Solomon KV, Song J, Srikitikulchai P, Stadler M, Suetrong S, Takahashi H, Takahashi T, Tanaka K, Tang LP, Thambugala KM, Theodorou MK, Thongbai B, Thummarukcharoen T, Tian Q, Tibpromma S, Verbeken A, Vizzini A, Vlasák J, Voigt K, Wanasinghe DN, Wang Y, Weerakoon G, Wen HA, Wen TC, Wijayawardene NN, Wongkanoun S, Wrzosek M, Xiao YP, Yan JY, Yang J, Yang SD, Young JT, Yu H, Zhang JF, Zhao J, Zhou LW. 2016 – Fungal diversity notes 253–370—Taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity* in press
- Liew ECY, Aptroot A, Hyde KD. 2002 – An evaluation of the monophyly of *Massarina* based on ribosomal DNA sequences. *Mycologia* 94, 803–813.
- Liu JK, Hyde KD, Jones EBG, Ariyawansa HA, Bhat DJ, Boonmee S, Maharachchikumbura SSN, McKenzie EHC, Phookamsak R, Phukhamsakda C, Shenoy BD, Abdel-Wahab MA, Buyck B, Chen J, Chethana KWT, Singtripop C, Dai DQ, Dai YC, Daranagama DA, Dissanayake AJ, Doilom M, D'souza MJ, Fan XL, Goonasekara ID, Hirayama K, Hongsan S, Jayasiri SC, Jayawardena RS, Karunarathna SC, Li WJ, Mapook A, Norphanphoun C, Pang KL, Perera RH, Peršoh D, Pinruan U, Senanayake IC, Somrithipol S, Suetrong S, Tanaka K, Thambugala KM, Tian Q, Tibpromma S, Udayanga D, Wijayawardene NN, Wanasinghe DN, Wisitrassameewong K, Zeng XY, Abdel-Aziz FA, Adamčík S, Bahkali AH, Boonyuen N, Bulgakov T, Callac P, Chomnunti P, Greiner K, Hashimoto A, Hofstetter V, Kang JC, Lewis D, Li XH, Liu XZ, Liu ZY, Matsumura M, Mortimer PE, Rambold G, Randrianjohany E, Sato G, Sri-Indrasutdhi V, Tian CM, Verbeken A, Von Brackel W, Wang Y, Wen TC, Xu JC, Yan JY, Zhao RL, Camporesi E. 2015 – Fungal diversity notes 1–110: taxonomic and phylogenetic contributions to fungal species. *Fungal Diversity* 72, 1–197. (DOI 10.1007/s13225-015-0324-y)
- Liu JK, Phookamsak R, Doilom M, Wiki S, Mei LY, Ariyawansa HA, Boonmee S, Chomnunti P, Dai DQ, Bhat DJ, Romero AI, Xhuang WY, Monkai J, Jones EBG, Chukeatirote E, KoKo TW, Zhao YC, Wang Y, Hyde KD. 2012 – Towards a natural classification of Botryosphaerales. *Fungal Diversity* 57, 149–210. (DOI 10.1007/s13225-012-0207-4)
- Liu JK, Phookamsak R, Jones EBG, Zhang Y, Ko-Ko TW, Hu HL, Boonmee S, Doilom M, Chukeatirote E, Bahkali AH, Wang Y, Hyde KD. 2011 – *Astrosphaeriella* is polyphyletic, with species in *Fissuroma* gen. nov., and *Neoastrsphaeriella* gen. nov. *Fungal Diversity* 51, 135–154. (DOI 10.1007/s13225-011-0142-9)

- Phookamsak R, Liu JK, Mckenzie EHC, Manamgoda DS, Chatpapamon C, Ariyawansa H, Thambugala KM, Dai DQ, Camporesi E, Chukeatirote E, Wijayawardene NN, Bahkali AH, Mortimer PE, Xu JC, Hyde KD. 2014 – Revision of *Phaeosphaeriaceae*. *Fungal Diversity* 68, 159–238. (DOI: 10.1007/s13225-014-0308-3)
- Phookamsak R, Norphanphoun C, Tanaka K, Dai DQ, Luo ZL, Liu JK, Su HY, Bhat DJ, Bahkali AH, Mortimer PE, Xu JC, Hyde KD. 2015 – Towards a natural classification of *Astrosphaeriella*-like species; introducing *Astrosphaeriellaceae* and *Pseudoastrosphaeriellaceae* fam. nov. and *Astrosphaeriellopsis*, gen. nov. *Fungal Diversity* 74, 143–197. (DOI 10.1007/s13225-015-0352-7)
- Posada D, Crandall KA. 1998 – Modeltest: testing the model of DNA substitution. *Bioinformatics* 14:817-818 (DOI 10.1093/bioinformatics/14.9.817).
