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Novelties in Fuscosporellaceae (Fuscosporellales): Two New *Parafuscosporella* from Thailand Revealed by Morphology and Phylogenetic Analyses

Nattawut Boonyuen [1,*], Charuwan Chuaseeharonnachai [1], Salilaporn Nuankaew [1], Papichaya Kwantong [1], Natapol Pornputtapong [2], Nakarin Suwannarach [3,*], E. B. Gareth Jones [4] and Sayanh Somrithipol [1]

1 Plant-Microbe Interaction Research Team (APMT), Integrative Crop Biotechnology and Management Research Group (ACBG), National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathum Thani 12120, Thailand; charuwan.chu@biotec.or.th (C.C.); salilaporn.nua@biotec.or.th (S.N.); papichaya.kwa@ncr.nstda.or.th (P.K.); sayanh@biotec.or.th (S.S.)
2 Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences and Research Unit of DNA Barcoding of Thai Medicinal Plants, Chulalongkorn University, Bangkok 10330, Thailand; natapol.p@chula.ac.th
3 Research Center of Microbial Diversity and Sustainable Utilization, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
4 Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia; torperadgj@gmail.com
* Correspondence: nattawut@biotec.or.th (N.B.); suwan.462@gmail.com (N.S.)

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Abstract: Asexual morphs of freshwater fungi have been mostly reported from tropical and subtropical regions. From our ongoing investigation of the diversity and taxonomy of freshwater microfungi in Thailand, a country with rich natural resources and diverse ecosystems, *Parafuscosporella ellipsoconidiogena* sp. nov. and *P. obovata* sp. nov., collected from decaying submerged twigs at Phalad Waterfall in a conserved forest in Chiang Mai Zoo, Chiang Mai Province, northern Thailand, are proposed. DNA phylogenies based on a combination of ITS and LSU datasets support the placement of these species in *Parafuscosporella* (Fuscosporellaceae, Fuscosporellales, Sordariomycetes), and these two novel species differ from known species in terms of morphology. Detailed descriptions, illustrations and a key to *Parafuscosporella* species are provided, as well as comparisons with other accepted *Parafuscosporella* species.

Keywords: two novel freshwater microfungi; phylogeny; systematics; Sordariomycetes; taxonomy

1. Introduction

Parafuscosporella belongs to Fuscosporellaceae (Fuscosporellales, Hypocreomycetidae, Sordariomycetes) [1]. The genus is characterized by sporodochial, black colonies; partly immersed, partly superficial, septate, hyaline to pale brown mycelium; semimacronematous, mononematous, simple or branched, mostly moniliform, smooth-walled, hyaline conidiophores; monoblastic, discrete or integrated, globose, subglobose, ellipsoidal or clavate, smooth-walled, hyaline conidiogenous cells; and conidia that are ellipsoidal to broadly obpyriform, transversely septate, smooth, dark brown to black and pale brown at the basal cell [1]. Based on morphological and molecular data, the type species without sexual morph, *P. moniliformis* Jing Yang, Bhat & K.D. Hyde, was described on dead and decaying submerged wood in Thailand. To date, five accepted species, namely, *P. aquatica* H. Yang & H. Zhang, *P. garethii* Boonyuen, Chuaseehar. & Somrith., *P. moniliformis*, *P. mucosa* Jing Yang, Bhat & K.D. Hyde, and *P. pyriformis* H. Yang, W. Dong & H. Zhang, have only been reported from decaying submerged wood in Thailand and China (http://www.speciesfungorum.org; accessed on 12 September 2021) [1–4]. In this study, we describe *P. ellipsoconidiogena* and *P. obovata* as the sixth and seventh species in the genus, respectively, collected from a waterfall

in Chiang Mai Zoo, Chiang Mai Province, Thailand. Morphological descriptions and illustrations of *P. ellipsoconidiogena* sp. nov. and *P. obovata* sp. nov., a key to the species and an updated combined gene phylogenetic tree (the internal transcribed spacer (ITS) region of ribosomal DNA and large subunit (LSU) of nuclear ribosomal DNA) are provided to reveal their taxonomic position among taxa in the Fuscosporellaceae (Fuscosporellales).

2. Materials and Methods

2.1. Sample Collection, Isolation and Morphological Data

Submerged woody material was randomly collected from Phalad Waterfall located in Chiang Mai Zoo (18°48′32.40′′ N; 98°56′49.20′′ E), Muang District, Chiang Mai Province, northern Thailand (http://www.chiangmai.zoothailand.org/en/accessed on 12 September 2021). The zoo is located on a 200-acre (81 ha) woody area at the foot of Doi Suthep-Pui National Park. Phalad Waterfall is within the Plant Genetic Conservation Project under the Royal Initiation of Her Royal Highness Princess Maha Chakri Sirindhorn (RSPG). Woody samples were placed into plastic bags and transferred to the mycological laboratory at the National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA, Pathum Thani, Thailand), for observation. Decaying wood specimens were incubated in plastic containers with sterile tissue paper soaked with sterile distilled water at room temperature (20–25 °C) for 7–14 days, according to the methods described by Boonyuen et al. [2]. The specimens were observed using a stereomicroscope (Olympus SZ61; Olympus Corporation, Tokyo, Japan) for the presence of freshwater microfungi, and permanent slides were prepared by adding lactoglycerol and sealing with clear nail polish. Morphological characteristics such as conidiophores, conidiogenous cells and conidial dimension were examined. Cultural characteristics such as colony appearance and colour over the plate were also studied. Axenic cultures were obtained by single spore isolation method, following the protocol in Chuaseeharonnachai et al. [5]. Germinated spores were transferred to a potato dextrose agar (PDA, Difco^{TM}, Sparks, MD, USA) plate and incubated at room temperature (20–25 °C). The type specimens were deposited at the FUNGARIUM BIOTEC Bangkok Herbarium (BBH; https://www.nbt-microbe.org accessed on 22 September 2021), as *Parafuscosporella ellipsoconidiogena* BBH 49158 (holotype) and *P. obovata* BBH 49160 (holotype). Pure cultures are maintained in the Thailand Bioresource Research Center (TBRC; https://www.tbrcnetwork.org accessed on 22 September 2021) as TBRC 15503 and TBRC 15505. The Index Fungorum numbers were registered as *P. ellipsoconidiogena* IF 555786 and *P. obovata* IF 555787, respectively [6].

2.2. DNA Extraction, PCR Amplification and Sequencing

Genomic DNA was extracted from pure fungal mycelium grown on PDA for 14 days at room temperature using cetyltrimethylammonium bromide (CTAB) lysis buffer as outlined by Sri-indrasutdhi et al. [7]. The ITS region of ribosomal DNA, LSU of nuclear ribosomal DNA, small subunit (SSU) of nuclear ribosomal DNA and RNA polymerase II second largest subunit (*RPB2*) were amplified via polymerase chain reaction (PCR) using the following primers: ITS1/ITS5/ITS4 [8] for the ITS, LR0R/LR5/LR7 [9] for the LSU, NS1/NS4 for the SSU [8] and fRPB2-5F2/fRPB2-7cR for *RPB2* [10].

PCR amplification was performed in a 50 µL reaction volume containing 25 µL of One PCR^{TM} Ultra (Bio-Helix, New Taipei City, Taiwan; a premix and ready-to-use solution, including Taq DNA polymerase, PCR buffer, dNTPs, gel loading dyes, enhancer, and fluorescence dye), 1 µL of each primer (10 µM), 1 µL of genomic DNA extract and 22 µL of sterile deionized water. The PCR thermal cycle programs of the ITS and LSU were as follows: 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, elongation at 72 °C for 2 min and a final extension at 72 °C for 10 min. The PCR thermal cycle program of the SSU was as follows: 95 °C for 5 min, followed by 34 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, elongation at 72 °C for 1.5 min and a final extension at 72 °C for 10 min. The PCR thermal cycle program of RPB2 was as follows: 95 °C for 5 min, followed by 34 cycles of denaturation at 95 °C for

1 min, annealing at 58 °C for 1 min, elongation at 72 °C for 1.5 min and a final extension at 72 °C for 10 min. The amplicons of the ITS and LSU were purified and sequenced by Macrogen Inc. (Seoul, South Korea) with the same PCR primer used for DNA amplification. The PCR products of *RPB2* were purified using a NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel, Düren, Germany) and sequenced at Macrogen Inc. (Seoul, South Korea).

