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Article

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Lignicolous freshwater fungi from China and Thailand: Multi-gene phylogeny reveals new species and new records in Lophiostomataceae

Bao DF[1,2,3], Su HY[1], Maharachchikumbura SSN[4], Liu JK[4], Nalumpang S[3], Luo ZL[1*] and Hyde KD[2]

[1]*College of Agriculture and Biological Sciences, Dali University, Dali 671003, Yunnan, P.R. China*
[2]*Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[3]*Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand*
[4]*School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu 611731, P.R. China*

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Abstract

We are investigating the diversity of lignicolous freshwater fungi from China and Thailand. In this study, six collections of Lophiostomataceae-like taxa were made from freshwater habitats in China and Thailand, of which three are identified as existing species *Biappendiculispora japonica*, *Neovaginatispora fuckelii* and *Vaginatispora armatispora*. While, the other collections are recognized as new species, *Flabellascoma aquaticum* sp. nov., *F. fusiforme* sp. nov. and *Sigarispora clavata* sp. nov. and these are introduced herein based on the morphological characters and multi-gene phylogenetic analyses of combined LSU, SSU, ITS, TEF1-α and RPB2 sequence data. Detailed descriptions and illustrations of these six species are provided.

Key words – 3 new species – Dothideomycetes – Pleosporales – sexual morphs – phylogeny taxonomy

Introduction

Lignicolous freshwater fungi grow on submerged woody debris in freshwater streams, ponds, lakes and tree hollows (Hyde 1995, Wong et al. 1998, Ho et al. 2002). They play an important role in ecosystem functioning and nutrient recycling of woody material (Palmer et al. 1997, Wong et al. 1998, Hyde and Goh 1998, Bucher et al. 2004). Lignicolous freshwater fungi are highly diverse and probably sensitive to environmental change and global warming (Hyde et al. 2016). We are studying the diversity of lignicolous freshwater fungi along the north-south gradient in the Asian/Australasian region (Hyde et al. 2016). By collecting these data, we are contributing to the knowledge of biogeographical diversity of fungi in freshwater habitats.

The family Lophiostomataceae was previously reported as “Lophiostomeae” by Nitschke (1869). Subsequently, Saccardo (1883) formally established Lophiostomataceae and placed it in

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Corresponding Author: Zong-Long Luo – e-mail – luozonglongfungi@163.com

Pleosporales. Species mostly occur as saprobes in terrestrial, freshwater and marine environments (Tibpromma et al. 2017, Hashimoto et al. 2018, Hyde et al. 2019). Their intergeneric relationships have been discussed by Kodsueb et al (2006) and Wang et al. (2007) and recently by Hashimoto et al. (2018). Members of this family are characterized by immersed to erumpent, carbonaceous to coriaceous ascomata with rounded or slit-like ostioles, mostly clavate asci and 1 to multi-septate, hyaline to dark brown ascospores with terminal appendages or mucilaginous sheaths (Hyde et al. 2013, 2017, Ariyawansa et al. 2015, Liu et al. 2015, Thambugala et al. 2015). Thambugala et al. (2015) revised the classification of this family, provided a backbone tree and accepted 16 genera. Wanasinghe et al. (2018) introduced a new genus, *Muritestudina* and Hashimoto et al. (2018) introduced seven genera in Lophiostomataceae. Presently, 24 genera are accepted in Lophiostomataceae (Wijayawardene et al. 2017, Hashimoto et al. 2018, Wanasinghe et al. 2018, Hyde et al. 2019).

During a survey of lignicolous freshwater fungi along the north-south gradient in the Asian/Australian region (Hyde et al. 2016), six lophiostomataceous lignicolous freshwater taxa were collected from Tibet and Yunnan provinces, China and southern Thailand. Three new species, *viz. Flabellascoma aquaticum*, *F. fusiforme* and *Sigarispora clavata* are introduced based on morphological characters and multi-gene phylogenetic analysis. Detailed descriptions and illustrations of the new species and records are also provided.

Materials & Methods

Isolation and morphological examination

Submerged decaying wood samples were collected from Tibet and Yunnan provinces, China and Sai khu waterfall, Thailand, and brought to the laboratory in plastic bags. The samples were incubated in plastic boxes lined with moistened tissue paper at room temperature for 1 week. Sample examination and morphological studies followed protocols outlined previously (Luo et al. 2018).

The fungal species present on the substrates were isolated using a single spore culture technique following the method described in Chomnunti et al. (2014). Germinating ascospores were transferred aseptically to fresh potato dextrose agar (PDA) media with antibiotics and incubated at room temperature for 2–4 weeks. Cultures were grown for 1–2 months and morphological characters such as colour, colony shape, texture and asexual morphs were recorded and checked after 30–60 days. The cultures were deposited in Mae Fah Luang University Culture Collection (MFLUCC) and Kunming Institute of Botany culture collection (KUMCC), herbarium specimens (dry wood with the fungi and slides) were deposited in Mae Fah Luang University (MFLU). Faces of fungi and Index Fungorum numbers were registered as detailed in Jayasiri et al. (2015) and in Index Fungorum (http://www.indexfungorum.org/names/nam-es.asp) respectively.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fungal mycelium grown on PDA or MEA at 25 °C using EZ gene^{TM} Fungal gDNA Kits (GD2416) according to the manufacturer's instructions. The regions of large subunit rRNA (LSU), internal transcribed spacers (ITS), small subunit rRNA (SSU), translation elongation factor (TEF1-α) and RNA polymerase II subunit 2 (RPB2) were amplified using the primer pairs LR0R/LR7 (Vilgalys & Hester 1990), ITS5/ITS4, NS1/ NS4 (White et al. 1990), 983F/2218R and fRPB2-5F/fRPB2-7cR (Liu et al. 1999) respectively. The amplification reactions were performed in 25 μL of PCR mixtures containing 12.5 μl of 2×Power Taq PCR Master Mix (a premix and ready to use solution, including 0.1 Units/μl Taq DNA Polymerase, 500 μm dNTP

Mixture each (dATP, dCTP, dGTP, dTTP), 20 mM Tris-HCl pH 8.3, 100 Mm KCl, 3 mM $MgCl_2$, stabilizer and enhancer), 1 µl of each primer, 1 µl DNA template and 9.5 µl deionized water. The PCR profile for the ITS, LSU, SSU and TEF1-α gene regions as follows: initial denaturation for 94 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 56 °C for 50 seconds, elongation at 72 °C for 1 minute and a final extension at 72 °C for 10 minutes. The RPB2 gene region was amplified with an initial denaturation of 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 1 min, annealing at 54 °C for 40 seconds, elongation at 72 °C for 90 seconds, and the final extension at 72 °C for 10 min. Purification and sequencing of PCR products were carried out using the above-mentioned PCR primers at Beijing Tsingke Biological Engineering Technology and Services Co., Ltd. (Beijing, P.R. China)

Sequence alignment and phylogenetic analyses

Sequence data for relevant strains were downloaded from GenBank following recent publications (Table 1) (Thambugala et al. 2015, Hashimoto et al. 2018, Wanasinghe et al. 2018, Hyde et al. 2019). Consensus sequences were assembled using BioEdit and aligned using MAFFT v.7.110 online program (http://mafft.cbrc.jp/alignment/server/) (Katoh & Standley 2013) and manually edited using BioEdit v7.2.3 (Hall 1999). The phylogenetic analyses were performed using Randomized Accelerated Maximum Likelihood (RAxML) and Bayesian analyses. Phylogeny website tool "ALTER" (Glez-Peña et al. 2010) was used to convert the alignment fasta file to Phylip format and Maximum likelihood (ML) analysis was performed using the CIPRES Science Gateway v.3.3 (http:// www.phylo.org/portal2/; Miller et al. 2010) using RAxML v.8.2.8 as part of the "RAxML-HPC2 on XSEDE" tool (Stamatakis 2006, Stamatakis et al. 2008). All free model parameters were estimated by RAxML with ML estimates of 25 per site rate categories. The final ML search was conducted using the GTRGAMMA + I substitution model.

Bayesian analysis was performed by MrBayes v3.1.2 (Ronquist et al. 2012). The model of evolution was estimated by using MrModeltest 2.2 (Nylander 2004). Posterior probabilities (Rannala & Yang 1996) were performed by Markov Chain Monte Carlo Sampling (BMCMC) in MrBayes v. 3.1.2. Six simultaneous Markov Chains were run for 1 million generations and trees were sampled every 100th generation (resulting in 10,000 trees). The first 2,000 trees representing the burn-in phase of the analyses were discarded and the remaining 8,000 (post burning) trees used for calculating posterior probabilities (PP) in the majority rule consensus tree.

Table 1 Isolates and sequences used in this study. The newly generated sequences are indicated in red and ex-type strains are indicated in bold.

