Five Novel Taxa from Freshwater Habitats and New Taxonomic Insights of Pleurotheciales and Savoryellomycetidae

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Abstract: Pleurotheciales is the largest order in Savoryellomycetidae with a large proportion of species known from freshwater habitats. In order to investigate the phylogenetic relationships of taxa within Pleurotheciales and contribute to their diversity, submerged wood was collected from freshwater habitats in China (Yunnan Province) and Thailand. Two dematiaceous, sporodochial taxa from Pleurotheciales, while O. guttulatum is referred to Savoryellomycetidae genera incertae sedis. The phylogenetic relationships are also presented for Coleodictyospora and Pseudocoleodictyospora, which raises an intriguing taxonomic issue. These two genera are positioned in two different classes, viz Sordariomycetes and Dothideomycetes, although they are quite similar except for the presence of a conidial sheath. This study expands our knowledge of the fungal diversity of freshwater fungi, and also indicates that Pleurotheciales species are mostly found in freshwater habitats.

Keywords: annulatacales-like; multi-locus phylogeny; Pseudocoleodictyospora; Sordariomycetes; submerged wood; Thailand; Yunnan

1. Introduction

Savoryellomycetidae currently accommodates four orders, Conioscyphales, Fuscosporellales, Pleurotheciales and Savoryellales. This is based on evidence from phylogenetic analyses and divergence time studies with the order having a stem age estimated as 268 MYA [1]. The four orders clustered as a robust clade in all studies [1–3]. Pleurotheciales, with a single-family Pleurotheciaceae [4], is the largest order in Savoryellomycetidae.
Pleurotheciaceae species have mostly been isolated from decaying wood or plant debris as saprobes, while few species were also identified as opportunistic human pathogens (*Phaeoisaria clematidis*) [5]. Multi-locus phylogenetic relationships of Pleurotheciaceae species were investigated to better understand their taxonomy [3,4,6–10] and as a result, eleven genera were accepted in the family [11].

Taxa of Pleurotheciaceae have perithecial ascomata with asexual dematiaceous hyphomycetous stages. Coelomyceteous asexual morphs have not been reported in the family. Pleurotheciaceae is an assemblage of genera representing a highly diverse morphology, especially in the asexual morphs. *Pleurothecia* and *Pleurothecium* (type) generally have macronematous, unbranched conidiophores, holoblastic, sympodially proliferating conidiogenous cells with a conspicuous rachis of denticles, and hyaline, septate conidia [4,9,10,12,13]. *Anapleurothecium* has macronematous, unbranched conidiophores, sympodial, denticulate conidiogenous cells which are the cases of *Pleurothecia* and *Pleurothecium*, but it has botuliform to cylindrical and brown conidia with a paler basal cell [7]. *Phaeoisaria* has synnematous conidiophores with tiny aseptate conidia [13]. *Phragmoccephala* also has synnematous conidiophores, but the conidia are relatively large, ellipsoidal to subglobose, with dark brown to black central cells and paler polar cells [14]. *Sterigmatobotrys* are distinct in the family by their well-defined stipe and a complex penicillate conidiophore head consisting of series of penicillate branches [6]. While some other genera lack conspicuous macronematous conidiophores, and the conidia directly arise from the hyphae on the host substrate or from micronematous, subhyaline conidiophores, such as *Neomonodictys* with subglobose to globose, muriform conidia [8] and *Helicoascolaiwania* with helicoid conidia [2,15].

The diversity of morphology is also reflected in some sexual morphs of Pleurotheciaceae. The genera *Adelosphaeria*, *Melanotrigonum*, *Pleurothecia* and *Pleurothecium* generally have superficial ascomata with a short papilla, narrowly or broadly clavate asci with a distinct, refractive apical ring and ellipsoidal-fusiform, septate ascospores [3,4,9], while *Phaeoisaria* has immersed ascomata with a quite long neck, cylindrical asci and filiform, multiseptate ascospores. *Helicoascolaiwania* is easily distinguished in the family in having generally immersed ascomata lying horizontally or obliquely to the host substrate and fusiform, versicolorous ascospores with darker central cells and paler polar cells [2,3].

The asexual–sexual morph connections were investigated based on cultural studies with a combination of molecular data. Some hyphomycetes were linked as the life cycle of known sexual morphs. The asexual morph of *Pleurothecium recurvatum* was first reported from the artificial medium (WA) inoculated by an ascomycetous species *Carpoligna pleurothecii* [16]. Réblová et al. [9] also found the asexual morphs from another medium (PCA) inoculated by ascomycetous species *Pleurothecia rivularia* and *Pleurothecium semifecundum*. With DNA sequence data, Luo et al. [10] linked the asexual–sexual morph of *Pleurothecia fusiformis* based on two specimens collected from Erhai Lake, Yunnan, China.

Luo et al. [6] used multi-locus analysis to first report a sexual morph in *Phaeoisaria*, namely *Ph. filiformis*, which was characterized by immersed ascomata with a long, cylindrical neck, and cylindrical asci containing hyaline and filiform ascospores. Although the morphological traits associate *Ph. filiformis* as allied with *Ceratosphaeria* and *Ophioceras* in Magnaporthales, phylogenetic analysis placed *Ph. filiformis* in Pleurotheciales. Neither conidia nor conidiomatal structures were observed from the culture of *Ph. filiformis* [6].

The main objectives of this study were to revise the taxon diversity within Pleurotheciaceae, report on novel taxa and provide new insights into the systematics of Savoryellomycetidae. Two specimens of dematiaceous hyphomycetes were preliminarily identified as Pleurotheciaceae species with micronematous conidiophores, holoblastic conidiogenous cells and dark muriform conidia, but the morphologies were rather unusual as compared to other members of the family. One specimen resembled annulatascaceae-like taxa in Diaportheomycetidae but is similar to the taxa of Pleurotheciaceae in Savoryellomycetidae. In order to clarify the placement of these specimens, a multi-locus analysis of a concatenated nuc 28S rDNA (LSU), nuc18S rDNA (SSU), internal transcribed spacer (ITS) and second-
largest subunit of RNA polymerase II (rpb2) dataset were performed, and phylogenetic relationships inferred.