2.3. Sequence Alignment and Phylogenetic Analyses

The SSU, ITS, LSU and *RPB2* sequences of our isolates are provided in this study. Based on previous phylogenetic studies on Fuscosporellaceae (Fuscosporellales) by Yang et al. [3], two combined analyses of the ITS and LSU sequences provided resolution at the species level. In addition, there are only a few SSU and *RPB2* sequences of Fuscosporellales available in GenBank. Thus, the ITS and LSU datasets were used only for the combined sequence data analyses in this study.

A maximum likelihood (ML) tree was constructed by RAxML-NG v. 1.0.3 using the GTR+G model and the all-in-one analysis option [11]. The best ML tree was identified using the two-step L-BFGS-B method [12], to optimize the parameters of the LG4X model [13]. ML branch support was obtained using nonparametric bootstrapping with 1000 replications.

A Bayesian inference (BI) phylogenetic tree was constructed with the GTR+G model using the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) method in MrBayes 3.2.7a [14]. The MCMCMC searches were run for 1,000,000 generations with sampling every 100 generations. BI posterior probabilities (BIPPs) were summarized and mapped on the best ML tree using the SumTrees program in DendroPy version 4.5.2 [15]. The first 100 trees were excluded as burn-in. The newly obtained sequences taxa used in phylogenetic analyses were deposited in the GenBank database and are provided in Table 1.

Table 1. Isolates used in this study with GenBank accession numbers.

Taxon Name	Strain Number	GenBank Accession Number		References
		ITS	LSU	
Ascotaiwania lignicola	NIL 00005	HQ446341	HQ446364	[16]
Ascotaiwania sawadae	SS 00051	HQ446340	HQ446363	[16]
Bactrodesmiastrum monilioides	FMR 10756 ^{T}	NR_152539	KF771879	[17]
Bactrodesmiastrum obovatum	FMR 6482	NR_152537	FR870266	[18]
Bactrodesmiastrum pyriforme	FMR 10747 ^{T}	NR_152536	FR870265	[18]
Bactrodesmiastrum pyriforme	FMR 11931	HE646636	HE646637	[18]
Canalisporium caribense	SS 03839	GQ390283	GQ390268	[7]
Canalisporium grenadoideum	BCC 20507 ^{T}	NR_111442	GQ390267	[7]
Conioscypha lignicola	CBS 335.93	-	AY484513	[19]
Conioscypha submersa	DLUCC 0904 ^{T}	NR_168820	MK835856	[20]
Conioscypha varia	CBS 436.70	MH859785	MH871548	[21]
Fuscosporella aquatica	MFLUCC 16-0859 ^{T}	NR_156398	NG_059853	[3]
Fuscosporella pyriformis	MFLUCC 16-0570 ^{T}	NR_152555	NG_059711	[1]
Leotia lubrica	AFTOL-ID 1	DQ491484	AY544644	[22]
Microglossum rufum	AFTOL-ID 1292	-	DQ470981	[23]
Mucispora infundibulata	MFLUCC 16-0866 ^{T}	NR_171733	NG_073625	[24]
Mucispora obscuriseptata	MFLUCC 15-0618 ^{T}	NR_152556	NG_059709	[1]
Mucispora phangngaensis	MFLUCC 16-0865 ^{T}	NR_156399	NG_059854	[3]
Parafuscosporella aquatica	KUMCC 19-0211 ^{T}	MN513034	MN512343	[4]
Parafuscosporella ellipsoconidiogena	**TBRC 15503 ^{T}**	**OK044749**	**OK044741**	**This study**
Parafuscosporella ellipsoconidiogena	**TBRC 15504**	**OK044750**	**OK044742**	**This study**
Parafuscosporella garethii	TBRC 6543 ^{T}	**OK135602**	KX958430	[2]
Parafuscosporella garethii	TBRC 6544	**OK135603**	KX958431	[2]

Table 1. *Cont.*

Taxon Name	Strain Number	GenBank Accession Number		References
		ITS	LSU	
Parafuscosporella moniliformis	MFLUCC 15-0626 ^{T}	NR_152557	NG_059710	[1]
Parafuscosporella mucosa	MFLUCC 16-0571 ^{T}	NR_152554	NG_059855	[1]
Parafuscosporella obovata	**TBRC 15505 ^{T}**	**OK044751**	**OK044743**	**This study**
Parafuscosporella pyriformis	MFLUCC 18-1400 ^{T}	MN513031	MN512340	[4]
Parafuscosporella pyriformis	KUMCC 19-0008	MN513030	MN512339	[4]
Phaeoisaria fasciculata	CBS 127885 ^{T}	NR_145395	NG_064241	[25]
Phaeoisaria sedimenticola	CGMCC 3.14949 ^{T}	JQ074237	JQ031561	[26]
Pleurotheciella centenaria	DAOM 229631 ^{T}	NR_111709	NG_060098	[27]
Pleurotheciella rivularia	CBS 125238 ^{T}	JQ429160	JQ429232	[27]
Pleurothecium recurvatum	CBS 138747	KT278728	KT278714	[25]
Pleurothecium semifecundum	CBS 131271 ^{T}	JQ429159	JQ429240	[27]
Pseudoascotaiwania persoonii	A57-14C	-	AY590295	[28]
Savoryella aquatica	SS 03801	-	HQ446372	[16]
Savoryella lignicola	NF 00204	-	HQ446378	[16]
Vanakripa minutiellipsoidea	CBS 112523	MH862895	MH874467	[21]

Note: The superscript T = ex-type isolates. "-" = sequence is unavailable. New sequences are listed in bold. Abbreviations. AFTOL-ID: Assembling the Fungal Tree of Life; BCC: BIOTEC Culture Collection, Pathum Thani, Thailand; CBS: Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; CGMCC: China General Microbiological Culture Collection Center; DLUCC: Dali University Culture Collection, Yunnan, China; FMR: mycology laboratory at the Faculty of Medicine in Reus, University Rovira i Virgili, Tarragona, Spain; KUMCC, Culture collection of Kunming Institute of Botany, Kunming, China; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; TBRC: Thailand Bioresource Research Center, Pathum Thani, Thailand.

3. Results

3.1. Molecular Phylogeny

The dataset for the phylogenetic analysis comprised 33 representative strains from Fuscosporellales, Savoryellales, Pleurotheciales and Conioscyphales. *Leotia lubrica* (AFTOL-ID 1) and *Microglossum rufum* (AFTOL-ID 1292) were used as outgroups. Based on the combined ITS and LSU sequence data, the phylogram (Figure 1) shows that *Parafuscosporella* is a monophyletic genus in Fuscosporellaceae (Fuscosporellales, Hypocreomycetidae, Sordariomycetes), with *P. ellipsoconidiogena* sp. nov. (TBRC 15503 and TBRC 15504) clustering with the closely related *P. moniliformis* MFLUCC 15-0626, with strong statistical support (ML-BS 100% and BYPP 1.00). The tree generated from ITS sequence data and combined ITS, LSU and *RPB2* sequence analyses had a somewhat similar topology (Figures S1 and S2). *Parafuscosporella obovata* sp. nov. (TBRC 15505) forms a sister clade, in relationship with the clade of *P. garethii*, *P. pyriformis* (MFLUCC 18-1400 and KUMCC 19-0008) and *P. mucosa* (MFLUCC 16-0571; ML-BS 98% and BIPP 0.99), with high statistical support.