Taxa	Strain number	GenBank accession numbers				
		SSU	ITS	LSU	TEF1-α	RPB2
Alpestrisphaeria terricola	**SC-12**	**JX985749**	**JN662930**	**JX985750**	**–**	**–**
Alpestrisphaeria jonesii	GAAZ 54–1	KX687755	KX687757	KX687753	KX687759	–
Alpestrisphaeria jonesii	GAAZ 54–2	KX687756	KX687758	KX687754	KX687760	–
Biappendiculispora japonica	**KT 573**	**AB618686**	**LC001728**	**AB619005**	**LC001744**	**–**
Biappendiculispora japonica	KT 686-1P	AB618687	LC001729	AB619006	LC001745	–
Biappendiculispora japonica	MFLUCC 17–2450	MN304834	MN304829	MN328900	–	–
Capulatispora sagittiformis	**KT 1934**	**AB618693**	**AB369268**	**AB369267**	**LC001756**	**–**
Coelodictyosporium muriforme	**MFLUCC 13–0351**	**KP899127**	**KP899136**	**KP888641**	**KR075163**	**–**

Table 1 Continued.

Taxa	Strain number	GenBank accession numbers				
		SSU	ITS	LSU	TEF1-α	RPB2
Coelodictyosporium pseudodictyosporium	**MFLUCC 13–0451**	**–**	**KR025858**	**KR025862**	**–**	**–**
Crassiclypeus aquaticus	**CBS 143641**	**LC312470**	**LC312499**	**LC312528**	**LC312557**	**LC312586**
Crassiclypeus aquaticus	**CBS 143642**	**LC312471**	**LC312500**	**LC312529**	**LC312558**	**LC312587**
Dimorphiopsis brachystegiae	**CPC 22679**	**–**	**KF777160**	**KF777213**	**–**	**–**
Flabellascoma minimum	**CBS 143645**	**LC312474**	**LC312503**	**LC312532**	**LC312561**	**LC312590**
Flabellascoma minimum	**CBS 143646**	**LC312475**	**LC312504**	**LC312533**	**LC312562**	**LC312591**
Flabellascoma aquaticum	**KUMCC15–0258**	**MN304832**	**MN304827**	**MN274564**	**MN328898**	**MN328895**
Flabellascoma cycadicola	**CBS 143644**	**LC312473**	**LC312502**	**LC312531**	**LC312560**	**LC312589**
Flabellascoma fusiforme	**MFLUCC 18–1584**	**–**	**MN304830**	**MN274567**	**MN328902**	**–**
Guttulispora crataegi	**MFLUCC 13–0442**	**KP899125**	**KP899134**	**KP888639**	**KR075161**	**–**
Guttulispora crataegi	MFLUCC 14–0993	KP899126	KP899135	KP888640	KR075162	–
Lentistoma bipolare	**CBS 143651**	**LC312483**	**LC312512**	**LC312541**	**LC312570**	**LC312599**
Lentistoma bipolare	**CBS 143652**	**LC312484**	**LC312513**	**LC312542**	**LC312571**	**LC312600**
Leptoparies palmarum	**CBS 143653**	**LC312485**	**LC312514**	**LC312543**	**LC312572**	**LC312601**
Lophiohelichrysum helichrysi	**MFLUCC 15–0701**	**KT333437**	**KT333435**	**KT333436**	**KT427535**	**–**
Lophiopoacea paramacrostoma	**MFLUCC 11–0463**	**KP899122**	**–**	**KP888636**	**–**	**–**
Lophiopoacea winteri	KT 740	AB618699	JN942969	AB619017	LC001763	JN993487
Lophiopoacea winteri	KT 764	AB618700	JN942968	AB619018	LC001764	JN993488
Lophiostoma caulium	CBS 623.86	GU296163	–	GU301833	–	GU371791
Lophiostoma crenatum	CBS 629.86	DQ678017	–	DQ678069	DQ677912	DQ677965
Lophiostoma heterosporum	CBS 644.86	AY016354	GQ203795	AY016369	DQ497609	DQ497615
Lophiostoma macrostomoides	CBS 123097	FJ795482	–	FJ795439	GU456277	FJ795458
Lophiostoma macrostomum	KT 635	AB521731	AB433275	AB433273	LC001752	JN993484
Lophiostoma quadrinucleatum	GKM 1233	–	–	GU385184	GU327760	–
Lophiostoma semiliberum	KT 828	AB618696	JN942970	AB619014	LC001759	JN993489
Lophiostoma alpigenum	GKM 1091b	–	–	GU385193	GU327758	–
Lophiostoma multiseptatum	**JCM17668**	**AB618684**	**LC001726**	**AB619003**	**LC001742**	
Neotrematosphaeria biappendiculata	KT 1124	GU205256	–	GU205227	–	–
Neotrematosphaeria biappendiculata	**KT 975P**	**GU205254**	**–**	**GU205228**	**–**	**–**
Neovaginatispora fuckelii	MFLUCC 17–1334	MN304833	MN304828	MN274565	MN328899	MN328896
Neovaginatispora fuckelii	CBS 101952	FJ795496	–	DQ399531	–	FJ795472
Neovaginatispora fuckelii	KH 161	AB618689	LC001731	AB619008	LC001749	–
Neovaginatispora fuckelii	KT 634	AB618690	LC001732	AB619009	LC001750	–
Parapaucispora pseudoarmatispora	**KT 2237**	**LC100018**	**LC100021**	**LC100026**	**LC100030**	**–**
Paucispora quadrispora	**KT 843**	**AB618692**	**LC001734**	**AB619011**	**LC001755**	**–**
Paucispora versicolor	**KH 110**	**LC001721**	**AB918731**	**AB918732**	**LC001760**	**–**

Table 1 Continued.

Taxa	Strain number	GenBank accession numbers				
		SSU	ITS	LSU	TEF1-α	RPB2
Paucispora quadrispora	KH 448P	LC001720	LC001733	LC001722	LC001754	–
Platystomum actinidiae	**KT 521**	**JN941375**	**JN942963**	**JN941380**	**LC001747**	**JN993490**
Platystomum compressum	MFLUCC 13–0343	KP899129	–	KP888643	KR075165	–
Platystomum crataegi	**MFLUCC 14–0925**	**KT026113**	**KT026117**	**KT026109**	**KT026121**	**–**
Platystomum rosae	**MFLUCC 15–0633**	**KT026115**	**-**	**KT026111**	**KT026119**	**–**
Platystomum salicicola	**MFLUCC 15–0632**	**KT026114**	**KT026118**	**KT026110**	**–**	**–**
Pseudolophiostoma obtusisporum	**CBS 143941**	**LC312490**	**LC312519**	**LC312548**	**LC312577**	**LC312606**
Pseudolophiostoma obtusisporum	**CBS 143658**	**LC312491**	**LC312520**	**LC312549**	**LC312578**	**LC312607**
Pseudolophiostoma tropicum	**CBS 143659**	**LC312492**	**LC312521**	**LC312550**	**LC312579**	**LC312608**
Pseudolophiostoma tropicum	**CBS 143660**	**LC312493**	**LC312522**	**LC312551**	**LC312580**	**LC312609**
Pseudolophiostoma vitigenum	**HH 26930**	**AB618697**	**LC001735**	**AB619015**	**LC001761**	**–**
Pseudolophiostoma vitigenum	HH 26931	AB618698	LC001736	AB619016	LC001762	–
Pseudopaucispora brunneospora	**CBS 143661**	**LC312494**	**LC312523**	**LC312552**	**LC312581**	**LC312610**
Pseudoplatystomum scabridisporum	BCC 22835	GQ925831	–	GQ925844	GU479857	GU479830
Pseudoplatystomum scabridisporum	BCC 22836	GQ925832	–	GQ925845	GU479856	GU479829
Sigarispora clavata	**MFLUCC 18–1316**	**MN304835**	–	**MN274566**	**MN328901**	–
Sigarispora arundinis	KT 651	AB618680	JN942965	AB618999	LC001738	JN993486
Sigarispora caryophyllacearum	**MFLUCC 17–0749**	**MG829176**	**MG828964**	**MG829076**	**MG829238.**	**–**
Sigarispora caudata	KT 530	AB618681	LC001723	AB619000	LC001739	–
Sigarispora caulium	**MFLUCC 15–0036**	**MG829177**	**MG828965**	**MG829077**	**MG829239**	**–**
Sigarispora coronillae	**MFLUCC 14–0941**	**KT026116**	**KT026120**	**KT026112**	**–**	**–**
Sigarispora junci	**MFLUCC 14–0938**	**MG829178**	**MG828966**	**MG829078**	**–**	**–**
Sigarispora medicaginicola	**MFLUCC 17–0681**	**MG829179**	**MG828967**	**MG829079**	**–**	**–**
Sigarispora muriformis	MFLUCC 13–0744	KY501110	KY496740	KY496719	–	–
Sigarispora ononidis	MFLUCC 14–0613	KU243126	KU243128	KU243125	KU243127	–
Sigarispora ravennica	**MFLUCC 14–0005**	**KP698415**	**KP698413**	**KP698414**	**–**	**–**
Sigarispora rosicola	**MFLU 15–1888**	**MG829180**	**MG828968**	**MG829080**	**MG829240**	**–**
Sigarispora scrophulariae	**MFLUCC 17–0689**	**MG829181**	**MG828969**	**MG829081**	**–**	**–**
Sigarispora thymi	**MFLU 15–2131**	**MG829182**	**MG828970**	**MG829082**	**MG829241**	**–**
Teichospora rubriostiolata	TR 7H	–	KU601590	KU601590	KU601609	KU601599
Teichospora trabicola	C 134E	–	KU601591	KU601591	KU601601	KU601600
Vaginatispora amygdali	**KT2248**	**LC312495**	**LC312524**	**LC312553**	**LC312582**	**LC312640**
Vaginatispora amygdali	MFLUCC 18–1586	MK085057	MK085055	MK085059	MK087657	–
Vaginatispora appendiculata	MFLUCC 16–0314	KU743219	KU743217	KU743218	KU743220	–
Vaginatispora aquatica	**MFLUCC 11–0083**	**KJ591575**	**KJ591577**	**KJ591576**	**–**	**–**

Table 1 Continued.