2. Materials and Methods

2.1. Herbarium Material, Isolation and Morphology

Decayed woody twigs and branches submerged in freshwater streams in forests were randomly collected in Yunnan Province, China, as well as Satun and Songkhla provinces in Thailand where all places are in the Greater Mekong Subregion. Specimens were placed in zip-lock plastic bags containing moistened cotton and taken to the laboratory. Sediment on separated specimens was washed off with tap water and incubated in plastic boxes lined with moistened tissue paper at room temperature (20–25 °C) for 1–2 weeks. The ascomata and sporodochia developed on the specimens were examined with a Nikon SMZ-171 dissecting microscope. Fungal structures were captured with a Nikon ECLIPSE Ni compound microscope fitted with a Canon EOS 750D digital camera. Single spore isolations were made from ascospore or conidium on potato dextrose agar (PDA, Shanghai Bio-way technology Co., Ltd., Shanghai, China) at room temperature (20–25 °C). All morphological approaches used herein were modified from Chomnunti et al. [17] and Senanayake et al. [18]. Tarosoft (R) Image Frame Work program was used to measure the fungal structures. Images were processed with Adobe Photoshop CS5 software (Adobe Systems, San Jose, CA, USA). Herbarium specimens (dry wood with fungal materials) were deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand and herbarium of Cryptogams, Kunming Institute of Botany Academia Sinica (HKAS), Kunming, China. Living cultures were deposited in the Mae Fah Luang University Culture Collection (MFLUCC) and Kunming Institute of Botany Culture Collection (KUMCC). The novel taxa were registered in the databases Facesoffungi (http://www.facesoffungi.org, accessed on 10 June 2021) [19] and Index Fungorum (http://www.indexfungorum.org/names/names.asp, accessed on 21 June 2021).

2.2. DNA Extraction, PCR Amplification and Sequencing

Fungal mycelia were scraped from the colonies on PDA. The Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Hangzhou, China) was used to extract total genomic DNA. The polymerase chain reaction (PCR) technique was utilized for the amplification of target DNA fragments. The primer pairs LR0R/LR5, NS1/NS4, ITS5/ITS4 and fRPB2-5F/fRPB2-7cR were used to amplify LSU, SSU, ITS and rpb2 [20–22]. The amplifications were carried out as detailed in Dong et al. [23]. The PCR thermal cycle program for the amplification of LSU, SSU and ITS was provided as initially 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 50 s, elongation at 72 °C for 90 s and a final extension at 72 °C for 10 min. The annealing was adjusted to 52 °C for rpb2. PCR products were checked on 1% agarose electrophoresis gels stained with Gel Red. The sequencing reactions were carried out by Shanghai Sangon Biological Engineering Technology and Services Co., Shanghai, China.

2.3. Molecular Phylogenetic Analyses

2.3.1. Sequence Selection and Phylogenetic Analyses Construction

The qualities of generated sequences were initially checked with Finch TV v. 1.4.0 and each gene was subjected to a BlastN search in NCBI’s GenBank to assess the confidence level. Phylogenetic placements of the unidentified fungi were resolved by analyzing four different datasets: (1) two multi-locus datasets of a concatenated LSU, SSU, ITS and rpb2 sequences; (2) two separate individual LSU and ITS datasets. The first multi-locus dataset was analysed to infer the phylogenetic positions of unidentified fungi within the Savoryellomycetidae along with sequences deposited from recent relevant publications [3,6,24]. In the second multi-locus dataset, we included the other subclasses in Sordariomycetes to infer the subclass status of an unidentified genus which could not be confirmed in any orders in the subclass Savoryellomycetidae. Besides, Pseudocoleodictyospora and its related taxa
were also included to show the phylogenetic relationships between *Coleodictyospora* and *Pseudocoleodictyospora*. The individual LSU and ITS phylogenetic analyses were utilized to auxiliarly assess the phylogenetic relationships of fungi in Savoryellomycetidae. All sequences used in this study were listed in Table 1.