3.2. Taxonomy

Parafuscosporella ellipsoconidiogena Chuaseehar., Somrith. & Boonyuen, sp. nov. (Figure 2).

Index Fungorum: IF 555786.

Etymology: Referring to the ellipsoidal shape of the conidiogenous cells.

Description: Asexual morph. Colonies on the natural substratum sporodochial, granular, scattered, black with a jelly-like covering. Mycelium mostly superficial, partially immersed, composed of branched, smooth-walled, septate, hyaline hyphae. Conidiophores semi- to macronematous, mononematous, compact, erect or flexuous, branched, 2–3-septate, mostly moniliform, smooth-walled, septate, hyaline, 25.2–68.8 × 3.6–7.9 µm (avg. 41.3 × 5.6 µm, n = 15), with each cell doliiform, ellipsoidal, fusiform, 7.5–17.5 × 3.6–7.9 µm. Conidiogenous cells holoblastic, monoblastic, integrated, terminal, smooth-walled, hyaline, doliiform, ellipsoidal, fusiform, 7.9–24.3 × 5.1–9.6 µm (avg. 15.3 × 7 µm, n = 20). Conidial secession rhexolytic. Conidia acrogenous, solitary, ellipsoidal to obovoid, smooth-walled, 2-celled with a transverse septum near the base, dark brown to black, 27.5–33 × 15–20 µm

(avg. 30.5 × 18 µm, *n* = 50), with a light brown, short and narrow, truncate basal cell and distinct, hyaline, 1.3−3.8 × 2.5−3 µm basal frills. Sexual morph unknown.

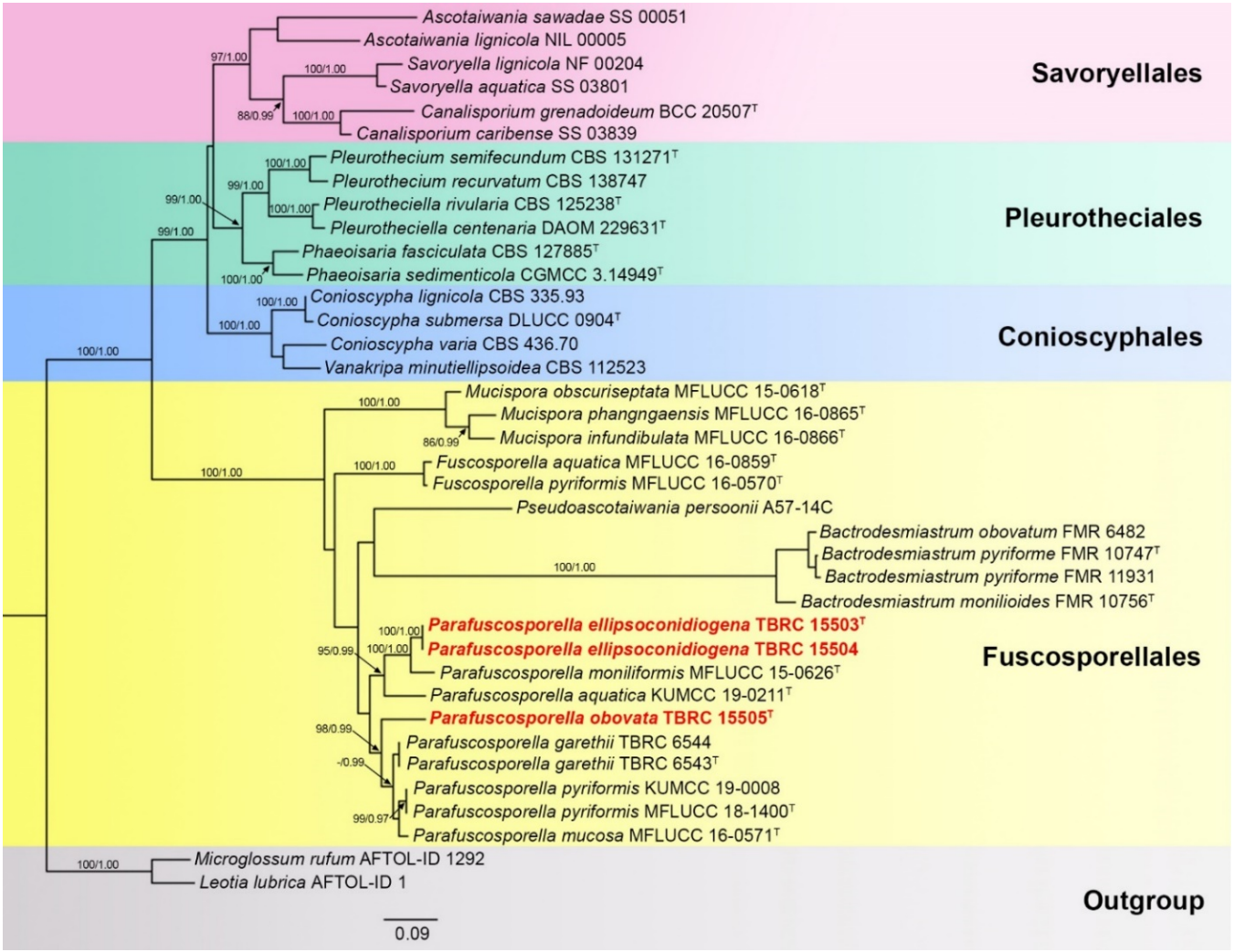

Figure 1. Maximum likelihood (ML) analysis based on combined ITS and LSU sequence data of *P. ellipsoconidiogena* sp. nov. and *P. obovata* sp. nov., together with representative species in Fuscosporellales (Hypocreomycetidae, Sordariomycetes) and other closely related orders in Hypocreomycetidae, Sordariomycetes. Bootstrap support values for ML (ML-BS) equal to or greater than 80% (left) and Bayesian posterior probabilities (BIPPs) equal to or greater than 0.95 (right) are given at the nodes. The new strains are in bold red, and the ex-type strains are indicated by ^{T}. *Leotia lubrica* (AFTOL-ID 1) and *Microglossum rufum* (AFTOL-ID 1292) in Leotiaceae (Leotiales) were used as outgroups.

Culture characteristics: On PDA, colonies growing on PDA at 20−25 °C for 30 days, dry, flat, circular, velvety, spreading, brown with beige-brown patches, with a prominent dark brown outer zone of submerged growth and serrate margin, reverse dark brown. Vegetative hyphae partly superficial and partially immersed, branched, smooth-walled, septate, subhyaline to light brown, 2–3.8 µm wide. Conidiophores micronematous, reduced to a single conidiogenous cell. Conidiogenous cells holoblastic, monoblastic, integrated, cylindrical or ellipsoidal, hyaline to pale brown, 3.75–12.5 × 3.75–6.4 µm (avg. 6.3 × 5.5 µm, *n* = 15). Conidial secession rhexolytic. Conidia acrogenous or pleurogenous, broadly obpyriform, ellipsoidal, obovoid, smooth-walled, 1- or 2-celled with a transverse septum near the base, medium brown to dark brown when mature, 15–27.5 × 10.5–17.5 µm (avg. 20.3 × 14.4 µm, *n* = 50), with a light brown, triangular basal cell; chlamydospores absent. Sexual morph absent.