Taxa	Strain number	GenBank accession numbers				
		SSU	ITS	LSU	TEF1-α	RPB2
Vaginatispora armatispora	MFLUCC 18–0247	MK085058	MK085056	MK085060	MK087658	MK087669
Vaginatispora armatispora	**HKTLCC1562**	**–**	**AF383955**	**–**	**–**	**–**
Vaginatispora armatispora	MFLUCC 18–0213	MN304831	MN304826	MN274563	MN328897	MN328894
Vaginatispora microarmatispora	MTCC 12733	MF142594	MF142592	MF142593	MF142595	MF142596
Vaginatispora scabrispora	**KT2443**	**LC312496**	**LC312525**	**LC312554**	**LC312583**	**LC312612**

Results

Phylogenetic analyses

The combined LSU, SSU, ITS, TEF1-α and RPB2 dataset comprised 82 taxa of Lophiostomataceae, with *Teichospora rubriostiolata* (TR7) and *Teichospora trabicola* (C134) as the outgroup taxa. The dataset comprised 4,188 characters after alignment including gaps (LSU: 1–826; SSU: 827–1767; ITS: 1768–2283; TEF1-α: 2284–3177; RPB2: 3178–4188). The RAxML analysis of the combined dataset yielded a best scoring tree (Fig. 1) with a final ML optimization likelihood value of –28080.438440. The matrix had 1551 distinct alignment patterns, with 26.56% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.250308, C = 0.245041, G = 0.267629, T = 0.237021; substitution rates AC = 1.632712, AG = 4.315873, AT = 1.313155, CG = 1.501540, CT = 9.264809, GT = 1.000000; gamma distribution shape parameter α = 0.180969. Support values for maximum likelihood (ML) above than 75% and Bayesian posterior probabilities (PP) greater than 0.95 are given at the nodes.

The phylogenetic analyses showed that newly isolates of *Vaginatispora armatispora*, *Biappendiculispora japonica* and *Neovaginatispora fuckelii* clustered with *V. armatispora* (MFLUCC 18–0247, HKTLCC 1562), *B. japonica* (KT 573, KT 686) and *N. fuckelii* (KT 634, KH 161, CBS 101952), respectively, with strong statistical support (100 ML/1.00 PP, 98 ML/1.00 PP and 100 ML/1.00 PP, respectively).

The novel species *Sigarispora clavata* grouped with members of the genus *Sigarispora* as a sister taxon to *S. caudata* with strong statistical support (76 ML/1.00 PP). The other new species, *Flabellascoma aquaticum* and *F. fusiforme* clustered together within the genus *Flabellascoma*, but they formed distinct lineages with significant statistical support (96 ML/1.00 PP and 100 ML/1.00 PP, respectively).

Flabellascoma aquaticum D.F. Bao, Z.L. Luo, K.D. Hyde & H.Y. Su, sp. nov. Fig. 2

Index Fungorum number: IF 556720; Facesoffungi number: FoF 06212

Etymology – Referring to the aquatic habitat

Holotype – MFLU 19–0992

Saprobic on submerged decaying wood. Sexual morph: *Ascomata* 280–440 μm high, 260–390 μm diam ($\bar{x}$ = 327 × 360 μm，n = 8), immersed, scattered, subglobose, dark brown to black, with a long, black neck. *Ostiole* crest-like, dark brown to black, elongated, composed of brown to black cells. *Peridium* 25–52 μm wide, uniform, comprising 2 zones, outer layers composed several dark brown cells, inner zone composed of 5–7 layers of rectangular, hyaline to pale brown cells of *textura*

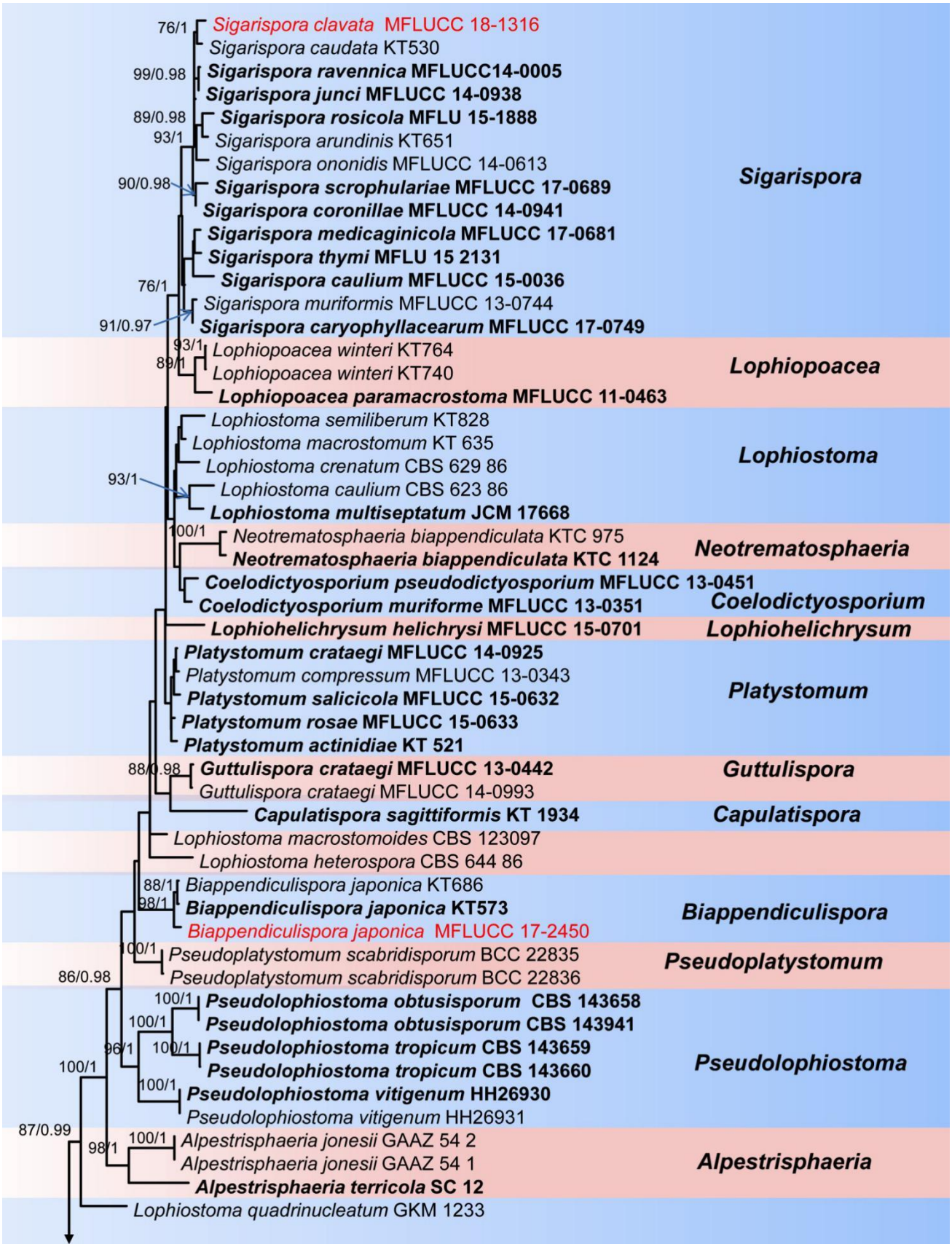

Figure 1 – Phylogenetic tree based on RAxML analyses of combined LSU, SSU, ITS, TEF1-α and RPB2 sequence data. Maximum likelihood bootstrap > 75 % and Bayesian posterior probabilities > 0.95 (BYPP) are indicated at the nodes. The ex-type strains are shown in bold and the newly obtained

isolates are shown in red. The tree is rooted with *Teichospora rubriostiolata* (TR7) and *Teichospora trabicola* (C134).