| Taxon                        | Voucher/Culture | GenBank Accession Numbers |
|------------------------------|-----------------|---------------------------|
|                              |                 | LSU | SSU | ITS | rpb2 |
| *Achroceratosphaeria potamia*| CBS 125414      | GQ996538 | GQ996541 | MH863679 | KM588908 |
| *Adelosphaeria catenata*     | CBS 138679<sup>T</sup> | KT278707 | KT278692 | KT278721 | KT278743 |
| *Anapleurothecium botulisporum* | FMR 11490<sup>T</sup> | KY853483 | - | KY853423 | - |
| *Arecophila bambusae*        | HKUCC 4794      | AF452038 | AY083802 | - | - |
| *Ascothriopsis latericola*   | ICMP 22739<sup>T</sup> | MN699407 | - | MN699390 | MN704312 |
| *Ascolithia xenicola*        | NIL 00005       | HQ446364 | HQ446284 | - | HQ446419 |
| *Bactrodesmastrum obvatum*   | FMR 6482        | FR870266 | - | - | - |
| *Bactrodesmastrum pyriforme* | FMR 11931       | HE646657 | - | - | - |
| *Bactrodesmium diversum*     | CBS 144080      | MN699415 | MN699371 | MN699355 | MN704294 |
| *Bactrodesmium obvatum*      | CBS 144078      | MN699425 | MN699376 | MN699396 | - |
| *Bactrodesmium pallidum*     | CBS 145349      | MN699429 | MN699380 | MN699364 | MN704302 |
| *Brachysporiella setosa*     | HKUCC 3713      | AFI132334 | - | - | - |
| *Canalisporium exiguum*      | SS 00809        | GQ390281 | GQ390266 | - | HQ446436 |
| *Canalisporium grenadoleum*  | BCC 20507       | GQ390267 | GQ390252 | - | HQ446420 |
| *Cercophora caudata*         | CBS 606.72      | AY999113 | DQ368659 | AY999135 | DQ368646 |
| *Cercophora neufeldiana*     | SMH 3303        | AY780062 | - | - | AY780167 |
| *Cercophora thailandica*     | MFLUCC 12-0845  | KU863127 | KU872131 | - | KU940176 |
| *Cheilymenia stercorosa*     | AFTOL 148       | AY544661 | AY544705 | - | - |
| *Coleodictyospora muriformis*| MFLUCC 18-1243<sup>T</sup> | MW981648 | MW981704 | MW981642 | - |
| *Conioscypha japonica*       | CBS 387.84      | AY484514 | JQ437438 | - | - |
| *Conioscypha lignicola*      | CBS 335.93<sup>T</sup> | AY484513 | JQ437439 | - | JQ429260 |
| *Conioscypha peruviana*      | ILL 41202<sup>T</sup> | KF781539 | - | - | - |
| *Conioscypha varia*          | CBS 604.70      | MH871656 | - | MH859869 | - |
| *Cosmospora arxii*           | CBS 748.69      | MH871181 | - | NR-145062 | HQ897725 |
| *Dematisporium aquaticum*    | MFLU 18-1641<sup>T</sup> | MK835855 | - | - | MN194029 |
| *Dematiapyrina aquilaria*    | CGMCC 3.17268<sup>T</sup> | KJ138623 | KJ138622 | KJ138621 | - |
| *Diaporthe cyatheae*         | YMJ 1364        | JX570891 | JX570890 | - | JX570893 |
| *Diaporthe cros*             | AR 3538         | AF408350 | - | - | - |
| *Diaporthe xishuangbanica*   | LC6744          | KY011862 | - | - | - |
| *Doratomyces stemonitis*     | AFTOL-ID 1380   | DQ836907 | DQ836901 | - | - |
| *Entosordaria perfdiosa*     | BW3             | MF488992 | - | - | MF489002 |
| *Fuscospora pyriformis*      | MFLUCC 16-0570<sup>T</sup> | KK550896 | KK550900 | - | KK576872 |
| *Fusicolla aqueductum*       | KUMCC 18-0015   | MH087221 | - | MH087219 | - |
| *Helicoascolithia farinosa*  | DAOMC 241947    | JQ429230 | - | JQ429145 | - |
Table 1. Cont.