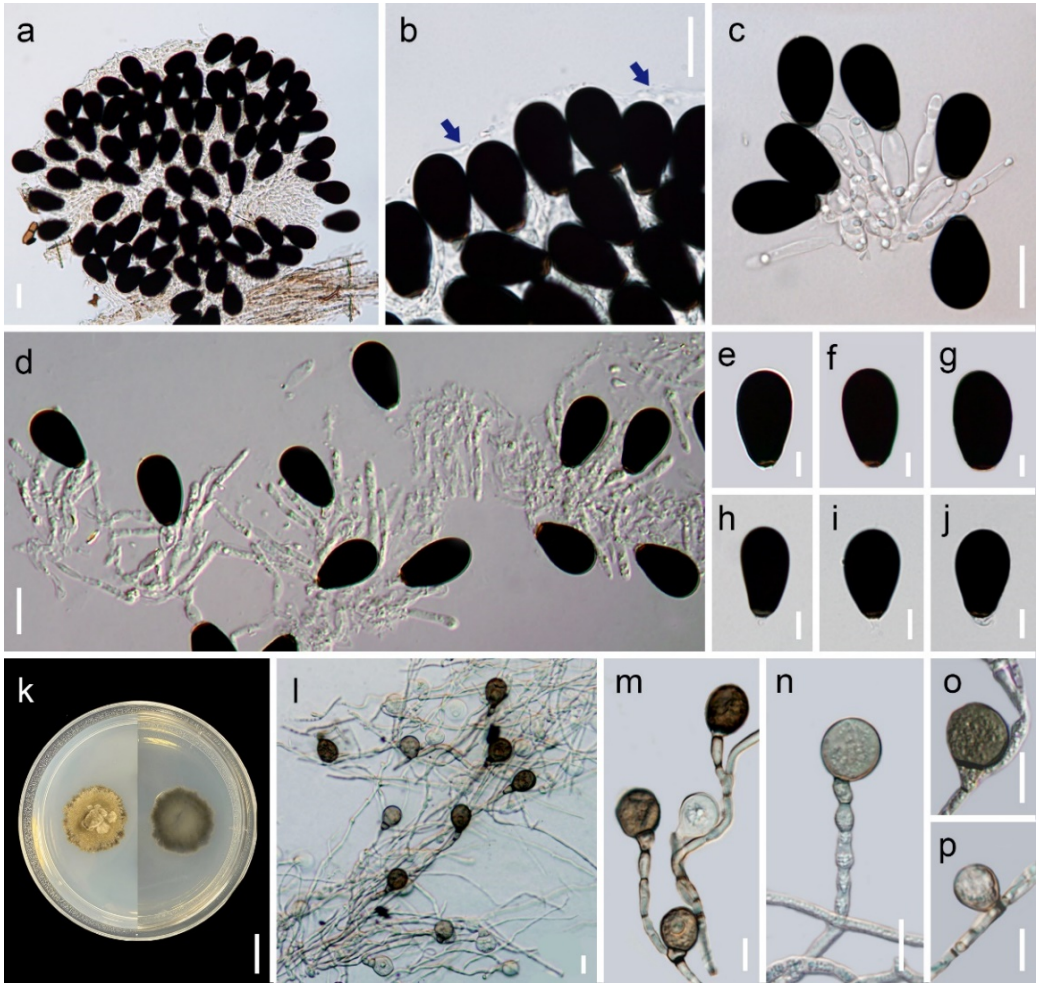

Figure 2. *Parafuscosporella ellipsoconidiogena* (BBH 49158, holotype). (**a**) Squash mount of a sporodochium. (**b**) Conidia with a jelly-like covering (arrows indicate a thin hyaline wall of jelly-like covering). (**c**,**d**) Conidiophores, conidiogenous cells and conidia. (**e**–**j**) Conidia. (**k**) Obverse (left) and reverse (right) views of a colony on PDA after 30 days. (**l**) Hyphae and conidia from culture. (**m**–**p**) Conidiogenous cells and conidia. Scale bars: a–d = 20 μm; e–j and l–p = 10 μm; and k = 1 cm.

Habitat and geographical distribution: Saprobe on submerged twigs, known from Thailand.

Type: Thailand, Chiang Mai Province, Mueang Chiang Mai District, Phalad Waterfall in Chiang Mai Zoo, 18°48′37″ N, 98°56′51″ E, on submerged twigs of an unidentified plant, 18 August 2018, N. Boonyuen, BBH 49158 holotype, TBRC 15503 ex-holotype living culture; BBH 49159 isotype, TBRC 15504 ex-isotype living culture.

Additional gene sequences: OK054346 (SSU), OK043808 (*RPB2*), OK054347 (SSU) and OK043809 (*RPB2*).

SSU: Based on BLAST analysis of the SSU sequences of TBRC 15503 (OK054346) and TBRC 15504 (OK054347), the data revealed that closely related strains with % identities were *Parafuscosporella moniliformis* MFLUCC 15-0626^{T} (100%), *P. mucosa* MFLUCC 16-0571^{T} (99.8%) and *P. garethii* TBRC 6544 (99.6%).

RPB2: BLAST analysis of the *RPB2* sequences of TBRC 15503 (OK043808) and TBRC 15504 (OK043809) revealed that the closely related strains with % identities were *Parafuscosporella garethii* TBRC 6543^{T} (92.7%), *P. garethii* TBRC 6544 (92.5–92.6%) and *P. pyriformis* KUMCC 19-0008 (92.0–92.1%).

LSU: BLAST analysis of the LSU sequences of TBRC 15503 (OK044741) and TBRC 15504 (OK044742) showed the most closely related strains with % identities were

Parafuscospora garethii TBRC 6543^{T} (97.4–97.6%), *P. garethii* TBRC 6544 (97.4–97.6%) and *P. moniliformis* MFLUCC 15-0626^{T} (98.9–99%).

ITS: BLAST analysis of the ITS sequences of TBRC 15503 (OK044749) and TBRC 15504 (OK044750) revealed the most closely related strains with % identities were *Parafuscosporella moniliformis* MFLUCC 15-0626^{T} (87.6–88.2%) and *Parafuscosporella aquatica* KUMCC 19–0211^{T} (87.8%).

Note: *Parafuscosporella ellipsoconidiogena* resembles species of *Vanakripa* Bhat, W.B. Kendr. & Nag Raj [29] in possessing a sporodochium; large and dark-pigmented conidia; and a narrow-long, hyaline conidiogenous cell resembling the separating cell of *Vanakripa*.

Parafuscosporella ellipsoconidiogena differs from *Vanakripa* species due to an absence of vermiform to obpyriform separating cells. Morphologically, *P. ellipsoconidiogena* is most similar to *P. mucosa* in having natural substratum colonies with a jelly-like covering, conidiophores arranging only one form in cylindrical or moniliform, ellipsoidal conidiogenous cells, and uniseptate, dark-pigmented conidia [1]. However, they mainly differ in the shape of conidiogenous cells and conidiophores. *Parafuscosporella ellipsoconidiogena* has doliiform or fusiform conidiogenous cells and moniliform conidiophores, while *P. mucosa* possesses globose, subglobose or clavate conidiogenous cells and cylindrical conidiophores. Conidiogenous cells of the new species are also longer (7.9–24.3 × 5.1–9.9 µm) than those of *P. mucosa*. *P. mucosa* produces cylindrical conidiophores and globose, subglobose, clavate and shorter (7–17 × 4–12 µm) conidiogenous cells that differ from those of *P. ellipsoconidiogena* [1].

In PDA culture, the sizes of the conidiogenous cells and conidia of both species somewhat overlap, and these two species mainly differ in the shape of the conidiogenous cells as well as the shape and colour of the conidia. *Parafuscosporella ellipsoconidiogena* has cylindrical or ellipsoidal conidiogenous cells and broadly obpyriform, ellipsoidal, obovoid, medium brown to dark brown conidia, while *P. mucosa* has doliiform or obovoid conidiogenous cells and globose to subglobose, olivaceous to pale brown conidia [1].

