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

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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.

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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
Alpestrisphaeria terricola	SC-12	JX985750	–	JX985749	JN662930
Biappendiculispora japonica	MAFF 239452	AB619005	LC001744	AB618686	LC001728
Biappendiculispora japonica	JCM 17671	AB619007	LC001746	AB618688	LC001730
Biappendiculispora japonica	JCM 17670	AB619006	LC001745	AB618687	LC001729
Capulatispora sagittiformis	JCM 15100	AB369267	LC001756	AB618693	AB369268
Coelodictyosporium muriforme	MFLUCC 13-0351	KP888641	KR075163	KP899127	KP899136
Coelodictyosporium pseudodictyosporium	MFLUCC 13-0451	KR025862	–	–	KR025858
Dimorphiopsis brachystegiae	CPC 22679	KF777213	–	–	KF777160
Floricola striata	JK 5603 K	GU479785	–	GU479751	–
Floricola striata	JK 5678I	GU301813	GU479852	GU296149	–
Guttulispora crataegi	MFLUCC 13-0442	KP888639	KR075161	KP899125	KP899134
Guttulispora crataegi	MFLUCC 14-0993	KP888640	KR075162	KP899126	KP899135
Lophiopoacea paramacrostoma	MFLUCC 11-0463	KP888636	–	KP899122	–
Lophiopoacea winteri	JCM 17648/KT 740	AB619017	LC001763	AB618699	JN942969
Lophiopoacea winteri	MAFF 239454	AB619018	LC001764	AB618700	JN942968
Lophiostoma alpigenum	GKM 1091b	GU385193	GU327758	–	–
Lophiostoma heterosporum	CBS 644.86	AY016369	DQ497609	AY016354	GQ203795
Lophiostoma macrostomoides	CBS 123097	FJ795439	GU456277	FJ795482	–
Lophiostoma macrostomum	JCM 13544	AB619010	LC001751	AB618691	JN942961
Lophiostoma macrostomum	JCM 13546/ MAFF 239447	AB433274	LC001753	AB521732	AB433276
Lophiostoma macrostomum	JCM 13545	AB433273	LC001752	AB521731	AB433275
Lophiostoma multiseptatum	JCM 17668	AB619003	LC001742	AB618684	LC001726
Lophiostoma multiseptatum	MAFF 239451	AB619004	LC001743	AB618685	LC001727
Lophiostoma quadrinucleatum	GKM1233	GU385184	GU327760	–	–
Lophiostoma semiliberum	JCM 13548	AB619012	LC001757	AB618694	JN942966
Lophiostoma semiliberum	JCM 13547	AB619013	LC001758	AB618695	JN942967
Lophiostoma semiliberum	JCM 13549/MAFF 239448	AB619014	LC001759	AB618696	JN942970
Lophiostoma triseptatum	SMH 2591	GU385183	–	–	–
Lophiostoma triseptatum	SMH 5287	GU385187	–	–	–
Lophiostoma viridarium	IFRDCC 2090	FJ795443	–	FJ795486	–
Melanomma pulvis-pyrius	CBS 124080	GU456323	GU456265	GU456302	–
Neotrematosphaeria biappendiculata	KTC 1124	GU205227	–	GU205256	–
Paucispora quadrispora	KH 448	LC001722	LC001754	LC001720	LC001733
Paucispora quadrispora	MAFF 239455/KT 843	AB619011	LC001755	AB618692	LC001734
Paucispora versicolor	MAFF 244508	AB918732	LC001760	LC001721	AB918731
Platystomum actinidiae	KT 521	JN941380	LC001747	JN941375	JN942963
Platystomum actinidiae	JCM 13125/ MAFF 239635	JN941379	LC001748	JN941376	JN942962
Platystomum actinidiae	IFRD 2014	FJ795437	–	FJ795480	–
Platystomum compressum	MFLUCC 13-0343	KP888643	KR075165	KP899129	–
Platystomum crataegi	MFLUCC 14-0925	KT026109	KT026121	KT026113	KT026117
Platystomum rosae	MFLUCC 15-0633	KT026111	–	KT026115	KT026119
Platystomum salicicola	MFLUCC 15-0632	KT026110	–	KT026114	KT026118