| Taxon                       | Voucher/Culture | GenBank Accession Numbers |
|-----------------------------|-----------------|---------------------------|
|                             |                 | **LSU** | **SSU** | **ITS** | **rpb2** |
| *Helicoascotaiwania farinosa* | ILLS 53605<sup>T</sup> | AY094189 | - | - | - |
| *Helicoascotaiwania farinosa* | P2-6 | AY316357 | - | - | - |
| *Helicoascotaiwania lacustris* | CBS 145963<sup>T</sup> | MN699430 | MN699382 | MN699399 | MN704304 |
| *Helicoascotaiwania lacustris* | CBS 145964 | MN699431 | MN699383 | MN699400 | MN704305 |
| *Helicoascotaiwania lacustris* | CBS 146144 | MN699432 | MN699384 | MN699401 | MN704306 |
| *Melanotrigonum ovale*       | CBS 138744 | KT278710 | KT278697 | KT278725 | KT278746 |
| *Melanotrigonum ovale*       | CBS 138815 | KT278711 | KT278698 | KT278722 | KT278747 |
| *Melanotrigonum ovale*       | CBS 138743<sup>T</sup> | KT278709 | KT278696 | KT278724 | KT278745 |
| *Microascus trigonosporus*   | AFTOL-ID 914 | DQ470958 | DQ471006 | DQ49106D | DQ491513 | - |
| *Mucispora obscuriseptata*   | MFLUCC 15-0618<sup>T</sup> | KX550892 | KX550897 | - | KX576870 |
| *Nectria nigrescens*         | AR 4211 | HM484720 | JN939647 | HM484707 | JQ041213 |
| *Neosacotaiwania fusiformis* | MFLUCC 15-0621<sup>T</sup> | KX550893 | - | MG398820 | KX576971 |
| *Neosacotaiwania limnetica*  | CBS 126576 | KY803513 | KT278689 | KY853452 | MN704308 |
| *Neosacotaiwania terestris*  | CBS 142291<sup>T</sup> | KY853515 | KY853515 | KY853454 | - |
| *Neonomadictys muriformis*   | MFLUCC 16-1136<sup>T</sup> | MN644485 | - | MN645309 | - |
| *Neorussosella alishanense*  | MFLUCC 11-0190 | MN028398 | - | MN028394 | - |
| *Neorussosella bambusae*     | MFLUCC 11-0124 | KJ474839 | - | KJ474827 | KJ474856 |
| *Neotorula submersa*         | KUMCC 15-0280 | KX789217 | - | KX789214 | - |
| *Neospora crassa*            | MUCL 19026 | AF284611 | X09471 | - | - |
| *Obliquifusidoideum guttulatum* | MFLUCC 18-1233<sup>T</sup> | MW981650 | MW981706 | MW981645 | - |
| *Paracremonium binnewijzendii* | CBS 143277 | MG250174 | - | NR-157491 | - |
| *Parathyridaria percutanea*  | CBS 868.95 | KF366449 | KF366451 | KF322118 | KF366452 |
| *Phaeoisaria annesophieae*   | MFLU 19-0531 | MT590804 | - | MT590190 | - |
| *Phaeoisaria aquatica*       | MFLUCC 16-1298<sup>T</sup> | MF399254 | - | MF399237 | MF401406 |
| *Phaeoisaria clematidis*     | MFLUCC 18-1017 | MW132065 | MW132063 | MW131990 | - |
| *Phaeoisaria fasciculata*    | CBS 127885<sup>T</sup> | KT278705 | KT278693 | KT278719 | KT278741 |
| *Phaeoisaria fasciculata*    | DAOM 230055 | KT278706 | KT278694 | KT278720 | KT278742 |
| *Phaeoisaria pseudoclematidis* | MFLUCC 11-0393<sup>T</sup> | KP744501 | KP753962 | KP744457 | - |
| *Phaeoisaria sedimentica*    | CGMCC 3.14949<sup>T</sup> | JQ031561 | - | JQ074237 | - |
| *Phragmocephala stemphylioides* | DAOM 673211 | KT278717 | - | KT278730 | - |
| *Pisorisporium cymbiforme*   | PRM 924378 | KM588952 | KM588899 | - | KM588905 |
| *Pleurothecia aquatica*      | MFLUCC 17-0464<sup>T</sup> | MF399253 | MF399220 | MF399236 | MF401405 |
| *Pleurothecia centenaria*    | DAOM 229631<sup>T</sup> | JQ429234 | JQ429246 | JQ429151 | JQ429265 |
| *Pleurothecia crumpens*      | CBS 142447<sup>T</sup> | MN699435 | MN699387 | MN699406 | MN704311 |
| *Pleurothecia fusiformis*    | MFLUCC 17-0113<sup>T</sup> | MF399250 | MF399218 | MF399233 | MF401403 |
| *Pleurothecia gattulata*     | KUMCC 15-0442 | MF399256 | MF399222 | MF399239 | MF401408 |
| *Pleurothecia gattulata*     | KUMCC 15-0296<sup>T</sup> | MF399257 | MF399223 | MF399240 | MF401409 |
| *Pleurothecia krabiiensia*   | MFLUCC 16-0852<sup>T</sup> | MG837013 | MG837023 | MG837018 | - |
| *Pleurothecia lunata*        | MFLUCC 17-0111<sup>T</sup> | MF399255 | MF399221 | MF399238 | MF401407 |
| Taxon                        | Voucher/Culture | GenBank Accession Numbers |
|-----------------------------|----------------|----------------------------|
|                            |                | LSU | SSU | ITS | rpb2 |
| Pleurothecilla riculata     | CBS 125238T    | JQ429232 | JQ429244 | JQ429160 | JQ429263 |
| Pleurothecilla saprophyltica| MFLUCC 16-1251T| MF399258 | MF399224 | MF399241 | MF401410 |
| Pleurothecilla submersa      | DLUCC 0739     | MF399259 | MF399225 | MF399242 | MF401411 |
| Pleurothecilla sympodia      | MFLUCC 17-1709T| MF399260 | MF399226 | MF399243 | MF401412 |
| Pleurothecia aquaticum      | MFLUCC 17-1331T| MF399263 | - | MF399245 | - |
| Pleurothecium aquaticum     | B-27           | MK835854 | MK834786 | - | - |
| Pleurothecium floriforme    | MFLUCC 15-0628 | KY697277 | KY697279 | KY697281 | - |
| Pleurothecium obvoideum     | CBS 209.95     | EU041841 | - | EU041784 | - |
| Pleurothecium pulneigense   | MFLUCC 16-1293 | MF399262 | MF399228 | - | MF401414 |
| Pleurothecium recurvatum    | DAOM 230069    | JQ429238 | JQ429252 | JQ429157 | JQ429269 |
| Pleurothecium semifecundum  | CBS 131271T    | JQ429240 | JQ429254 | JQ429159 | JQ429270 |
| Podosordaria tulasnei       | CBS 128.80     | KT281897 | - | - | - |
| Pseudocoleodictyospora      | A57-14C        | - | - | - | - |
| Psuedoascotaiwania persoonii|                 | A57-14C | - | - | - |
| Pseudocoleodictyospora      | MFLUCC 12-0554 | KU764710 | KU712471 | KU712440 | KU712493 |
| Pseudocoleodictyospora      | MFLUCC 12-0385 | KU764709 | KU712461 | KU712443 | KU712491 |
| Pseudocoleodictyospora      | MFLUCC 12-0387 | KU764704 | KU712462 | KU712444 | KU712492 |
| Pseudocoleodictyospora      | MFLUCC 12-0565 | KU764701 | KU712472 | KU712441 | KU712494 |
| Pseudoneurospora amorphoporcata | CBS 626.80 | FR774287 | - | - | - |
| Rhexoacrodictys erecta      | HSAUPmyr6489   | KX033555 | KX033555 | KU999963 | - |
| Rhexoacrodictys erecta      | HSAUPmyr6489   | KX033555 | KX033555 | KU999963 | - |
| Rhexoacrodictys erecta      | KUMCC 20-0194  | MT559123 | - | MT555421 | - |
| Rhexoacrodictys fimbicola   | HMAS47737      | KX033553 | KX033522 | KU999960 | - |
| Rhexoacrodictys fimbicola   | HMAS43690      | KX033550 | KX033519 | KU999957 | - |
| Roussoella nitidula         | MFLUCC 11-0182 | KJ474843 | - | KJ474835 | KJ474859 |
| Saprodesmium dematisporum   | KUMCC 18-0059T | MW981647 | MW981707 | MW981646 | - |
| Savoryella aquatica         | SS 03801       | HQ446372 | HQ446290 | - | HQ446441 |
| Savoryella lignicola        | NF00204        | HQ446378 | HQ446300 | HQ446357 | - |
| Savoryella longispora       | SAT 00322      | HQ446380 | HQ446300 | HQ446359 | HQ446450 |
| Savoryella paucispora       | SAT 00866      | HQ446381 | HQ446303 | - | HQ446451 |
| Savoryella verrucosa        | SS 00052       | HQ446374 | - | HQ446353 | HQ446445 |
Table 1. Cont.