In the phylogenetic tree inferred from the two combined ITS and LSU sequences (Figure 1), *P. ellipsoconidiogena* is closely related to *P. moniliformis*. Morphologically, *P. ellipsoconidiogena* and *P. moniliformis* share a similar morphology of the sporodochial conidiomata and conidiophores that are mostly moniliform with ellipsoidal moniliform conidiogenous cells [1]. However, *P. ellipsoconidiogena* differs from *P. moniliformis* in having natural substratum colonies with a jelly-like covering, doliiform or fusiform, shorter and narrower (7.9–24.3 × 5.1–9.6 µm) conidiogenous cells with obovoid conidia, while in *P. moniliformis*, conidiomatal colonies without a jelly-like covering, globose, subglobose, clavate, longer and wider (5.5–36 × 5–21 µm) conidiogenous cells with broadly obpyriform conidia [1]. In PDA culture, the differences between *P. ellipsoconidiogena* and *P. moniliformis* are in the shape of conidiogenous cells and conidia. In *P. ellipsoconidiogena*, it has ellipsoidal conidiogenous cells with broadly obpyriform, ellipsoidal or obovoid conidia, while *P. moniliformis* has subglobose or dumbbell-shaped conidiogenous cells with globose to subglobose conidia [1]. The comparison of *Parafuscosporella* species on natural substrates and on PDA culture are presented in Tables 2 and 3.

Table 2. Descriptions of *Parafuscosporella* species on natural substrate. The new taxa described in this study are indicated in bold.

Species	Conidioma	Conidiophore	Conidiogenous Cell	Conidium	Habitat and Geographical Distribution	Reference
P. aquatica	Sporodochial without jelly-like covering	Mostly globose to subglobose in moniliform	Globose to subglobose, 7–14 × 8–11 µm	Ellipsoidal to obovoid, 1-septate, apical cell dark brown to black, basal cell paler, 20–29 × 13–19 µm	Decaying submerged wood, Mukdahan, Thailand	[4]
P. ellipsoconidiogena	**Sporodochial with jelly-like covering**	**Mostly doliiform, ellipsoidal, fusiform in moniliform, with each**	**Doliiform, ellipsoidal, fusiform, 7.9 – 24.3 × 5.1 – 9.6 µm**	**Ellipsoidal to obovoid, 1-septate, apical cell dark brown to black, basal cell light brown, 27.5 – 33 × 15 – 20 µm**	**Submerged twigs, Chiang Mai, Thailand**	**This study**
P. garethii	Sporodochial with jelly-like covering	Cylindrical in single or mostly globose to subglobose in moniliform	Cylindrical, 1.25–2.5 µm wide, mostly globose to subglobose, 8–12.5 µm diam., or ellipsoidal, 10–15 × 7.5–8 µm	Obpyramidal, coronate apex with 4–9 conical projections, 5 –7.5 × 5 µm, 1–2-septate, distal cell black, lower cells light brown, 37.5–47.5 × 25–42.5 µm	Decaying submerged wood, Chiang Mai, Thailand	[2]
P. moniliformis	Sporodochial without jelly-like covering	Mostly globose to subglobose, ellipsoidal or clavate in moniliform	Globose, subglobose, ellipsoidal or clavate, 5.5–36 × 5–21 µm	Ellipsoidal to broadly obpyriform, 1-septate, dark brown to black, basal cell pale brown, 28–37 × 14–21 µm	Decaying submerged wood, Prachuap Khiri Khan, Thailand	[1]
P. mucosa	Sporodochial with jelly-like covering	Cylindrical in single	Globose, subglobose, ellipsoidal or clavate, 7–17 × 4–12 µm	Obovoid to obpyriform, 1-septate, brown to dark brown, basal cell paler, 26.5–36 × 12–26 µm	Decaying submerged wood, Prachuap Khiri Khan, Thailand	[1]
P. obovata	**Sporodochial without jelly-like covering**	**Mostly globose to subglobose or ellipsoidal in moniliform**	**Globose to subglobose, 9.5–11.2 µm diam., or obovoid, 9.6–10.1 × 7.1–7.8 µm**	**Obovoid, broadly obovoid to subglobose, 1-septate, apical cell dark brown to black, basal cell light brown, 22.5– 36.3 × 13 – 32.5 µm**	**Submerged twigs, Chiang Mai, Thailand**	**This study**

Table 2. *Cont.*

Species	Conidioma	Conidiophore	Conidiogenous Cell	Conidium	Habitat and Geographical Distribution	Reference
P. pyriformis	Sporodochial with jelly-like covering	Cylindrical in single or globose to subglobose or ellipsoidal in moniliform	Cylindrical to clavate, 2–3 × 0.5–1 µm, or globose to subglobose, 8–13 µm diam.	Obovoid to obpyriform, 1–2 septate, dark brown to black, basal cells brown, 23–30 × 16–26 µm	Decaying submerged wood, Nakhon Si Thammarat, Thailand (Holotype); Yunnan, China (Paratype)	[4]

Table 3. Descriptions of *Parafuscosporella* species in the PDA culture. The new taxa described in this study are indicated in bold.

Species	Colony	Conidiogenous Cell	Conidium		Reference
			Shape and Colour	Size	
P.aquatica	Brown, dense and tight mycelia, sparse margin	Integrated	Obovoid to obpyriform, mostly 1-septate, brown to dark brown	16–24 × 9–16 µm	[4]
P. ellipsoconidiogena	**Brown with beige-brown patches, with a dark brown outer zone, flat, circular, velvety, serrate margin**	**Integrated, cylindrical, ellipsoidal, 3.75–12.5 × 3.75–6.4 µm**	**Broadly obpyriform, ellipsoidal, obovoid, 0 – 1-septate, medium brown to dark brown**	**15–27.5 × 10.5–17.5 µm**	**This study**
P. garethii	Rounded, floccose, grey to dark grey	Integrated, cylindrical	Obovoid to obpyriform, 1-2-septate, upper cell(s) brown to dark brown, basal cell light brown	22.5–30 × 15–25 µm	[2]
P. moniliformis	Dark brown, irregularly layered (on MEA)	Integrated or cylindrical, subglobose or dumbbell-shaped, 5–15 × 2–10 µm	Globose to subglobose, 0–1-septate, medium brown to dark brown	15.5–24.5 × 13–18.5 µm	[1]
P. mucosa	Dark brown, irregular, sparse aerial hyphae, undulate margin, producing chlamydospores	Integrated, doliiform or obovoid, 4–9.5 × 2–5 µm	Globose to subglobose, 0–1-septate, olivaceous to pale brown	16.5–29 × 13–19 µm	[1]
P. obovata	**Olivaceous brown with a beige-brown outer zone, raised, circular, lanose, floccose, entire margin**	**Integrated, cylindrical**	**Ellipsoidal, obovoid to broadly obovoid, obpyriform, 0 – 1-septate, brown to dark brown**	**11–17.5 × 7.5–13.8 µm**	**This study**
P. pyriformis	Grey to dark grey, rounded, floccose, undulate margin	Integrated or cylindrical, 1.5–3 µm wide	Globose to subglobose, sometimes moniliform, aseptate, light brown to brown	8–12 × 7–12 µm	[4]

Parafuscosporella obovata Chuaseehar., Somrith. & Boonyuen, sp. nov. (Figure 3).