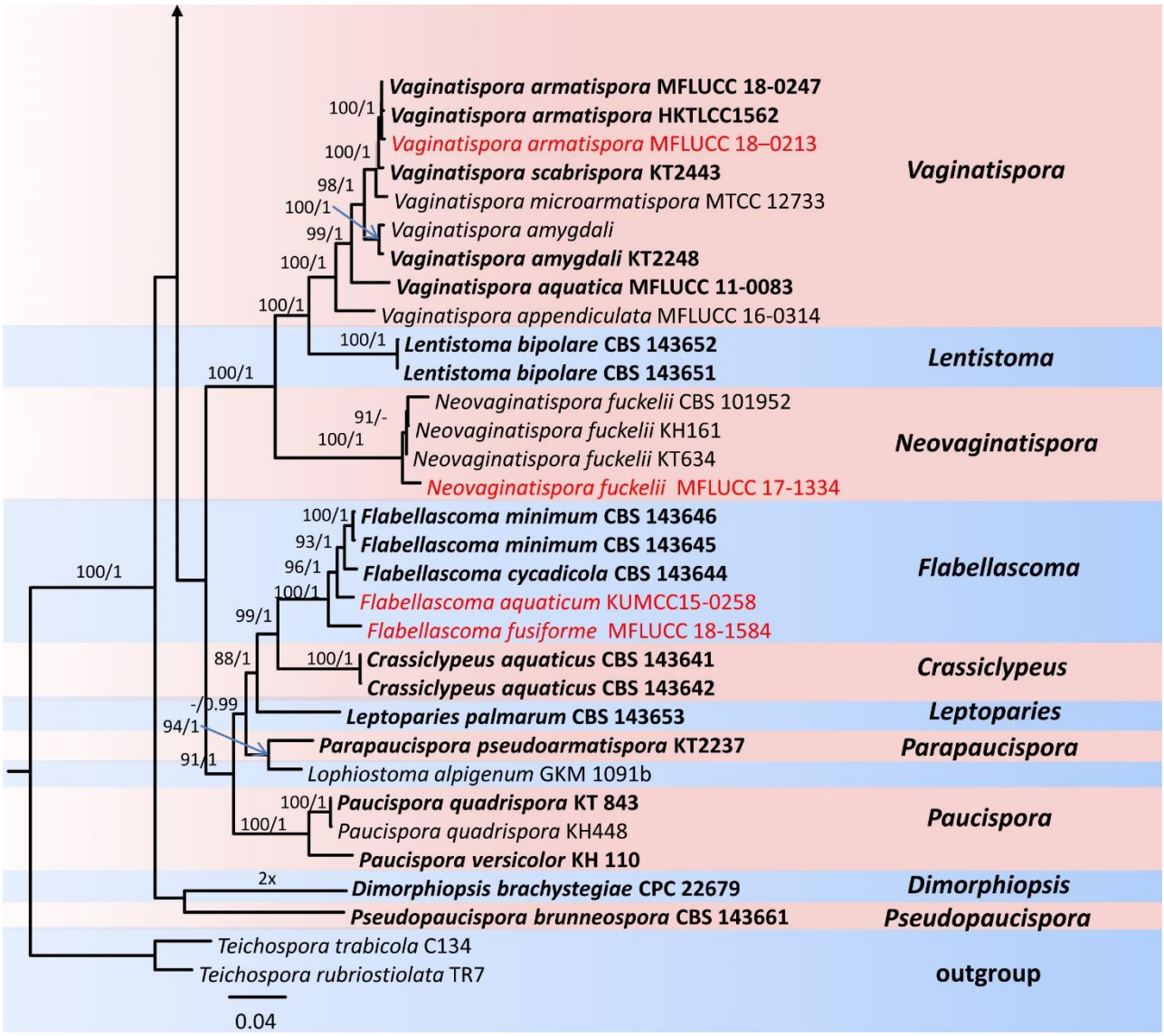

Figure 1 – Continued.

angularis and *globulosa*. *Hamathecium* comprising numerous, 1.2–2 μm wide, cellular pseudoparaphyses, septate, branched and anastomosed. *Asci* 48–72 × 8–9 μm ($\bar{x}$ = 60 × 8.6 μm，n = 20), 8-spored, bitunicate, fissitunicate, cylindrical-clavate, short pedicellate, apically rounded with an ocular chamber. *Ascospores* 16–18 × 4.3–5.3 μm ($\bar{x}$ = 17 × 5 μm, n = 30), fusiform with narrow and obtuse ends, hyaline, uniseptate, slightly constricted at the septum, 4-guttules, 2 middle ones larger than end ones, with a narrow sheath. Appendages drawn out from sheath at both of ends (4.7–7 μm wide, n = 30), hyaline, unbranched. Asexual morph: Undetermined.

Material examined – CHINA, Yunnan Province, saprobic on decaying wood submerged in Dulong River (27°53'51.50" N, 98°20'13.10" E), May 2015, Z.L. Luo, S-390 (MFLU 19–0992, holotype), ex-type living culture, KUMCC 15–0258.

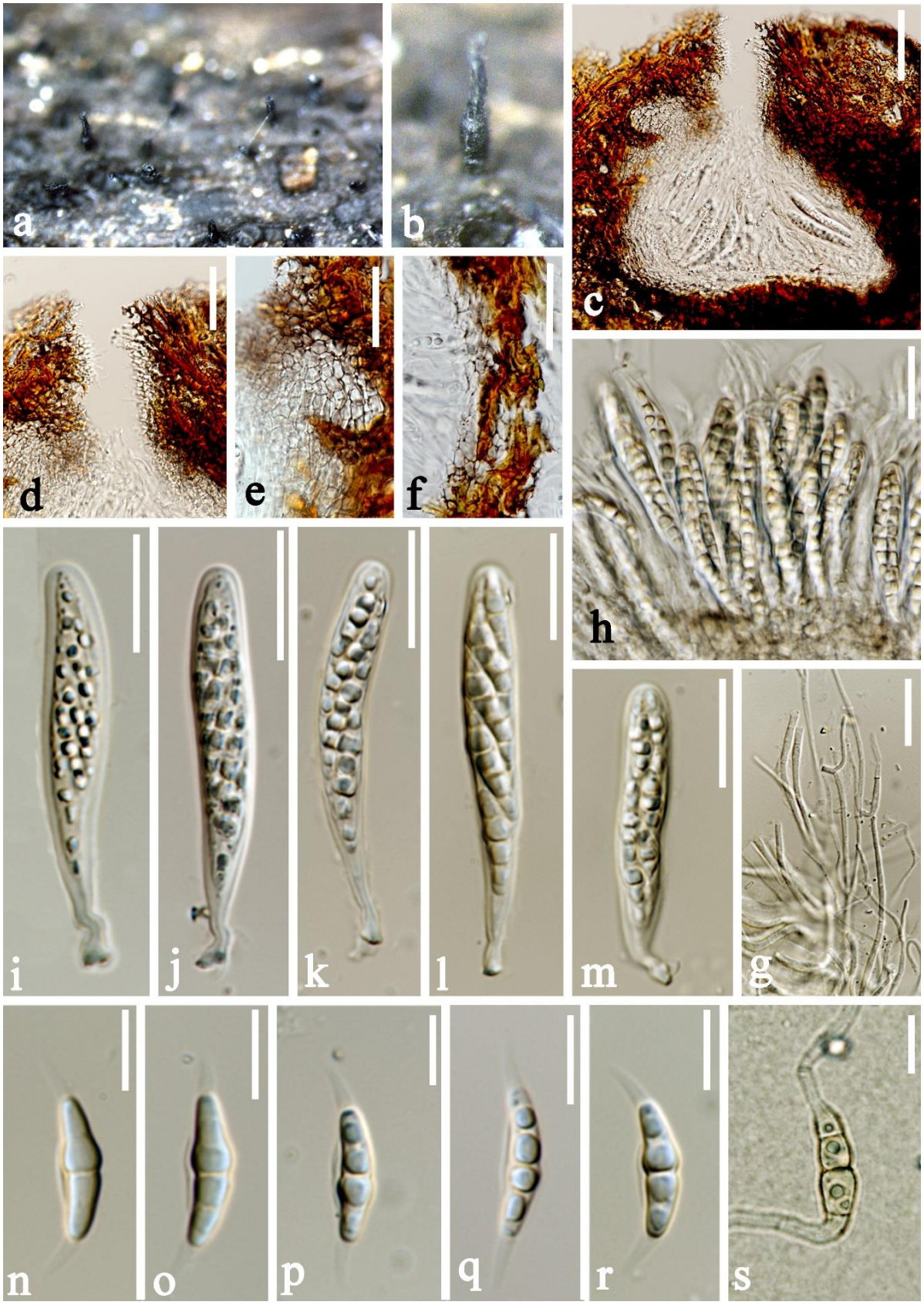

Figure 2 – *Flabellascoma aquaticum* (MFLU 19–0992, holotype). a Ascomata on submerged wood. b Neck of ascoma. c Section of ascoma. d Ostiolar neck of ascoma. e, f Peridium of ascomata. g Pseudoparaphyses. h–m Asci. n–r Ascospore. s Germinating ascospore. Scale bars: c = 50 μm, d–f = 30 μm, g–m = 20 μm, n–s = 10 μm.