| Taxon                     | Voucher/Culture | GenBank Accession Numbers |
|---------------------------|-----------------|---------------------------|
| **Savoryella yunnanensis** | MFLUCC 18-1395⁰ | LSU: MK411422 SSU: MK411423 ITS: - rpb2: - |
| **Sordaria fimicola**      | CBS 508.50      | LSU: - SSU: - ITS: - rpb2: - |
| **Sterigmatobotrys macrocarpa** | PRM 915682    | LSU: GU017317 SSU: JQ429255 ITS: JQ429153 rpb2: - |
| **Sterigmatobotrys macrocarpa** | DAOM 230059    | LSU: GU017316 SSU: - ITS: JQ429154 rpb2: JQ429271 |
| **Sterigmatobotrys rudis** | DAOM 229838     | LSU: JQ429241 SSU: JQ429256 ITS: JQ429152 rpb2: JQ429272 |
| **Sterigmatobotrys uniseptata** | MFLUCC 15-0358⁰ | LSU: MK835850 SSU: MK834784 ITS: MK878379 rpb2: - |
| **Subglobosporium tectonae** | MFLUCC 12-0393 | LSU: KU764703 SSU: KU712464 ITS: KU712445 rpb2: KU712485 |
| **Subglobosporium tectonae** | MFLUCC 12-0390 | LSU: KU764702 SSU: KU712463 ITS: KU712446 rpb2: KU712495 |
| **Thyridaria broussonetiae** | CBS 141481      | LSU: - SSU: - ITS: - rpb2: - |
| **Torula aquatica**        | MFLUCC 16-1115 | LSU: MG208146 SSU: - ITS: MG208167 rpb2: MG207977 |
| **Torula herbarum**        | CPC 24114       | LSU: KR873288 SSU: - ITS: KR873260 rpb2: - |
| **Triadelphia uniseptata** | TA06NZ-142     | LSU: KT278718 SSU: - ITS: - rpb2: - |
| **Tubakia seoraksanensis** | CBS 127490      | LSU: KP260499 SSU: - ITS: - rpb2: - |
| **Xylaria hypoxylon**      | CBS 122620      | LSU: KY610495 SSU: - ITS: KY610407 rpb2: KY624231 |
| **Zalerion maritima**      | FCUL280207CP1   | LSU: JN886806 SSU: KT347203 ITS: KT347216 rpb2: - |
| **Zalerion xylestrix**     | 309156          | LSU: EU848592 SSU: EU848591 ITS: - rpb2: - |

The ex-type cultures are indicated using "⁰" after strain numbers and newly generated sequences are indicated in **bold**.

2.3.2. Maximum Likelihood Analyses

Each dataset was aligned with MAFFT v. 7.409 online version [25] and manually verified with BioEdit v. 7.2.5 Biological Sequence Alignment Editor (Ibis BioSciences, Carlsbad, CA, USA), and then concatenated with Mesquite v. 3.11. The maximum likelihood (ML) analyses were performed with RAxML-HPC v. 8 on XSEDE in CIPRES Science Gateway [26,27], with the following changes from the default settings: maximum hours to run: 5; model for bootstrapping phase: GTRGAMMA; analysis type: rapid bootstrap analysis/search for best-scoring ML tree (-f a); bootstrapping type: rapid bootstrapping (-x); bootstrap iterations: 1000 (the maximum value allowed).

2.3.3. Bayesian Inference Analyses

The Bayesian inference (BI) analyses were performed with MrBayes on XSEDE also in CIPRES Science Gateway [26,27]. In the first analysis of Savoryellomycetidae, the best-fit model was GTR+I+G for LSU, ITS and rpb2, and SYM+I+G for SSU. Six simultaneous Markov chains were run for 965,100 generations and trees were sampled every 100th generation. In total, 9651 trees were sampled and the first 25% of sampled trees representing the burn-in phase of the analyses were discarded and the remaining 7239 trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree (critical value for the topological convergence diagnostic is 0.01) [28].

In the second analysis, the best-fit model was GTR+I+G for all datasets. Six simultaneous Markov chains were run for 685,100 generations and trees were sampled every 100th generation. In total, 6851 trees were sampled and the first 25% of sampled trees representing the burn-in phase of the analyses were discarded and the remaining 5139 trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree (critical value for the topological convergence diagnostic is 0.01) [28].

Phylogenetic trees were viewed with FigTree v. 1.4.03 (http://tree.bio.ed.ac.uk/ accessed on 5 May 2021) and edited with Microsoft Office PowerPoint 2007 (Microsoft Corporation, WA, USA).
3. Results
3.1. Phylogenetic Analyses

In the first phylogenetic analysis (Figure 1), the representative homologous sequences of Conioscyphales, Fuscosporellales and Savoryellales and sequences from all genera of Pleurotheciales representing 90 isolates and two outgroup taxa (Doratomyces stemonitis AFTOL-ID 1380 and Microascus trigonosporus AFTOL-ID 914) were included. The matrix had 2205 distinct alignment patterns, with 41.06% of completely undetermined characters and gaps. In the RAxML tree, three distinct independent lineages were identified: (1) one new genus *Obliquifusoideum* (no bootstrap support); (2) one new genus *Saprodesmium* (100% ML BS/1.00 BI PP); (3) *Coleodictyospora* (62% ML BS/–) with one new species *C. muriformis*.

Figure 1. Cont.
The relationships of *Cheilymenia stercorea* as well as *Pseudocoleodictyospora* were weak with four orders in Savoryellomycetidae (Figure 1), but it was shown to be a genus in Savoryellomycetidae with strong bootstrap support. In the second multi-locus phylogenetic analysis (Figure 2), a total of seven subclasses (Diaporthomycetidae, Hypocreomycetidae, Lulworthiomycetidae, Pisorisporiomycetidae, Savoryellomycetidae, Sordariomycetidae and Xylariomycetidae) in Sordariomycetes, as well as *Pseudocoleodictyospora* and its relatives in Dothideomycetes were included in the dataset, representing 55 isolates and one outgroup taxon (*Cheilymenia stercorea* AFTOL-ID 148). The ex-type cultures are indicated using "T" after strain numbers and the new species introduced in this study are indicated in **bold**.