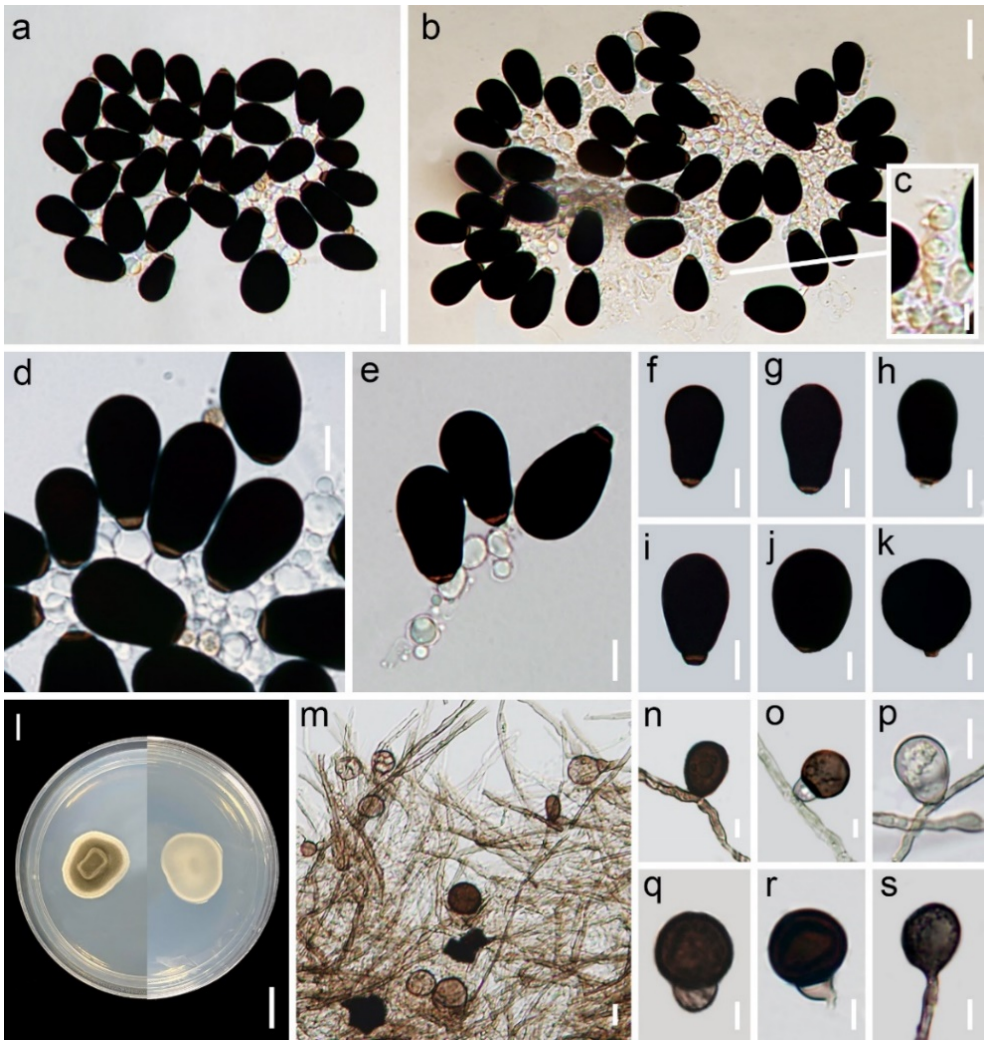

Figure 3. *Parafuscosporella obovata* (BBH 49160, holotype). (**a**,**b**) Squash mount of the sporodochia. (**c**) Conidiophore. (**d**,**e**) Conidiogenous cells and conidia. (**f**–**k**) Conidia. (**l**) Obverse (left) and reverse (right) views of a colony on PDA after 30 days. (**m**) Hyphae and conidia from culture. (**n**–**s**) Conidiogenous cells and conidia. Scale bars: a,b = 20 μm; c–k and m = 10 μm; l = 1 cm; and n–s = 5 μm.

Index Fungorum: IF 555787.

Etymology: Referring to the presence of obovoid conidia.

Description: Asexual morph. Colonies on the natural substratum sporodochial, granular, scattered, black. Mycelium mostly superficial, partially immersed, composed of branched, smooth-walled, septate, hyaline hyphae. Conidiophores semi- to macronematous, mononematous, compact, erect or flexuous, branched, 3–4-septate, mostly moniliform, smooth-walled, septate, hyaline, 25.2–42.1 × 5.8–9.7 μm (avg. 31 × 8.9 μm, n = 10), with each cell globose to subglobose, 7.1–9.5 μm diam., or ellipsoidal, 7.1–11.2 × 5.8–9.7 μm. Conidiogenous cells holoblastic, monoblastic, integrated, terminal, smooth-walled, hyaline, globose to subglobose, 9.5–11.2 μm diam. (avg. 8.6 μm diam, n = 20), or obovoid, 9.6–10.1 × 7.1–7.8 μm (avg. 9.9 × 7.2 μm, n = 20). Conidial secession rhexolytic. Conidia acrogenous, solitary, obovoid, broadly obovoid to subglobose, often slightly bent, smooth-walled, 2-celled with a transverse septum near the base, dark brown to black, 22.5–36.3 × 13–32.5 μm (avg. 28.5 × 18.3 μm, n = 50), with a light brown, short and

narrow, truncate basal cell and distinct, hyaline, 0.5−5 × 2.5−5 µm basal frills. Sexual morph unknown.

Culture characteristics: On PDA, colonies after 30 days at 20−25 °C, dry, raised, circular, lanose, floccose, spreading, with an olivaceous brown center, a beige-brown outer zone and entire margin, reverse light olivaceous brown center and beige-brown outer zone. Vegetative hyphae partly superficial and partially immersed, branched, smooth-walled, septate, subhyaline to light brown, becoming dark brown with age, 2–5 µm wide.

Conidiophores micronematous, reduced to a single conidiogenous cell. Conidiogenous cells holoblastic, monoblastic, integrated or cylindrical, hyaline to pale brown. Conidial secession rhexolytic. Conidia acrogenous or pleurogenous, ellipsoidal, obovoid to broadly obovoid, obpyriform, smooth-walled, 1- or 2-celled with a transverse septum near the base, brown to dark brown when mature, 11–17.5 × 7.5–13.8 µm (avg. 14.5 × 11.5 µm, *n* = 50); with a light brown, triangular basal cell; chlamydospores absent. Sexual morph absent.

Habitat and geographical distribution: Saprobe on submerged twigs, known from Thailand.

Type: Thailand, Chiang Mai Province, Mueang Chiang Mai District, Phalad Waterfall in Chiang Mai Zoo, 18°48′37″ N, 98°56′51″ E, on submerged twigs of an unidentified plant, 30 August 2019, N. Boonyuen, (BBH 49160 holotype, TBRC 15505 ex-holotype living culture).

Additional gene sequences: OK054348 (SSU) and OK043810 (*RPB2*).

SSU: BLAST analysis of the SSU sequence of TBRC 15505 (OK054348) revealed the most closely related strains were *Parafuscosporella mucosa* MFLUCC 16-0571 (99.7% identity), *P. garethii* TBRC 6544 (99.6%) and TBRC 6543^{T} (99.6%).

RPB2: BLAST analysis of the *RPB2* sequence of TBRC 15505 (OK043810) revealed the mostly closely related strains with % identities were *Parafuscospora garethii* TBRC 6544 (94.6%), *P. pyriformis* KUMCC 19–0008 (94.5%) and *P. garethii* TBRC 6543 (94.3%).

LSU: BLAST analysis of the LSU sequence of TBRC 15505 showed the most closely related strains with % identities were *Parafuscosporella mucosa* MFLUCC 16-0571 (97.9%) and *P. garethii* TBRC 6543^{T} (97.7%).

ITS: BLAST analysis of the ITS sequence of TBRC 15505 showed the most closely related strains with % identities were *Parafuscosporella mucosa* MFLUCC 16-0571 (89.7%) and *Parafuscosporella* sp. MAW-2020a (89.7%).

Note: *Parafuscosporella obovata* is clearly distinct from other members of the genus based on molecular data. Morphologically, *P. obovata* is most similar to *P. aquatica* and *P. moniliformis* in natural substratum colonies without a jelly-like covering, moniliform conidiophores, globose to subglobose conidiogenous cells and dark-pigmented conidia with a transverse septum [1,4]. However, *P. moniliformis* differs from *P. obovata* in having larger (5.5–36 × 5–21 µm), ellipsoidal or clavate conidiogenous cells [1]. Moreover, *P. moniliformis* has ellipsoidal to broadly obpyriform and narrower (28–37 × 14–21 µm) conidia [1], while *P. obovata* produces obovoid, broadly obovoid to subglobose and wider (22.5–36.3 × 13–32.5 µm) conidia. *Parafuscosporella obovata* differs from *P. aquatica* in having broadly obovoid to subglobose and larger conidia, while the smaller conidia of *P. aquatica* (20–29 × 13–19 µm) are ellipsoidal to obovoid [4].