Notes – *Flabellascoma* species have similar shaped asci and ascospores. *F. cycadicola* and *F. minimum* are distinguishable from each other based on ascospore size. *F. aquaticum* differs from *F. cycadicola* in ascus and ascospore size (48–72 × 8–9 vs. 67.5–88 × 9–12 μm and 16–18 × 4.3–5.3 vs. 17–23 × 4.5–7 μm). Moreover, *F. aquaticum* can be morphologically distinguished from *F. cycadicola* and *F. minimum* based on ascomata characters. *F. aquaticum* has ascomata with a cylindrical, long, black neck (Fig. 2b). Whereas, ascomata of *F. cycadicola* and *F. minimum* have short, elongated, crest-like, ostiolar neck. In the phylogenetic analysis, *F. aquaticum* and *F. fusiforme* grouped with other existing *Flabellascoma* species, but formed distinct lineages, and thus are distinct species (Fig. 1).

Flabellascoma fusiforme D.F. Bao, Z.L. Luo, K.D. Hyde & H.Y. Su, sp. nov. Fig. 3

Index Fungorum number: IF 556721; Facesoffungi number: FoF 06213

Etymology – referring to the fusiform ascospores.

Holotype – MFLU 19–0993

Saprobic on submerged decaying wood. Sexual morph: *Ascomata* 310–420 μm high, 320–380 μm diam ($\overline{x}$ = 358 × 345 μm, n = 5), scattered, immersed, subglobose, dark brown to black. *Ostiole* crest-like, central, with a reduced crest and a pore-like opening, composed dark brown to black cells. *Peridium* 25–50 μm wide, composed two strata, outer stratum comprising brown to dark brown, thick-walled cells, inner stratum composed of several layers with lightly pigmented to hyaline cells. *Hamathecium* comprising 1.5–3 μm wide, septate, branched, cellular pseudoparaphyses. *Asci* 66–80 × 10–12 μm ($\overline{x}$ = 72.8 × 10.8 μm, n = 20), 8-spored, bitunicate, fissitunicate, cylindrical-clavate with a furcate pedicel, apically rounded with a broad ocular chamber. *Ascospores* 15–18 × 4–5 μm ($\overline{x}$ = 16.6 × 4.7 μm, n = 30), fusiform, with narrow and obtuse to acute ends, hyaline, uniseptate, 4-guttules, 2 middle ones larger than end ones, constricted at septum, smooth, with a thin sheath, appendages drawn out from sheath at both of ends (5.4–8 μm wide, n = 30), hyaline, unbranched. Asexual morph：Undetermined.

Material examined – CHINA, Yunnan Province, saprobic on decaying wood submerged in Nujiang River (27°37'29.92" N, 98°45'38.10" E), October 2016, Z.L. Luo, S-1583 (MFLU 19–0993, holotype), ex-type culture, MFLUCC 18–1584

Notes – Currently, two species are accepted in *Flabellascoma*. In this study, we introduce two additional species in this genus. The new species *Flabellascoma fusiforme* fits well with the morphological characters of *Flabellascoma*, such as immersed ascomata, bitunicate, fissitunicate, cylindrical-clavate asci and fusiform, hyaline, 1-septate ascospores with a narrow bipolar sheath. We herein compare the morphological differences between new species *Flabellascoma fusiforme* with other *Flabellascoma* species.

Flabellascoma fusiforme is phylogenetically close to *F. aquaticum* (Fig. 1). However, *Flabellascoma fusiforme* can be easily distinguished from *F. aquaticum* by its ascomatal shape and ascus size. *Flabellascoma fusiforme* has ascomata with a short, elongated, crest-like ostiolar neck (Fig. 3a, b, c), while the ascomata in *F. aquaticum* has a cylindrical, long, black neck (Fig. 2b) and the asci of *F. fusiforme* are larger than *F. aquaticum* (66–80 × 10–12 vs. 48–72 × 8–9 μm).

Flabellascoma fusiforme can be distinguished from *F. cycadicola* in having smaller ascospores (15–18 × 4–5 vs. 17–23 × 4.5–7 μm) and longer ascus pedicel. In addition, both the ascospores ends of *F. cycadicola* are narrower than *F. fusiforme*. Furthermore, *F. fusiforme* differs from *F. minimum* in its shape of ascomata and ascospores; *F. fusiforme* has subglobose ascomata and straight ascospores. However, *F. minimum* ascomata are ellipsoidal to lageniform and ascospores are slightly curved.

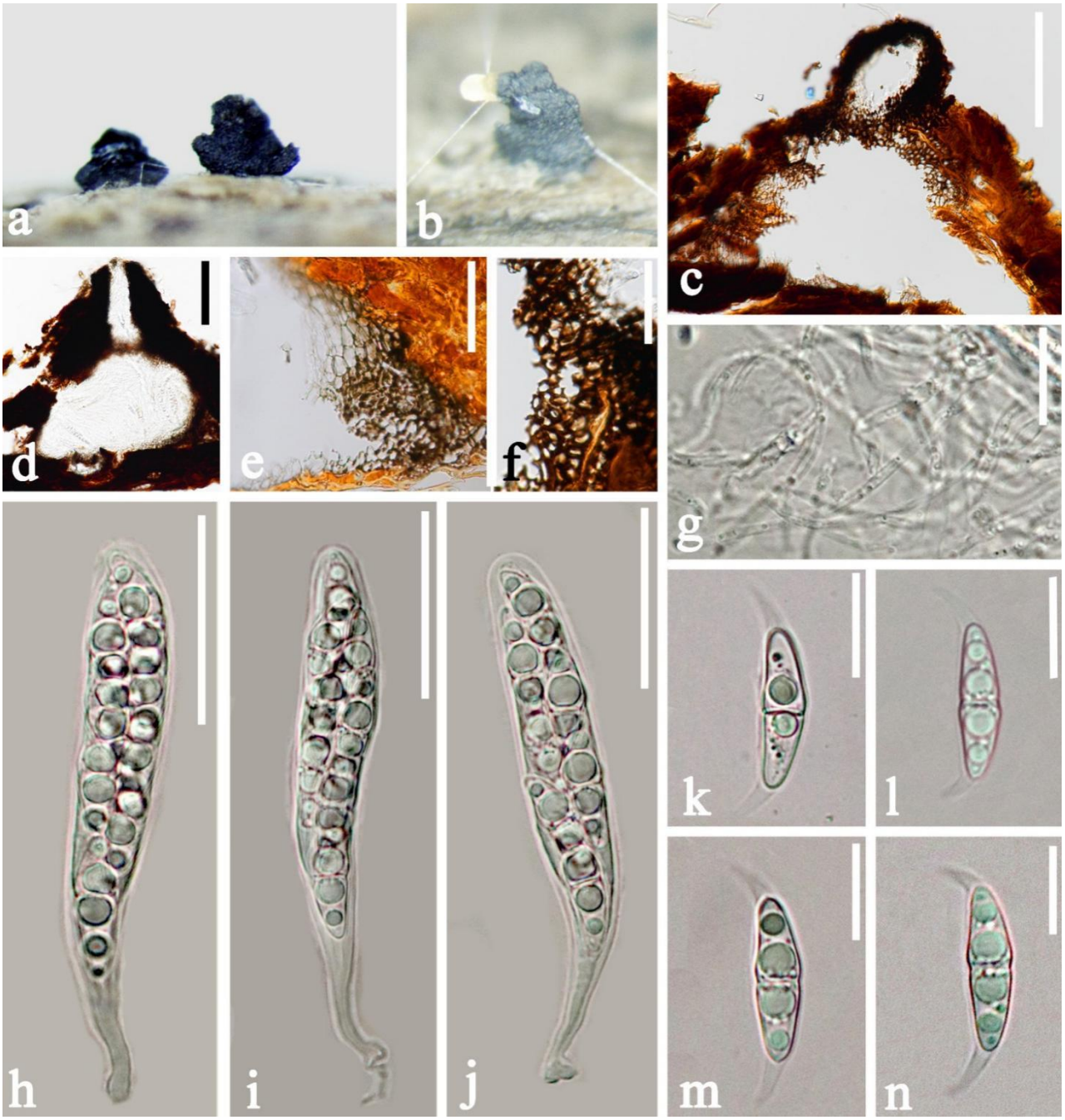

Figure 3 – *Flabellascoma fusiforme* (MFLU 19–0993, holotype). a–c Ascomata on submerged wood. c, d Section of ascoma. e, f Peridium of ascomata. g Pseudoparaphyses. h–j Asci. k–n Ascospore. Scale bars: c, d = 100 μm, e = 50 μm, f, h–j = 30 μm, g = 20μm, k–n = 10 μm.