In the second multi-locus phylogenetic analysis (Figure 2), a total of seven subclasses (Diaporthomycetidae, Hypocreomycetidae, Lulworthiomycetidae, Pisorisporiomycetidae, Savoryellomycetidae, Sordariomycetidae and Xylariomycetidae) in Sordariomycetes, as well as *Pseudocoleodictyospora* and its relatives in Dothideomycetes were included in the dataset, representing 55 isolates and one outgroup taxon (*Cheilymenia stercorea* AFTOL-ID 148). The matrix had 2068 distinct alignment patterns, with 45.12% of completely undetermined characters and gaps. In the RAxML tree, *Coleoidictyospora* was phylogenetically distant from *Pseudocoleodictyospora*, although their morphology was quite similar [29]. The relationships of *Obliquifusisoides* were weak with four orders in Savoryellomycetidae (Figure 1), but it was shown to be a genus in Savoryellomycetidae with strong bootstrap support.

**Figure 1.** RAxML tree of Savoryellomycetidae with four orders, Conioscyphales, Fuscosporellales, Pleurotheciales and Savoryellales. The multi-locus tree is generated from combined LSU, SSU, ITS and rpb2 sequence data. Bootstrap support values for maximum likelihood (the first value) equal to or greater than 60% and Bayesian posterior probabilities (the second value) equal to or greater than 0.95 are placed near the branches as ML BS/BI PP. The asterisk "*" represents bootstrap support values with 100% ML BS and 1.00 BI PP. The tree is rooted to *Obliquifusisoides gutulatum* MLFUC 18-1233*.”
Figure 2. RAxML tree with taxa from two classes, Dothideomycetes and Sordariomycetes, to show the phylogenetic relationships between *Coleodictyospora* and *Pseudocoleodictyospora*. The illustrations of species in *Coleodictyospora* and *Pseudocoleodictyospora* are displayed near the generic names. The multi-locus tree is generated from combined LSU, SSU, ITS and *rpb2* sequence data. Bootstrap support values for maximum likelihood (the first value) equal to or greater than 60% and Bayesian posterior probabilities (the second value) equal to or greater than 0.95 are placed near the branches as ML BS/BI PP. The asterisk "*" represents bootstrap support values with 100% ML BS and 1.00 BI PP. The tree is rooted to *Cheilymenia stercorea* AFTOL 148. The ex-type cultures are indicated using "T" after strain numbers and the new species introduced in this study are indicated in **bold**.
In order to assess the phylogenetic position of *Obliquifusoideum* in Savoryellomycetidae, we constructed individual LSU and ITS phylogenetic trees (shown as Supplementary Figures S1 and S2) to enable topological comparison with those derived from the multi-locus datasets. The matrix of the LSU sequence comprised 92 isolates and had 537 distinct alignment patterns, with 14% of undetermined characters or gaps. The matrix of ITS sequence comprised 70 isolates and had 615 distinct alignment patterns, with 30.9% of undetermined characters or gaps. The phylogenetic position of *Obliquifusoideum* was different in all RAxML trees: it clustered with Savoryellales in individual LSU tree (Supplementary Figure S1), clustered with Savoryellales and *Pleurothecium* species of Pleurotheciales in individual ITS tree (Supplementary Figure S2), and clustered with Pleurotheciales in the multi-locus phylogenetic tree (Figure 1); but without bootstrap support in all trees.

3.2. Taxonomy of Fungi Colonising Decaying Submerged Wood

3.2.1. Novel Taxa in Pleurotheciaceae

In this section, one new genus and two new species are introduced in Pleurotheciaceae. These taxa are described alphabetically below.

*Sordariomycetes* O.E. Erikss. and Winka, Myconet 1(1): 10 (1997)

*Savoryellomycetidae* Hongsanan, K.D. Hyde and Maharachch., Fungal Diversity 84: 35 (2017)

*Pleurotheciales* Réblová and Seifert, in Réblová, Seifert, Fournier and Štěpáněk, Persoonia 37: 63 (2016)

*Pleurotheciaceae* Réblová and Seifert, in Réblová, Seifert, Fournier and Štěpáněk, Persoonia 37: 63 (2016)

*Coleodictyospora* Charles ex Matsush., Matsushima Mycological Memoirs 5: 8 (1987)

*Type species*: *Coleodictyospora cubensis* Charles ex Matsush.

*Notes*: *Coleodictyospora* was introduced by Charles [30] with a single species *C. cubensis*, but it lacked a Latin diagnosis. Matsushima [31] validated this genus and characterized it as having cylindrical, simple, septate and hyaline conidiophores, monoblastic conidiogenous cells, and transversely oblong or inverse reniform, muriform conidia surrounded by a semi-gelatinous, hyaline sheath. *Berkleasmium micronesicum* was then transferred to *Coleodictyospora* as *C. micronesiaca* based on its very similar morphological traits with *C. cubensis*, but it differs in having smaller conidia (30–42 × 15–18 µm vs. 42–50 × 20–22 µm) and reduced conidiophores [31].

*Coleodictyospora cubensis* was initially collected from North America [30] and subsequently recorded in Brunei [32] and Japan [33]. Nakagiri and Ito [33] named their specimen IFO 32,660 as *C. cubensis* based on the dimensions of conidiophores, conidia and conidial sheaths, as well as the number of conidial septa and the conidiophore attaching point, although it had overlapping conidial size with *C. micronesiaca*. Nakagiri and Ito [33] emphasized that IFO 32,660 might be a novel species considering the thinner conidia (28–48 × 13–19 µm vs. 42–50 × 20–22 µm) and less number of septa (7–11 vs. 8–14) than the type specimen of *C. cubensis*. However, this hypothesis could not be tested without the re-examination of the specimen IFO 32,660 or the molecular data from similar specimens in the same locality (Ishigaki Island, Japan).

*Coleodictyospora micronesiaca* is likely to be a cosmopolitan species as it was recorded in several countries worldwide, including China (Hong Kong, Taiwan) [31,34], Cuba [35], USA (Florida) [36], Mauritius [37], Mexico [38], Micronesia [39], Peru [40], Philippines [37] and Thailand [41]. However, these records were diagnosed solely based on the morphology and lack of support from molecular data, and the descriptions were omitted or briefly noted.

In this study, we isolated a *Coleodictyospora* species from decaying wood submerged in freshwater and provide sequence data for it. Since *C. cubensis* and *C. micronesiaca* lack sequence data in GenBank, we identified our new collection as a novel species in *Coleodictyospora* based on the comparison of their morphology.