The comparison among these three species in PDA culture, *P. obovata* has ellipsoidal, obovoid to broadly obovoid, or obpyriform conidia, whereas *P. moniliformis* has larger globose to subglobose conidia, and *P. aquatica* has obovoid to obpyriform uniseptate conidia [1,4]. The conidia of *P. obovata* are smaller (11–17.5 × 7.5–13.8 µm) than those of *P. moniliformis* (15.5–24.5 × 13–18.5 µm) and narrower than those of *P. aquatica* (16–24 × 9–16 µm) [1,4]. In addition, *P. moniliformis* has subglobose or dumbbell-shaped conidiogenous cells [1], whereas *P. obovata* and *P. aquatica* do not present such forms of conidiogenous cells [4]. A key to *Parafuscosporella* species, including *P. ellipsoconidiogena* and *P. obovata*, is provided based on morphological characters on natural substrates and in PDA culture observations.

3.3. Key to the Species of Parafuscosporella

1a. Colonies on natural substrate with jelly-like covering 2
1b. Colonies on natural substrate without jelly-like covering 3
2a. Conidiophores composed of two forms: (a) cylindrical and (b) moniliform 4
2b. Conidiophores composed of one form 5
3a. Conidia from PDA culture globose to subglobose, 15.5–24.5 × 13–18.5 µm . . . . . *P. moniliformis* [1]
3b. Conidia from PDA culture ellipsoidal, obovoid to broadly obovoid, obpyriform 6
4a. Conidia obpyramidal, coronate apex, 37.5–47.5 × 25–42.5 µm . . . . . *P. garethii* [2]
4b. Conidia obovoid to obpyriform, 23–30 × 16–26 µm . . . . . *P. pyriformis* [4]
5a. Conidiophores cylindrical . . . . . *P. mucosa* [1]
5b. Conidiophores mostly moniliform . . . . . *P. ellipsoconidiogena* sp. nov.
6a. Conidia from PDA culture mostly 1-septate, 16-24 × 9-16 µm . . . . . *P. aquatica* [4]
6b. Conidia from PDA culture 0–1-septate, 11–17.5 × 7.5–13.8 µm . . . . *P. obovata* sp. nov.

4. Discussion

In this study, phylogenetic analyses based on the combined ITS and LSU coupled with morphology placed *Parafuscosporella* species, together with two novel taxa of *P. ellipsoconidiogena* [1–3], and *P. obovata* within Fuscosporellaceae (Fuscosporellales), in agreement with a previous study [4]. In addition, both novel species described here are clearly separate from the known species in terms of phylogeny and morphology. Thus, two species, *P. ellipsoconidiogena* and *P. obovata*, found in Thailand, are newly introduced.

The morphological characters of *Parafuscosporella* in culture are different from natural material. The culture characteristic of these taxa on PDA is characterized by the absence of conidiomatal colonies; conidiophores reduced to a single conidiogenous cell; integrated or often cylindrical, ellipsoidal, subglobose or dumbbell-shaped conidiogenous cells; and 0–2-septate, pigmented, *Humicola*-like or *Trichocladium*-like conidia, as described in Table 3 [1,2,4].

Based on conidial characters, the significant distinctiveness of *Parafuscosporella* spp. in species identification is mainly on natural material and synthetic media, such as shape, size, septation and conidial formation. To identify *Parafuscosporella* spp., both morphological description and DNA sequences analyses are needed (i.e., ITS data or the combined analyses of ITS and LSU sequences), so that they can be resolved at the species level [3].

The geographical distribution of *Parafuscosporella* species show they are only known from Thailand and potentially in China. All *Parafuscosporella* species are freshwater fungi living on decaying woody material [1–4]. *Parafuscosporella ellipsoconidiogena* and *P. obovata*, introduced here with morphological descriptions and molecular phylogenetic analyses of a multigene DNA sequence dataset, were discovered in Chiang Mai Province in northern Thailand, where previous studies (i.e., from Chiang Dao District, Mae Teang District and Doi Suthep-Pui National Park) have also discovered novel microfungi and new freshwater fungi (i.e., [2,30–33]). Compared to other provinces and parts of Thailand, Chiang Mai Province has a tropical savanna climate with low latitudes and moderate elevations and is characterized by days that range between warm and hot year-round and nights that are cool with tolerable temperatures. Furthermore, Chiang Mai has three major seasons, including the cool (November to February), dry-hot (March to May) and rainy (June to October) seasons. In this study, two species, *P. ellipsoconidiogena* and *P. obovata*, were collected during the rainy season in August 2018 and August 2019, respectively. This season is characterized by a high level of flowing water and abundant decaying submerged wood at Phalad Waterfall located in Chiang Mai Zoo. Located in a conserved and undisturbed forest in Chiang Mai Zoo, the aquatic environment of Phalad Waterfall is undisturbed by humans; as a consequence, it is probably conducive to the discovery of novel fungal species. In addition, regarding fungal distribution, our results are in accordance with earlier studies [2,4,31], showing that *Parafuscosporella* species are freshwater hyphomycetes

on woody substrates. The main advantage of these fungi on submerged woods is that they have the ability to maintain activity at low temperatures and degrade submerged organic matter under various climatic conditions. These new freshwater asexual fungi add to the increasing number of microfungi known from Thailand, and suggests that numerous new species await discovery in other conserved and undisturbed forests of Thailand. As most *Parafuscosporella* species are documented from Thailand, wider sampling from other global locations is required.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/d13110517/s1, Figure S1: ITS sequence data and, Figure S2: combined ITS, LSU and *RPB2* sequence analyses.

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References

1. Yang, J.; Maharachchikumbura, S.S.N.; Bhat, D.J.; Hyde, K.D.; McKenzie, E.H.C.; Jones, E.B.G.; Al-Sadi, A.M.; Lumyong, S.J. Fuscosporellales, a new order of aquatic and terrestrial Hypocreomycetidae (Sordariomycetes). *Cryptogam. Mycol.* **2016**, *37*, 449–475. [CrossRef]
2. Boonyuen, N.; Chuaseeharonnachai, C.; Suetrong, S.; Sujinda, S.; Somrithipol, S. *Parafuscosporella garethii* sp. nov. (Fuscosporellales) from a rivulet in a community-based northern forest, in Thailand. *Mycosphere* **2016**, *7*, 1265–1272. [CrossRef]
3. Yang, J.; Liu, J.K.; Hyde, K.D.; Jones, E.B.G.; Liu, Z.Y. Two new species in Fuscosporellaceae from freshwater habitats in Thailand. *Mycosphere* **2017**, *8*, 1893–1903. [CrossRef]
4. Yang, H.; Dong, W.; Yu, X.D.; Bhat, D.J.; Boonmee, S.; Zhang, H. Four freshwater dematiaceous hyphomycetes in Sordariomycetes with two new species of *Parafuscosporella*. *Phytotaxa* **2020**, *441*, 19–34. [CrossRef]
5. Chuaseeharonnachai, C.; Somrithipol, S.; Boonyuen, N. A new species of *Fusticeps* from Thailand. *Mycosphere* **2014**, *5*, 313–317. [CrossRef]
6. Jayasiri, S.C.; Hyde, K.D.; Ariyawansa, H.A.; Bhat, D.J.; Buyck, B.; Cai, L.; Dai, Y.C.; Abd-Elsalam, K.A.; Ertz, D.; Hidayat, I.; et al. The faces of fungi database: Fungal names linked with morphology, phylogeny and human impacts. *Fungal Divers.* **2015**, *74*, 3–18. [CrossRef]