Taxon	Culture Accession No *	GenBank Accession No.			
		LSU	TEF	SSU	ITS
Pseudolophiostoma vitigenum	JCM 13534/MAFF 239459	AB619015	LC001761	AB618697	LC001735
Pseudolophiostoma vitigenum	JCM 17676	AB619016	LC001762	AB618698	LC001736
Pseudoplatystomum scabridisporum	BCC 22835	GQ925844	GU479857	GQ925831	–
Pseudoplatystomum scabridisporum	BCC 22836	GQ925845	GU479856	GQ925832	–
Sigarispora arundinis	JCM 13550	AB618998	LC001737	AB618679	JN942964
Sigarispora arundinis	JCM 13551/MAFF 239449	AB618999	LC001738	AB618680	JN942965
Sigarispora caudata	MAFF 239453	AB619000	LC001739	AB618681	LC001723
Sigarispora caulium	MAFF 239450	AB619001	LC001740	AB618682	LC001724
Sigarispora caulium	JCM 17669	AB619002	LC001741	AB618683	LC001725
Sigarispora coronillae	MFLUCC 14-0941	KT026112	–	KT026116	KT026120
Sigarispora ravennica	MFLUCC 14-0005	KP698414	–	KP698415	KP698413
Vaginatispora appendiculata	**MFLUCC 16-0314**	**KU743218**	**KU743220**	**KU743219**	**KU743217**
Vaginatispora aquatica	MFLUCC 11-0083	KJ591576	–	KJ591575	KJ591577
Vaginatispora armatispora	HKLTCC 1562	–	–	–	AF383955
Vaginatispora fuckelii	JCM 17672	AB619008	LC001749	AB618689	LC001731
Vaginatispora fuckelii	MAFF 239458	AB619009	LC001750	AB618690	LC001732
Vaginatispora 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 LuangUniversity 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: Π(A): 0.249198, Π(C): 0.239722, Π(G): 0.266913 and Π(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 μm high, 200–400 μm diam. ($\overline{x}$ = 387.3 × 266.2 μm, n = 10), scattered, immersed, coriaceous, black, globose to subglobose, ostiolate. *Ostiole* 100–200 μm high, 50–70 μm ($\overline{x}$ = 165.3 × 63.9 μm, n = 10) diam., slit-like, central, with a crest-like papilla, with an irregular, pore-like opening,