- Rambaut A. 2012 – FigTree version 1.4.0. Available at <http://tree.bio.ed.ac.uk/software/figtree/>
- Rannala B, Yang Z. 1996 – Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution* 43, 304–311. (DOI 10.1007/BF02338839)
- Read SJ, Jones EBG, Moss ST. 1997 – Ultrastructural observation of asci, ascospores and appendages of *Massarina armatispora* (Ascomycota). *Mycoscience* 38, 141–146. (DOI 10.1007/BF02460849)
- Rehner S. 2001 – *Primers for Elongation Factor 1- $\alpha$  (EF1- $\alpha$ )*. <http://ocid.NACSE.ORG/research/deephyphae/EF1primer.pdf>.
- Silvestro D, Michalak I. 2012 – raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution* 12, 335–337. (DOI 10.1007/s13127-011-0056-0)
- Stamatakis A. 2006 – RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688–2690. (DOI 10.1093/bioinformatics/btl446)
- Swofford DL. 2002 – PAUP: phylogenetic analysis using parsimony, version 4.0 b10. Sinauer Associates, Sunderland (DOI 10.1111/j.0014-3820.2002.tb00191.x)
- Thambugala KM, Ariyawansa HA, Li YM, Boonmee S, Hongsan S, Tian Q, Singtripop C, Bhat DJ, Camporesi E, Jayawardena R, Liu ZY, Chukeatirote E, Hyde KD. 2014 – Dothideales. *Fungal Diversity* 68, 105–158. (DOI 10.1007/s13225-014-0303-8)
- Thambugala KM, Hyde KD, Tanaka K, Tian Q, Wanasinghe DN, Ariyawansa HA, Jayasiri SC, Boonmee S, Camporesi E, Hashimoto A, Hirayama K, Schumacher RK, Promputtha I, Liu ZY. 2015 – Towards a natural classification and backbone tree for *Lophiostomataceae*, *Floricolaceae* and *Amorosiaceae* fam. nov. *Fungal Diversity* 74, 199–266. (DOI 10.1007/s13225-015-0348-3)
- Tian Q, Liu JK, Hyde KD, Wanasinghe DN, Boonmee S, Jayasiri SC, Luo ZL, Taylor JE, Phillips AJL, Bhat DJ, Li WJ, Ariyawansa HA, Thambugala KM, Jones EBG, Chomnunti P, Bahkali AL, Xu JC, Camporesi E. 2015 – Phylogenetic relationships and morphological reappraisal of *Melanommataceae* (Pleosporales). *Fungal Diversity* 74, 267–324. (DOI 10.1007/s13225-015-0350-9)
- Vilgalys R, Hester M. 1990 – Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172, 4238–4246.