7. Sri-indrasutdhi, V.; Boonyuen, N.; Suetrong, S.; Chuaseeharonnachai, C.; Sivichai, S.; Gareth Jones, E.B. Wood-inhabiting freshwater fungi from Thailand: *Ascothailandia grenadoidia* gen. et sp. nov., *Canalisporium grenadoidia* sp. nov. with a key to *Canalisporium* species (Sordariomycetes, Ascomycota). *Mycoscience* **2010**, *51*, 411–420. [CrossRef]
8. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*; Elsevier: Amsterdam, The Netherlands, 1990; pp. 315–322. [CrossRef]
9. Vilgalys, R.; Hester, M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J. Bacteriol.* **1990**, *172*, 4238–4246. [CrossRef]
10. Liu, Y.J.; Whelen, S.; Hall, B.D. Phylogenetic relationships among ascomycetes: Evidence from an RNA polymerase II subunit. *Mol. Biol. Evol.* **1999**, *16*, 1799–1808. [CrossRef]
11. Kozlov, A.M.; Darriba, D.; Flouri, T.; Morel, B.; Stamatakis, A. RAxML-NG: A fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics* **2019**, *35*, 4453–4455. [CrossRef]
12. Fletcher, R. *Practical Methods of Optimization*; John Wiley & Sons: Hoboken, NJ, USA, 2013.
13. Le, S.Q.; Dang, C.C.; Gascuel, O. Modeling protein evolution with several amino acid replacement matrices depending on site rates. *Mol. Biol. Evol.* **2012**, *29*, 2921–2936. [CrossRef]
14. Ronquist, F.; Teslenko, M.; Mark, P.; Ayres, D.L.; Höhna, A.D.S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **2012**, *61*, 539–542. [CrossRef]
15. Sukumaran, J.; Holder, M.T. DendroPy: A Python library for phylogenetic computing. *Bioinformatics* **2010**, *26*, 1569–1571. [CrossRef] [PubMed]
16. Boonyuen, N.; Chuaseeharonnachai, C.; Suetrong, S.; Sri-indrasutdhi, V.; Sivichai, S.; Gareth Jones, E.B.; Pang, K.L. Savoryellales (Hypocreomycetidae, Sordariomycetes): A novel lineage of aquatic ascomycetes inferred from multiple-gene phylogenies of the genera *Ascotaiwania*, *Ascothailandia*, and *Savoryella*. *Mycologia* **2011**, *103*, 1351–1371. [CrossRef] [PubMed]
17. Hernández-Restrepo, M.; Gené, J.; Castañeda-Ruiz, R.F.; Mena-Portales, J.; Guarro, J. Emendation of the genus *Bactrodesmiastrum* (Sordariomycetes) and description *of Bactrodesmiastrum monilioides* sp. nov. from plant debris in Spain. *Mycol. Prog.* **2015**, *14*, 48. [CrossRef]
18. Hernández-Restrepo, M.; Mena-Portales, J.; Gené, J.; Cano, J.; Guarro, J. New *Bactrodesmiastrum* and *Bactrodesmium* from decaying wood in Spain. *Mycologia* **2013**, *105*, 172–180. [CrossRef]
19. Réblová, M.; Seifert, K. *Conioscyphascus*, a new ascomycetous genus for holomorphs with *Conioscypha* anamorphs. *Stud. Mycol.* **2004**, *50*, 95–108.
20. Luo, Z.L.; Hyde, K.D.; Liu, J.K.; Maharachchikumbura, S.S.N.; Jeewon, R.; Bao, D.F.; Bhat, D.J.; Lin, C.G.; Li, W.L.; Jing, Y. Freshwater Sordariomycetes. *Fungal Divers.* **2019**, *99*, 451–660. [CrossRef]
21. Vu, D.; Groenewald, M.; de Vries, M.; Gehrmann, T.; Stielow, B.; Eberhardt, U.; Al-Hatmi, A.; Groenewald, J.Z.; Cardinali, G.; Houbraken, D.; et al. Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. *Stud. Mycol.* **2019**, *92*, 135–154. [CrossRef]
22. Lutzoni, F.; Kauff, F.; Cox, C.J.; McLaughlin, D.; Celio, G.; Dentinger, B.; Padamsee, M.; Hibbett, D.; James, T.J.; Baloch, E.; et al. Assembling the fungal tree of life: Progress, classification, and evolution of subcellular traits. *Am. J. Bot.* **2004**, *91*, 1446–1480. [CrossRef]
23. Spatafora, J.W.; Sung, G.H.; Johnson, D.; Hesse, C.; O'Rourke, B.; Serdani, M.; Spotts, R.; Lutzoni, F.; Hofstetter, V.; Miadlikowska, J.; et al. A five-gene phylogeny of Pezizomycotina. *Mycologia* **2006**, *98*, 1018–1028. [CrossRef] [PubMed]
24. Hyde, K.D.; Norphanphoun, C.; Maharachchikumbura, S.S.N.; Bhat, D.J.; Jones, E.B.G.; Bundhun, D.; Chen, Y.J.; Bao, D.F.; Boonmee, S.; Calabon, M.S.; et al. Refined families of Sordariomycetes. *Mycosphere* **2020**, *11*, 305–1059. [CrossRef]
25. Réblová, M.; Seifert, K.A.; Fournier, J.; Štepánek, V. Newly recognised lineages of perithecial ascomycetes: The new orders Conioscyphales and Pleurotheciales. *Pers. Mol. Phylogeny Evol. Fungi* **2016**, *37*, 57–81. [CrossRef]
26. Cheng, X.L.; Li, W.; Zhang, T.Y. A new species of *Phaeoisaria* from intertidal marine sediment collected in Weihai, China. *Mycotaxon* **2014**, *127*, 17–24. [CrossRef]
27. Réblová, M.; Seifert, K.A.; Fournier, J.; Štěpánek, V. Phylogenetic classification of *Pleurothecium* and *Pleurotheciella* gen. nov. and its dactylaria-like anamorph (Sordariomycetes) based on nuclear ribosomal and protein-coding genes. *Mycologia* **2012**, *104*, 1299–1314. [CrossRef]
28. Campbell, J.; Shearer, C.A. *Annulusmagnus* and *Ascitendus*, two new genera in the Annulatascaceae. *Mycologia* **2004**, *96*, 822–833. [CrossRef]
29. Bhat, D.J.; Kendrick, B. Twenty-five new conidial fungi from the Western Ghats and the Andaman Islands (India). *Mycotaxon* **1993**, *49*, 19–90.
30. Bussaban, B.; Lumyong, S.; Lumyong, P.; Hyde, K.D.; McKenzie, E.H.C. Three new species of *Pyricularia* are isolated as zingiberaceous endophytes from Thailand. *Mycologia* **2003**, *95*, 519–524. [CrossRef] [PubMed]
31. Zhang, H.; Jones, G.E.B.; Zhou, D.; Bahkali, A.H.; Hyde, K.D. Checklist of freshwater fungi in Thailand. *Cryptogam. Mycol.* **2011**, *32*, 199–217. [CrossRef]
32. Perera, R. New species of *Thozetella* and *Chaetosphaeria* and new records of *Chaetosphaeria* and *Tainosphaeria* from Thailand. *Mycosphere* **2016**, *7*, 1201–1321. [CrossRef]
33. Calabon, M.S.; Jones, E.B.G.; Boonmee, S.; Doilom, M.; Lumyong, S.; Hyde, K.D. Five novel freshwater Ascomycetes indicate high undiscovered diversity in lotic habitats in Thailand. *J. Fungi.* **2021**, *7*, 117. [CrossRef] [PubMed]