Sigarispora clavata D.F. Bao, Z.L. Luo, K.D. Hyde & H.Y. Su, sp. nov. Fig. 4

Index Fungorum number: IF 556722; Facesoffungi number: FoF 06214

Etymology – Referring to the clavate ascospores.

Holotype – MFLU 19–0994

Saprobic on submerged decaying wood in freshwater habitats. Sexual morph: *Ascomata* 327–470 μm high, 280–460μm diam ($\overline{x}$ = 380 × 400 μm, n = 5), semi-immersed to immersed, subglobose, coriaceous, black, ostiolate. Ostiole slit-like, variable in shape, with a crest-like apex and a pore-like opening, plugged by gelatinous tissue, made up of lightly pigmented, pseudoparenchymatous cells.

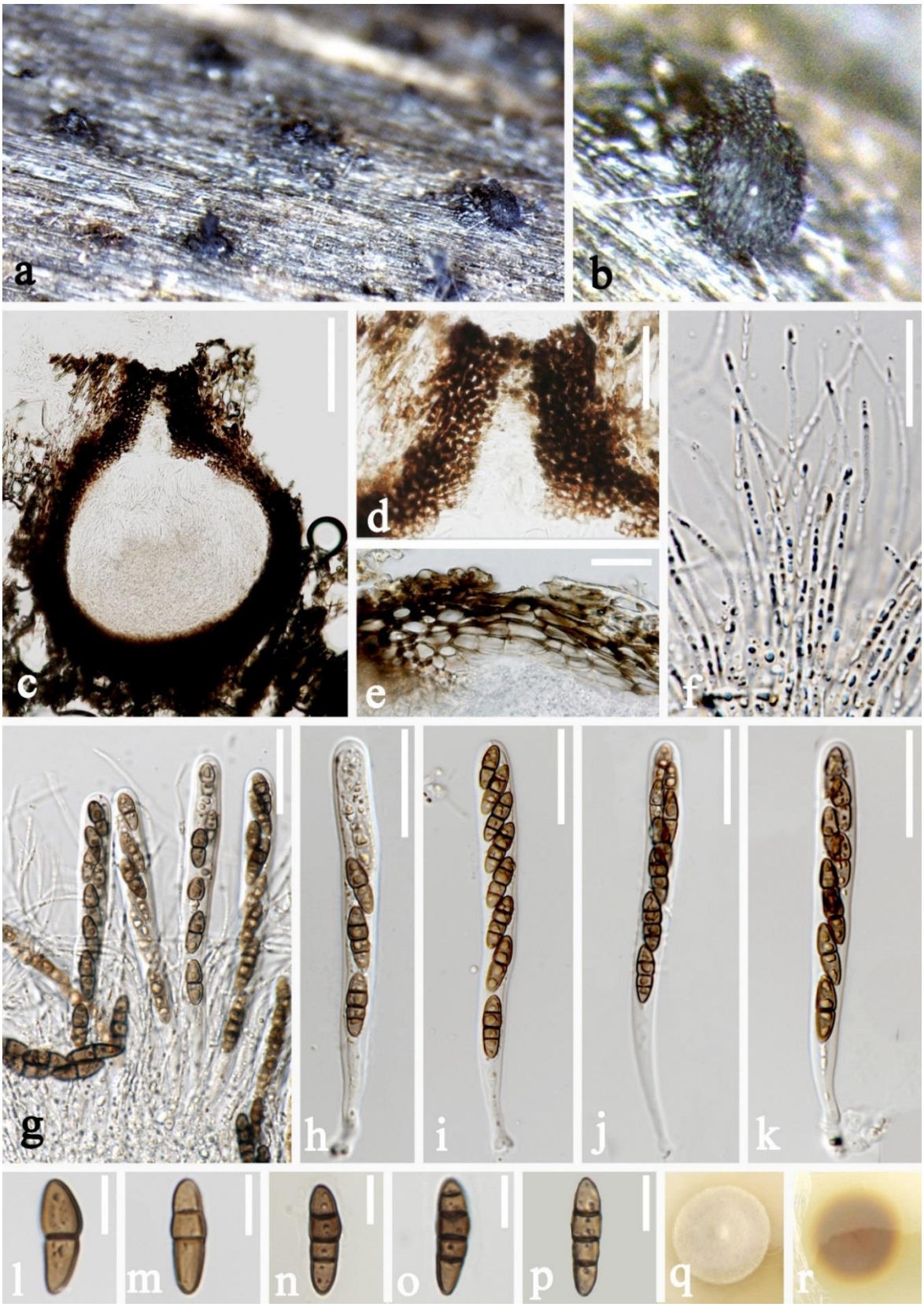

Figure 4 – *Sigarispora clavata* (MFLU 19–0994, holotype). a, b Ascomata on submerged wood. c Section of ascoma. d Ostiolar neck of ascoma. e Peridium. f Pseudoparaphyses. g–k Asci. l–p Ascospore. q, r culture on PDA. Scale bars: c = 100 μm, d = 50 μm, e, g–k = 30 μm, f = 20 μm, l–p = 10 μm.

Peridium 32–55 μm wide, comprises a single stratum, with brown to dark brown cells of *textura globulosa* and *textura angularis*. *Hamathecium* comprising 1.5–2 μm wide, filiform, hyaline, septate, guttulate, smooth cellular pseudoparaphyses. *Asci* 91–117 × 9–12 μm ($\overline{x}$ = 104 × 11 μm, n = 20), cylindrical, round at the apex, bitunicate, long pedicellate, with a small ocular chamber at the apex. *Ascospores* 13–17 × 4–6 μm ($\overline{x}$ = 15.4 × 5.3 μm, n = 30), uni to bi-seriate, overlapping, ellipsoidal to clavate, with obtuse ends, straight or slightly curved, hyaline when young, dark brown to yellowish brown at maturity, 1–4-septate, slightly constricted at the septum, guttules, smooth walled. Asexual morph: Undetermined.

Material examined – CHINA, Tibet Autonomous Region (30°15'08.02" N, 82°58'27.77" E), on submerged decaying wood, May 2017, Z.L. Luo, 2XZ A 1–2–1, S-1483 (MFLU 19–0994, holotype), ex-type culture, MFLUCC 18–1316.

Notes – Species of *Sigarispora* are characterized by immersed, papillate, ostiolate ascomata, a peridium of pseudoparenchymatous cells, bitunicate, fissitunicate asci and ellipsoidal-fusiform or muriform ascospores, with or without sheaths, and appendages. Our species fits well within the species concept of genus *Sigarispora*. However, our species can be distinguished from other species of *Sigarispora* by its 1–4-septate, ellipsoidal to clavate ascospores with obtuse ends.

In our study, *Sigarispora clavata* is sister to *S. caudata* with good support (76 ML/1.00 PP). *Sigarispora clavata* shares similar morphological characteristics with *S. caudate* in having immersed to semi-immersed, coriaceous ascomata, bitunicate, cylindrical asci with ocular chamber and brown to dark brown ascospores. However, *S. clavata* differs from *S. caudate* in ascomatal size (380–400 × 280–460 vs. 145–210 × 210–305 μm) and ascospores shape, septations and size (1–4 vs. 4–6 septate, 13–17 × 4–6 vs. 23.5–34.5 × 5.5–7 μm, respectively), *S. clavata* has ellipsoidal to clavate ascospores with obtuse ends, whereas ascospores of *S. caudata* are fusiform with narrow and acute ends.

Biappendiculispora japonica Thambug., Wanas., Kaz. Tanaka & K.D. Hyde, in Thambugala et al., Fungal Diversity, 74: 214 (2015) Fig. 5

Index Fungorum Number: IF551529; Facesoffungi number: FoF01097

Saprobic on submerged decaying wood. Sexual morph: *Ascomata* 330–420 μm high, 240–300 μm diam ($\overline{x}$ = 383 × 276 μm, n = 5), semi-immersed to immersed, solitary to gregarious, subglobose, papillate, coriaceous, black. *Ostiole* slit-like, central, brown to dark brown. *Peridium* 15–25 μm wide composed one stratum, with several layers of brown to dark brown, cells of *textura angularis*, cells towards the inside lighter, outside is darker. *Hamathecium* comprising numerous, branched, septate, guttulate, cellular pseudoparaphyses, embedded in a gelatinous matrix. *Asci* 108–130 × 15–18 μm ($\overline{x}$ = 119 × 16.4 μm, n = 20), 8-spored, bitunicate, fissitunicate, cylindric-clavate, with short pedicel, rounded at the apex, with an ocular chamber. *Ascospores* 29.8–39 × 6–8 μm ($\overline{x}$ = 34.2 × 7 μm, n = 30), overlapping uni to bi-seriate, fusiform with acute ends, mostly curved, 7–8-septate, constricted at the septa, guttulate, hyaline when young, becoming yellowish to brown at maturity, smooth, with appendages at both ends. Asexual morph: Undetermined.