*Coleodictyospora muriformis* W. Dong, Doilom and K.D. Hyde sp. nov. (Figures 3 and 4a,b)
Figure 3. Coleodictyospora muriformis (MFLU 18-1544, holotype). (a,b) sporodochia with a mass of conidia on natural substrate; (c) conidia, conidiogenous cells and conidiophores (arrow shows branched conidiophore); (d,e) conidia with conidiogenous cells; (f,g) conidiophore bearing conidia; (h,i) reniform conidia with semi-gelatinous sheaths (h clearly shows the dark brown bands at the conidial transverse septa); (j) conidium in Indian Ink showing an irregular sheath; (k,l) colony on PDA (left-front, right-reverse). Scale bars, (c–i) 20 μm; (j) 30 μm.
Figure 4. Coleodictyospora spp. (a, b from MFLU 18-1544, holotype. c–e redrawn from Matsushima [31] and Seifert et al. [42]). (a, b) C. muriformis (conidia with branched conidiophores); (c) C. micronesica (conidia growing on a short subulate conidiogenous cell which directly ascends from the basal mat of sporodochia); (d, e) C. cubensis (d sporodochia bearing a mass of conidia. e conidia with a long conidiogenous cell). Scale bars, (a–e) 30 µm.

Index Fungorum number: IF558195; Facesoffungi number: FoF 09872
Etymology: in reference to the muriform conidia of the fungus
Holotype: MFLU 18-1544
Saprobic on decaying wood submerged in freshwater. Sexual morph: undetermined. Asexual morph: hyphomycetous. Colonies on natural substrate, effuse, gregarious, punctiform, sporodochial, raised, black. Mycelium partly immersed in natural substrate, consisting
of branched, septate, thin-walled, smooth, pale brown to brown hyphae. Conidiophores up to 55 μm long, 3 μm wide, micronematous, mononematous, ascending from the basal mat of sporodochia, subcylindrical, branched, septate, hyaline to pale brown, smooth, thin-walled. Conidiogenous cells 7.5–20 × 2.5–3.5 μm (X = 13.5 × 3 μm, n = 10), holoblastic, monoblastic, integrated, determinate, terminal, cylindrical, hyaline, smooth, thin-walled. Conidia 32–44 × 15.5–19 μm (X = 38.5 × 17 μm, n = 100), solitary, acrogenous, generally produced in the middle position, occasionally laterally on conidiophores, and perpendicularly to the conidiophores, mostly cylindro-ellipsoidal, sometimes reniform, muriform, dictyoseptate, with (7–)8–9 transverse and (2–)3 longitudinal septa, deeply constricted and with dark brown bands at the transverse septa, slightly constricted and brown to dark brown at the longitudinal septa, often distinctly constricted at the middle where conidiophore attaches to form reniform, brown, smooth, thin-walled, with a hyaline, semi-gelatinous sheath. Sheaths well-defined, ellipsoidal, thin at the beginning, 2 μm thick; becoming irregular-shaped, uneven, larger after being mounted in the water, up to 55 μm thick in Indian Ink.

Culture characteristics: on PDA, colony irregular, reaching 15 mm diam. in 25 days at room temperature (25–30 °C), surface rough, with dense mycelia, velvety, dry, umbonate in the middle from the side view, edge undulate; from above, dark gray at the margin, pale gray at the middle; from below, dark brown to black at the margin, pale gray at the middle; not producing pigmentation in culture.

Material examined: THAILAND, Satun Province, Khuan Kalong District, Thung Nui Sub-District (6°55′19″ N 100°08′17″ E), on decaying wood submerged in Chang stream originated from Panan Waterfall, 10 May 2018, W. Dong, hat284 (MFLU 18-1544, holotype), ex-type living culture MFLUCC 18-1243 = MFLUCC 18-1279; ibid., HKAS 105018, isotype, ex-isotype living culture KUMCC 19-0034 = KUMCC 19-0052.

Habitat and distribution: stream is located in tropical rainforest in Southern Thailand with hot and humid climate conditions, shallow and clear, flowing slowly from the Panan Waterfall, surrounded by angiosperms.

Notes: Coleodictyospora muriformis belongs in Coleodictyospora based on the punctiform, sporodochial colonies on the natural substrate, monoblastic conidiogenous cells, and cylindro-ellipsoidal, muriform conidia produced perpendicularly to the conidiophores and with a hyaline, semi-gelatinous sheath [30,31]. Coleodictyospora muriformis is easily distinguished from the type species C. cubensis by its shorter conidiophores (up to 55 μm long vs. 70–85 μm long), smaller conidia (32–44 × 15.5–19 μm vs. 42–50 × 20–22 μm) and fewer conidial transverse septa ((7–)8–9 vs. 8–14). The transverse septa of the conidia of C. muriformis are filled with dark brown bands, while they were neither described nor illustrated in C. cubensis [30,31,33,42]. Coleodictyospora muriformis has overlapping conidial dimensions with C. micronesiaca, but it differs in having long, branched conidiophores (up to 55 μm long) and longer conidiogenous cells (7.5–20 × 2.5–3.5 μm), and the conidiophores attach to the middle of the conidia. In contrast, C. micronesiaca lacks conidiophores and the conidiogenous cells are shorter (2–8 × 3–4 μm), which directly ascend from the basal mat of sporodochia; the conidiogenous cells often attach to the end of the conidia. In addition, the conidiogenous cells in C. micronesiaca are mostly short subulate, while they are long cylindrical in C. muriformis. We therefore introduce C. muriformis as new to the genus. A morphological comparison of Coleodictyospora species is summarized in Table 2 and a combined figure plate of three species is illustrated in Figure 4.

Saprodessium W. Dong and Doilom gen. nov.