- Wanasinghe DN, Jones EBG, Camporesi E, Boonmee S, Ariyawansa HA, Wijayawardene NN, Mortimer PE, Xu JC, Yang JB, Hyde KD. 2014a – An exciting novel member of *Lentitheciaceae* in Italy from *Clematis vitalba*. *Cryptogamie Mycologie* 35, 323–337. (DOI 10.7872/crym.v35.iss4.2014.323)
- Wanasinghe DN, Jones EBG, Camporesi E, Boonmee S, Karunarathna SC, Thines M, Mortimer PE, Xu J, Hyde KD. 2014b – *Dematiopleospora mariae* gen. sp. nov., from *Ononis spinosain* Italy. *Cryptogamie Mycologie* 35, 105–117. (DOI 10.7872/crym.v35.iss2.2014.105)
- Wanasinghe DN, Jones EBG, Camporesi E, Mortimer PE, Xu JC, Bahkali Ah, Hyde KD. 2015 – The genus *Murispora*. *Cryptogamie Mycologie* 36, 419–448. (DOI 10.7872/crym/v36.iss4.2015.419)

- White TJ, Bruns T, Lee J, Taylor SB. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), PCR protocols: a guide to methods and applications: 315–322. Academic Press, San Diego, California, USA.
- Wijayawardene NN, Crous PW, Kirk PM, Hawksworth DL, Boonmee S, Braun U, Dai DQ, D'souza MJ, Diederich P, Dissanayake A, Doilom M, Hongsanan S, Jones EBG, Groenewald JZ, Jayawardena R, Lawrey JD, Liu JK, Luecking R, Madrid H, Manamgoda DS, Muggia L, Nelsen MP, Phookamsak R, Suetrong S, Tanaka K, Thambugala KM, Wanasinghe DN, Wikee S, Zhang Y, Aptroot A, Ariyawansa HA, Bahkali AH, Bhat DJ, Gueidan C, Chomnunti P, De Hoog GS, Knudsen K, Li WJ, McKenzie EHC, Miller AN, Phillips AJL, Piatek M, Raja HA, Shivas RS, Slippers B, Taylor JE, Tian Q, Wang Y, Woudenberg JHC, Cai L, Jaklitsch WM, Hyde KD. 2014a – Naming and outline of Dothideomycetes-2014 including proposals for the protection or suppression of generic names. *Fungal Diversity* 69, 1–55. (DOI 10.1007/s13225-014-0309-2)
- Wijayawardene NN, Hyde KD, Bhat DJ, Camporesi E, Schumacher RK, Chethana KWT, Wikee S, Bahkali AH, Wang Y. 2014b – *Camarosporium*-like species are polyphyletic in *Pleosporales*; introducing *Paracamarosporium* and *Pseudocamarosporium* gen. nov. in *Montagnulaceae*. *Cryptogamie Mycologie* 35, 177–198. (DOI 10.7872/crym.v35.iss2.2014.177)
- Wijayawardene NN, Hyde KD, Camporesi E, Bhat DJ, Goonasekara ID, Nadeeshan D, Camporesi E, Schumacher RK, Wang Y. 2015 – Additions to brown spored coelomycetous taxa in *Massarinae*, *Pleosporales*: introducing *Phragmocamarosporium* gen. nov. and *Suttonomyces* gen. nov. *Cryptogamie Mycologie* 36, 213–224. (DOI 10.7872/crym/v36.iss2.2015.213)
- Wijayawardene NN, Hyde KD, Wanasinghe DN, Papizadeh M, Goonasekara ID, Camporesi E, Bhat DJ, McKenzie EHC, Phillips AJL, Diederich P, Tanaka K, Li WJ, Tangthirasunun N, Phookamsak R, Dai DQ, Dissanayake AJ, Weerakoon G, Maharachchikumbura SSN, Hashimoto A, Matsumura M, Wang Y. 2016 – Taxonomy and phylogeny of dematiaceous coelomycetes. *Fungal Diversity in press*
- Wu ZH, Wang TH, Huang W, Qu YB. 2001 – A simplified method for chromosome DNA preparation from filamentous fungi. *Mycosystema* 20, 575–577.
- Zhang H, Hyde KD, Zhao Y, McKenzie EH, Zhou D. 2014 – Freshwater ascomycetes: *Lophiostoma vaginatispora* comb. nov. (Dothideomycetes, Pleosporales, Lophiostomaceae) based on morphological and molecular data. *Phytotaxa* 176, 184–191. (DOI 10.11646/phytotaxa.176.1.18)
- Zhaxybayeva O, Gogarten JP. 2002 – Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. *BMC Genomics* 3:4 (DOI 10.1186/1471-2164-3-4)