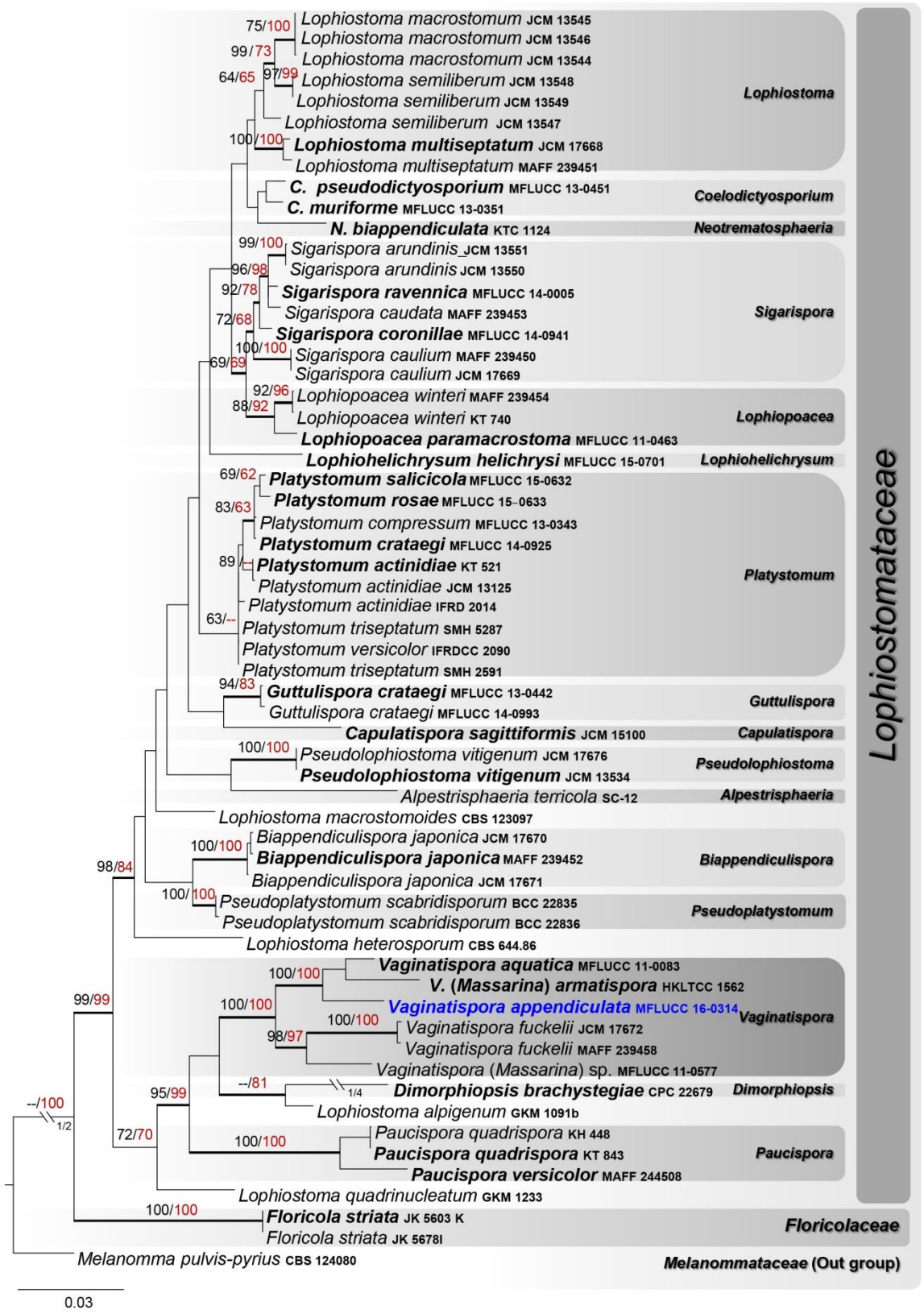

Fig. 1 – RAxML tree based on a combined dataset of LSU, TEF, SSU and ITS partial sequences. Bootstrap support values for maximum likelihood (ML, black) and maximum parsimony (MP, red) higher than 60 % are defined as above the nodes and branches with Bayesian posterior probabilities (BYPP) greater than 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 ($\overline{x}$ = 158.9 × 29 µm, n = 35), 8-spored, bitunicate, fissitunicate, cylindrical-clavate, with a developed pedicel (10–30 µm long; $\overline{x}$ = 21 µm, n =30), apically rounded with an ocular chamber. *Ascospores* 40–45 × 10–15 µm ($\overline{x}$ = 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; $\overline{x}$ = 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***