Material examined – CHINA, Yunnan Province, Dali city, Midu County, saprobic on decaying wood submerged in a stream, May 2015, H.W. Shen S–787 (DLU 787), living culture, MFLUCC 17–2450.

Notes – *Biappendiculispora japonica* was introduced by Tanaka & Harada (2003) and it was previously placed in the genus *Lophiostoma*. Subsequently, Thambugala et al. (2015) showed that *L. japonica* grouped as a sister clade to *Pseudolophiostoma*. Therefore, Thambugala et al. (2015) introduced a new genus *Biappendiculispora* to accommodate *Lophiostoma japonica*. Morphologically, our fresh collection fits well with *B. japonica*, such as immersed, coriaceous

ascomata, bitunicate, cylindric-clavate asci with an ocular chamber and hyaline to brown, fusiform ascospores with acute ends. Phylogenetic analysis showed that our isolate clustered together with *B. japonica* with strong bootstrap support (98 ML/1.00 PP) (Fig. 1). Therefore, we identify our collection as *Biappendiculispora japonica* and it is a new record from China which was previously reported in Japan from terrestrial habitats.

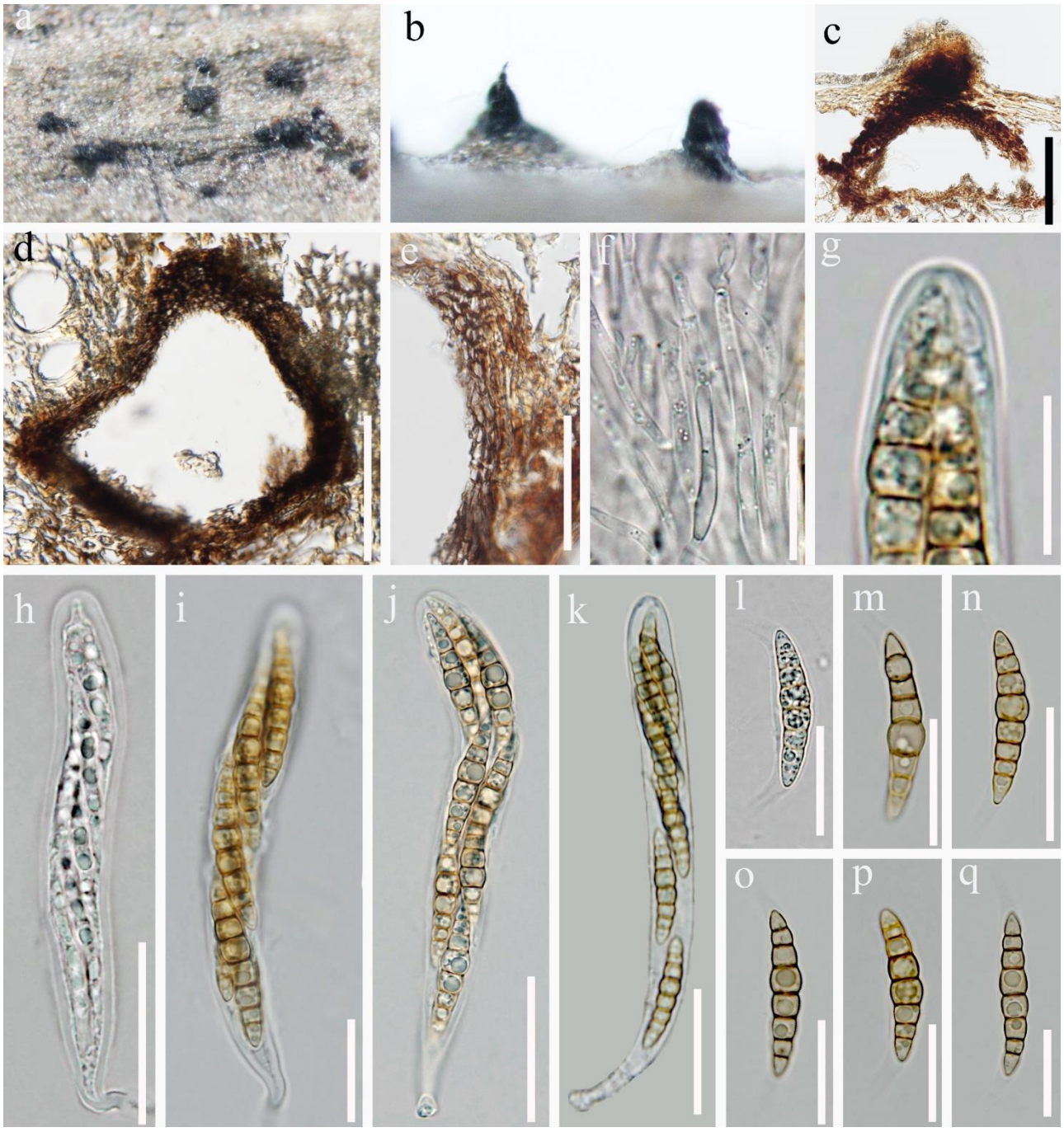

Figure 5 – *Biappendiculispora japonica* (DLU 787) a, b Ascomata on submerged wood. c, d Section of ascoma. e Peridium. f Pseudoparaphyses. g Ascus apex. h–k Asci. l–q Ascospore. Scale bars: c, d = 100 μm, e = 50 μm, f–g, l–q = 20 μm, f = 20 μm, h–k = 30 μm.

Neovaginatispora fuckelii (Sacc.) A. Hashim., K. Hiray. & Kaz. Tanaka, Studies in Mycology 90: 188. 2018. Fig. 6

Index Fungorum Number: IF551535; Facesoffungi number: FoF00829

Basionym: *Lophiostoma fuckelii* Sacc., Michelia 1(no. 3): 336 (1878)

= *Lophiostoma pulveraceum* Sacc., Michelia 1: 336, 1878

= *Didymosphaeria lophospora* Sacc. & Speg., Michelia 1: 376, 1878

= *Lophiosphaera mendax* Rehm, Ann. Myc. 5: 544,1907

= *Vaginatispora fuckelii* (Sacc.) Thambugala, Fungal diversity 74: 242, 2015

Saprobic on submerged decaying wood in freshwater habitats. Sexual morph: *Ascomata* 230–270 μm high, 180–220μm diam ($\bar{x}$ = 238 × 203 μm, n = 5), semi-immersed, coriaceous, black, subglobose, ostiolate. Ostiole rounded or slit-like, variable in shape, central, periphysate, with a porelike opening. *Peridum* 25–40 μm wide, comprise two strata, outer stratum stratum comprising brown to dark brown flattened *cells*, inner stratum comprising several layers of hyaline cells of *textura angularis*. *Hamathecium* comprising 1.5–2.0 μm wide, septate, cellular *Pseudoparaphyses*, filiform, septate with small guttules, hyaline. *Asci* 47–54 × 6.5–8.5 μm ($\bar{x}$ = 50 × 7.5 μm, n = 20), 8-spored, bitunicate, cylindrical, round at the apex, short pedicellate, with an indistinct ocular chamber. *Ascospores* 12–13 × 3–4 μm ($\bar{x}$ = 12 × 3.5 μm, n = 30), biseriate, overlapping, fusiform with acute ends, hyaline, straight or slightly curved, 1-septate, strongly constricted at the septum, mostly 4-guttules, smooth-walled, with hyaline appendages at both ends. Asexual morph: Undetermined.

Material examined – CHINA, Yunnan Province, Binchuan county, saprobic on decaying wood submerged in a stream, May 2015, H.W. Shen, S–784 (DLU 784), living culture, MFLUCC 17–1334.

Notes – The new collection is identified as *Neovaginatispora fuckelii* based on morphology and phylogeny. Morphologically, our new collection fits well with the description of *Neovaginatispora fuckelii* (Thambugala et al. 2015, Tennakoon et al. 2018). In our phylogenetic analysis, our newly collected isolate clustered with *N. fuckelii* with high bootstrap support (100 ML/1.00 PP). We therefore, identified the newly isolate as *N. fuckelii*.

Neovaginatispora fuckelii has a wide distribution and it has been reported from terrestrial habitats in China, Japan, Germany, Sweden, Switzerland and UK (Wang & Lin 2004, Thambugala et al. 2015, Tennakoon et al. 2018). In this study, our new collection was collected from a freshwater habitat. Thus, we report our collection as a new record from freshwater habitat.

