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

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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. obvata* 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; semimacrornematous, mononematous, simple or branched, mostly moniliform, smooth-walled, hyaline conidiofores; monoblastic, discrete or integrated, globose, subglobose, ellipsoidal or clavate, smooth-walled, hyaline conidiogenous cells; and conidia that are ellipsoidal to broadly obovate, 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. obvata* 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\textsuperscript{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\textsuperscript{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 |
|-------------------------------|---------------|--------------------------|------------|
| Ascotaiwania lignicola        | NIL 00005     | HQ446341 HQ446364        | [16]       |
| Ascotaiwania sawadae          | SS 00051      | HQ446340 HQ446363        |            |
| Bactrodesmiastrum monilioides | FMR 10756 T   | NR_152539 KF771879       | [12]       |
| Bactrodesmiastrum obovatum    | FMR 6482      | NR_152537 FR870266       | [18]       |
| Bactrodesmiastrum pyriforme   | FMR 10747 T   | NR_152536 FR870265       | [18]       |
| Bactrodesmiastrum pyriforme   | FMR 11931     | HE646636 HE646637        | [18]       |
| Canalrisporiaceae carinense   | SS 03839      | GQ390283 GQ390268        | [7]        |
| Canalrisporiaceae grenadoideum| BCC 20507 T   | NR_111442 GQ390267       | [7]        |
| Conioscypha lignicola         | CBS 335.93    | -                        | [19]       |
| Conioscypha submera           | DLUCC 0904 T  | NR_168820 MK35856        | [20]       |
| Conioscypha varia             | CBS 436.70    | MH859785 MH871548        | [21]       |
| Fuscosporella aquatica        | MFLUCC 16-0889 T | NR_156398 NG_059853     | [3]        |
| Fuscosporella pyriformis      | MFLUCC 16-0570 T | NR_152555 NG_059711     | [1]        |
| Lessia lubrica                | AFTOL-ID 1    | DQ491484 AY344644        | [22]       |
| Microglossum rufum            | AFTOL-ID 1292 | -                        | [23]       |
| Miculspora infundibulata      | MFLUCC 16-0866 T | NR_171733 NG_073625    | [24]       |
| Miculspora obscurisepata      | MFLUCC 15-0618 T | NR_152556 NG_059709     | [1]        |
| Miculspora phangngaensis       | MFLUCC 16-0865 T | NR_156399 NG_059854     | [3]        |
| Parafuscosporella aquatica    | KUMCC 19-0211 T | MN513034 MN512343       | [4]        |
| Parafuscosporella ellipsocodiogaena | TBRC 15503 T | OK044749 OK044741      | This study |
| Parafuscosporella ellipsocodiogaena | 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 |
|--------------------------------|---------------|--------------------------|------------|
| Parafuscosporella moniliformis  | MFLUCC 15-0626<sup>T</sup> | NR_152557 NG_059710      | [1]        |
| Parafuscosporella mucosa        | MFLUCC 16-0571<sup>T</sup> | NR_152554 NG_059855      | [1]        |
| **Parafuscosporella obovata**   | TBRC 15505<sup>T</sup>   | OK044751 OK044743        | This study |
| Parafuscosporella pyriformis    | MFLUCC 18-1400<sup>T</sup> | MNS153031 MNS12340       | [4]        |
| Parafuscosporella pyriformis    | KUMCC 19-0008  | MNS153030 MNS12339       | [4]        |
| Phaeoisaria fasciculata         | CBS 127885<sup>T</sup>  | NR_145395 NG_064241      | [25]       |
| Phaeoisaria sedimenticola       | CGMCC 3.14949<sup>T</sup> | JQ074237 JQ031561        | [26]       |
| Pleurotheciella centenaria      | DAOM 229631<sup>T</sup> | NR_111709 NG_060098      | [27]       |
| Pleurotheciella rivularia       | CBS 125238<sup>T</sup>  | JQ429160 JQ429232        | [27]       |
| Pleurothecium recurvatum        | CBS 138747       | KT278728 KT278714        | [25]       |
| Pleurothecium semifecundum      | CBS 131271<sup>T</sup> | JQ429159 JQ429240        | [27]       |
| Pseudoascotaiwania persoonii    | A57-14C         | -                        | [28]       |
| Savoryella aquatica             | SS 03801        | -                        | [16]       |
| Savoryella lignicola            | NF 00204        | -                        | [16]       |
| Vanakripa minutiellipsoididea   | CBS 112523<sup>T</sup> | 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 Fuscosporales, 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-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 distict, hyaline, 1.3–3.8 × 2.5–3 µm basal frills. Sexual morph unknown.

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, chlamydosporas 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 15-0626T (100%), P. mucosa MFLUCC 16-0571T (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 6543T (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 6543T (97.4–97.6%), P. garethii TBRC 6544 (97.4–97.6%) and P. moniliformis MFLUCC 15-0626T (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 Parafuscospora moniliformis MFLUCC 15-0626T (87.6–88.2%) and Parafuscospora aquatica KUMCC 19–0211T (87.8%).

Note: Parafuscospora ellipsacoconidiogena 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.

Parafuscospora ellipsacoconidiogena differs from Vanakripa species due to an absence of vermiform to obpyriform separating cells. Morphologically, P. ellipsacoconidiogena 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 conidigenous cells, and uniseptate, dark-pigmented conidia [1]. However, they mainly differ in the shape of conidigenous cells and conidiophores. Parafuscospora ellipsacoconidiogena has doliiform fusiform conidigenous cells and moniliform conidiophores, while P. mucosa possesses globose, subglobose or clavate conidigenous cells and cylindrical conidiophores. Conidigenous 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, subgloboso, clavate and shorter (7–17 × 4–12 µm) conidigenous cells that differ from those of P. ellipsacoconidiogena [1].

In PDA culture, the sizes of the conidigenous cells and conidia of both species somewhat overlap, and these two species mainly differ in the shape of the conidigenous cells as well as the shape and colour of the conidia. Parafuscospora ellipsacoconidiogena has cylindrical or ellipsoidal conidigenous cells and broadly obpyriform, ellipsoidal, obovoid, medium brown to dark brown conidia, while P. mucosa has doliiform or obovoid conidigenous 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. ellipsacoconidiogena is closely related to P. moniliformis. Morphologically, P. ellipsacoconidiogena and P. moniliformis share a similar morphology of the sporodochial conidiomata and conidiophores that are mostly moniliform with ellipsoidal moniliform conidigenous cells [1]. However, P. ellipsacoconidiogena 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) conidigenous 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) conidigenous cells with broadly obpyriform conidia [1]. In PDA culture, the differences between P. ellipsacoconidiogena and P. moniliformis are in the shape of conidigenous cells and conidia. In P. ellipsacoconidiogena, it has ellipsoidal conidigenous cells with broadly obpyriform, ellipsoidal or obovoid conidia, while P. moniliformis has subglobose or dumbbell-shaped conidigenous cells with globose to subglobose conidia [1]. The comparison of Parafuscospora 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 × 7.5–8.8 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                                                      | Size                              | Reference          |
|-----------|----------------------------------------------------------|-----------------------------------------------------|---------------------------------------------------------------|-----------------------------------|--------------------|
| 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. ellipoconidiogena | 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, doliform 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 6543T (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 6543T (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 obvoid 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. ellipsococonidiogena 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. ellipsocconiidiognia 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. ellipsocconiidiognia [1–3], and P. obovata within Fuscosporallaceae (Fuscosporallales), 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. ellipsocconiidiognia 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 ellipsocconiidiognia 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. ellipsocconiidiognia 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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