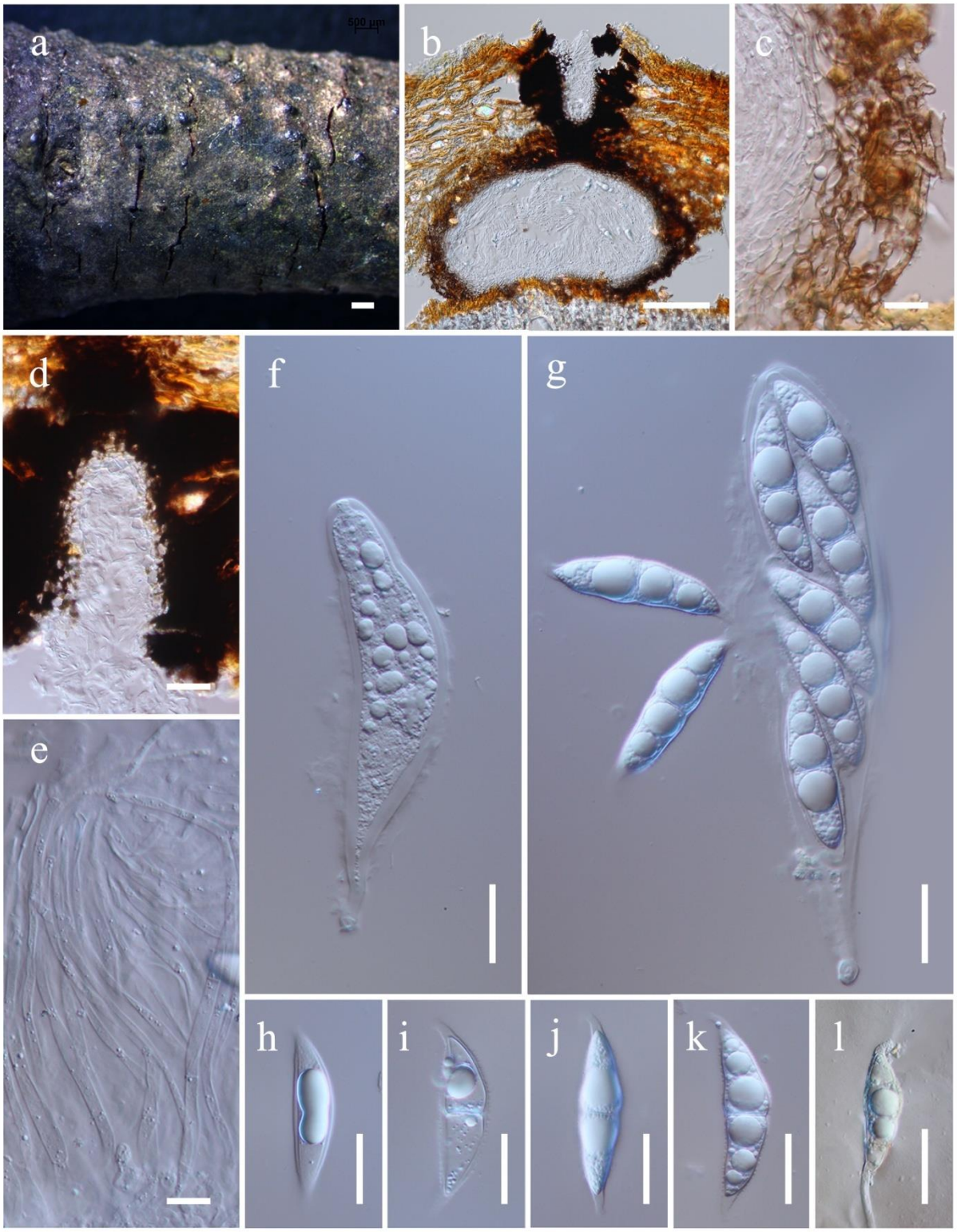

Fig. 2 – ***Vaginatispora appendiculata*** (**holotype**). **a** Appearance of ascomata on host substrate. **b** Section of ascoma. **c** Peridium. **d** Close up of ostiole. **e** Pseudoparaphyses. **f, g** Asci. **h-k** Ascospores. **l** Germinated spore. Scale bars: a = 500 µm, b = 100 µm, c, e = 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 (40–45 × 10–15 μm) than those of *V. armatispora* (28–38 × 7–9.8 μm). 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 μm 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.

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