Vaginatispora armatispora (K.D. Hyde, Vrijmoed, Chinnaraj & E.B.G. Jones) Wanas., E.B.G. Jones & K.D. Hyde Stud. Fung. 1(1): 62 (2016) Fig. 7

Index Fungorum number: IF 819870; Facesoffungi number: FoF 05060

Saprobic on submerged decaying wood in freshwater and marine habitats. Sexual morph: *Ascomata* 240–340 μm diam. 270–335 μm high, ($\bar{x}$ = 300 × 288 μm, n = 5), scattered, immersed or semi-immersed, globose to subglobose, black, base flatted, coriaceous to carbonaceous. *Ostiole* black, elongated, crest-like, central, with hyaline periphyses. *Peridium* 25–40 μm wide, composed of several pale brown to brown cells of *textura angularis*, cells towards the inside hyaline to pale brown, at the outside, darker, somewhat flattened, fusing and with the host tissues. *Hamathecium* comprising 1.5–2 μm wide, septate, hyaline pseudoparaphyses composing situated between and above the asci, embedded in a gelatinous matrix. *Asci* 82–115 × 13.5–15.7 μm ($\bar{x}$ = 108 × 14.3 μm, n = 20), 8-spored, bitunicate, fissitunicate, cylindrical-clavate, short pedicellate, apically rounded with an ocular chamber, hyaline. *Ascospores* 22–30 × 5.5–8 μm ($\bar{x}$ = 25.8 × 6.7 μm, n = 30), fusiform, with obtuse ends, hyaline when young, pale brown at mature, smooth-walled, 1-septate, strongly constricted at the septum, distinct large guttules, mostly 6-guttules, rarely 4-guttules, 2–3-guttules in each cells, surrounded by a narrow mucilaginous sheath, with distinct hyaline appendages at both ends. Asexual morph: undetermined.

Figure 6 – *Neovaginatispora fuckelii* **(**DLU 784) a, b Ascomata on submerged wood. c, f Peridium d Section of ascoma. e Ostiole. g Pseudoparaphyses. h Ascus apex. i–k Asci. l–s Ascospores. t Germinating ascospore. u, v Culture on PDA. Scale bars: c, i = 50 μm, d, e = 100 μm, f = 30 μm, g, i–k = 20 μm, h = 5 μm, l–t = 10 μm.

Material examined – THAILAND, Sai khu waterfall, Prachuap khiri Khan, on submerged decaying wood, August 2017, V. Kumar, B29, (DLU B29), living culture, MFLUCC 18–0213.

Notes – *Vaginatispora armatispora* was previously introduced as *Massarina armatispora* by

Hyde et al. (1992), that was collected from Mangroves in India and China. The placement of this species was updated by Wanasinghe et al. (2016) and, transferred to *Vaginatispora* based on both phylogeny and morphology.

In this study, our new isolate (MFLUCC 18–0213) clustered with strains of *Vaginatispora armatispora* with strong bootstrap (100 ML/1.00 PP). Morphology of our new isolate overlaps with *Vaginatispora armatispora* (HKTLCC1562, MFLUCC 18–0247) (Hyde et al. 1992, 2019). Therefore, we identified this new isolate as *Vaginatispora armatispora*.

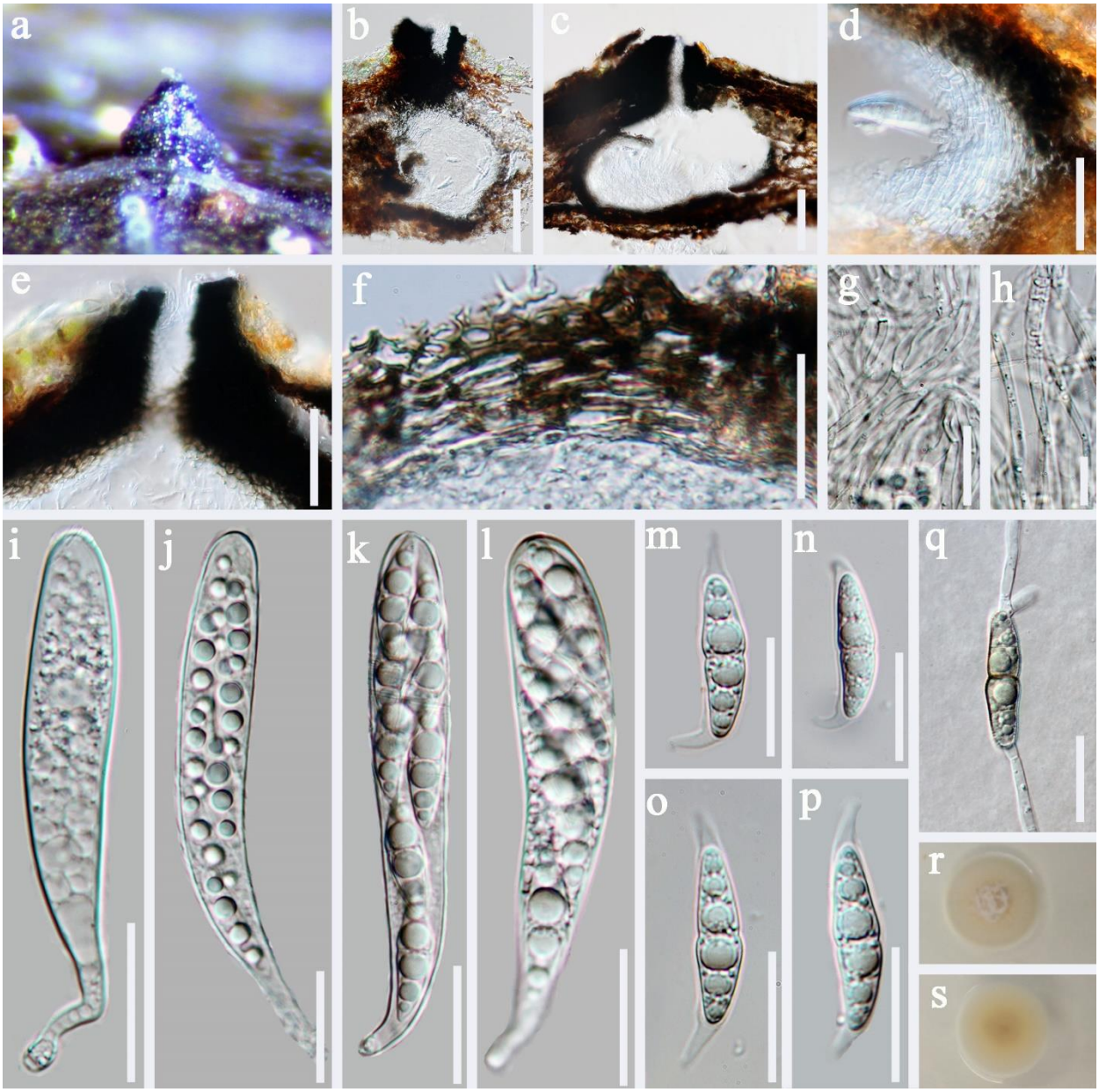

Figure 7 – *Vaginatispora armatispora* **(**DLU B29) a Ascomata on submerged wood. b, c Section of ascoma. d, f Peridium. e Ostiole. g, h Pseudoparaphyses. i–l Aci. i–k Asci. m–p Ascospore. q Germinating ascospore. r, s Culture on PDA. Scale bars: b, c = 100 μm, d = 30 μm, e = 50 μm, f, i–q = 20 μm, g, h = 10 μm.

Discussion

Lophiostomataceae, with over 150 species, is a highly speciose family in Pleosporales,

(Wijayawardene et al. 2018). They are cosmopolitan and distributed in many countries (China, France, Hungary, Italy, India, Japan, Russia and Thailand) (Thambugala et al. 2015, Wijayawardene et al. 2017, Hashimoto et al. 2018, Wanasinghe et al. 2018). However, only two Lophiostomataceae species *viz. Lophiostoma bipolare* and *L. proprietunicatum* have been reported in Yunnan, China (Luo et al. 2004). In this study, we carried out research on lignicolous freshwater fungi in Tibet and Yunnan provinces, China and Southern Thailand. Six Lophiostomataceae species have been collected and among them, five species are reported from China showing that members of this family are also widely distributed in China.

Species of Lophiostomataceae are highly diverse in their ascomata, peridium, pseudoparaphyses and ascospores characters. It is difficult to distinguish the species based on morphological characters alone and many species lack sequence data (Thambugala et al. 2015). Furthermore, some genera in Lophiostomataceae such as *Lophiostoma* are paraphyletic (Hashimoto et al. 2018). Therefore, further collections and phylogenetic studies are recommended for better understand the species boundaries within these genera.

Flabellascoma species are so far reported from terrestrial habitats (Hashimoto et al. 2018), while the two new species, *F. aquaticum* and *F. fusiforme* were collected from freshwater. This expands the habitat range of *Flabellascoma* species to freshwater. Species of *Flabellascoma* have similar shape and size of asci and ascospores and it is therefore difficult to distinguish them. but ascomatal features appear to be informative to delineate them. *Flabellascoma aquaticum* ascomata has a long black neck, whereas, *F. fusiforme* has ascomata with a short, elongated, crest-like ostiolar neck (Hashimoto et al. 2018). *Sigarispora* is a well-resolved genus in Lophiostomataceae. Species in this genus are known from terrestrial habitats in Russia, Italy and Japan (Li et al. 2016, Thambugala et al. 2015, Wanasinghe et al. 2018) and in this study, the new species *Sigarispora clavata* is reported from freshwater habitats in China.

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