Index Fungorum number: IF558196; Facesoffungi number: FoF 09873

Etymology: “saprus” = saprobic, referring to the saprobic lifestyle of the fungus; “desmóś” = bond, link, referring to the aggregated conidia in sporodochia

Saprobic on decaying wood submerged in freshwater. Sexual morph: undetermined. Asexual morph: hyphomycetous. Colonies on natural substrate, effuse, gregarious, punctiform, sporodochial, raised, black. Mycelium partly immersed in natural substrate, consisting of branched, septate, thin-walled, smooth, pale brown to brown hyphae. Conidiophores micronematous, mononematous, unbranched, vesiculate, septate. Conidiogenous cells...
holoblastic, monoblastic, integrated, determinate. Conidia solitary, obovoid to ellipsoidal, clearly muriform, olivaceous when young, becoming quite blackish with age and obscuring the septa, with several subhyaline basal cells, smooth, thin-walled. Conidial secession schizolytic.

Table 2. Morphological comparison of Coleodictyospora species (on natural substrate) (update from Nakagiri and Ito [33]).

|                          | C. cubensis                  | C. micronesica               | C. muriformis             |
|--------------------------|------------------------------|------------------------------|----------------------------|
| Conidiophores            | 70–85 × 3.5–5 µm             | Reduced, 2–8 × 3–4 µm (on CMA) | Up to 55 µm long, 3 µm wide |
| Conidiophore attaching point | Middle                      | End                          | Middle                     |
| Conidia                  | 42–50 × 20–22 µm             | 30–40 × 13–16 µm             | 32–44 × 15.5–19 µm         |
| Conidial sheaths         | 55–60 × 40–45 µm             | Present, but not measured    | Up to 55 µm thick in Indian Ink (7–)8–9 |
| Conidial transverse septa| 8–14                         | 6–9                          |                            |
| Conidial septa with or without dark bands | Not mentioned               | Not mentioned               | With dark brown bands      |

Type species: Saprodesmium dematiosporum W. Dong, Doilom and K.D. Hyde

Notes: The BlastN search of NCBI’s GenBank using the LSU sequence shows Saprodesmium dematiosporum has the closest hits with several genera in Pleurotheciaceae, i.e., Rhexoacrodictys erecta (KUMCC 20-0194, similarity = 96.68%), Neomonodictys muriformis (MFLUCC 16-1136, similarity = 94.11%) and Pleurothecium obovoideum (CBS 209.95, similarity = 93.73%). The closest hits using SSU sequence are Rhexoacrodictys erecta (KUMCC 20-0194, similarity = 99.54%), Dematipyriforma aquilaria (3-11-1, similarity = 99.49%) and Pleurothecium aquaticum (B-27, similarity = 99.06%). Based on ITS BlastN search, the closest relatives are however Phaeoisaria sp. (BAB-4787, similarity = 97.11%) and Pleurothecium recurvatum (CBS 138686, similarity = 96.11%). Saprodesmium dematiosporum clusters as an independent branch between Dematipyriforma and Rhexoacrodictys with high bootstrap support in concatenated LSU-SSU-ITS-rpb2 phylogeny (100% ML BS/1.00 BI PP, Figure 1) and individual ITS phylogeny (100% ML BS/1.00 BI PP, Supplementary Figure S2). Saprodesmium dematiosporum clusters with Rhexoacrodictys species in individual LSU phylogeny, but no bootstrap support (Supplementary Figure S1).

Dematipyriforma is an endophytic genus comprising a single species D. aquilaria [43]. Dematipyriforma shares similar morphological characteristics with Saprodesmium in having micronematous conidiophores, holoblastic conidiogenous cells and septate conidia. However, they are entirely different genera in the following aspects. The conidiophores of Dematipyriforma are hypha-like [43], while they are vesiculate in Saprodesmium which are also unique in the family Pleurotheciaceae. The conidia of Dematipyriforma are elongate pyriform, 4–5 transverse septe, sometimes 1–2 longitudinal septe, pale grey olivaceous to pale brown, and has rhexolytic conidial secession [43]. In contrast, the conidia of Saprodesmium are obovoid to ellipsoidal, irregularly muriform and olivaceous when young, becoming quite blackish with age and obscuring the septa, with several subhyaline basal cells, and has schizolytic conidial secession. In addition, Saprodesmium species is saprobe, while Dematipyriforma species is endophyte [43].

Rhexoacrodictys, typified by R. erecta, was introduced for several hyphomycetes characterized by macronematous, long cylindrical conidiophores with percurrent proliferating, monoblastic, integrated, terminal conidiogenous cells, and obovoid, oval or subspherical, muriform, brown to dark brown conidia often with a paler basal cell bearing a small marginal frill derived from the upper portion of the conidiophores and with rhexolytic conidial secession [44]. Saprodesmium shares some morphological traits with Rhexoacrodictys especially with regards to its muriform and obovoid conidia. Saprodesmium, however, has olivaceous conidia and when mature it has quite a blackish pigmentation obscuring the conidial septa. Rhexoacrodictys is featured by rhexolytic conidial secession with conidia that have a conspicuous paler basal cell bearing a small marginal frill, while the conidia of Saprodesmium secede schizolytically and it instead has several subhyaline, depressed
subglobose cells at the base. *Rhexoacrodictys* has macronematous, long cylindrical conidiophores with percurrent proliferating [44,45], whereas *Saprodesmium* has micronematous, short, vesiculate, determinate conidiophores.

Based on the multi-locus and individual phylogenetic analyses, as well as the morphological comparison with the similar taxa in the family, we introduce *Saprodesmium* as a novel genus in Pleurotheciaceae.

*Saprodesmium dematiosporum* W. Dong, Doilom and K.D. Hyde sp. nov. (Figure 5)

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**Figure 5.** *Saprodesmium dematiosporum* (HKAS 101710, holotype). (a) sporodochia with a mass of conidia and scattered conidia on natural substrate; (b) conidia and conidiophores; (c) conidia attach on pseudoparenchyma of sporodochia; (d,e) conidia with conidiogenous cells (arrows); (i) conidial surface showing muriform pattern; (j,k) colony on PDA (left-front, right-reverse). Scale bars, (b–e,h) 15 µm; (f,g,i) 10 µm.

*Index Fungorum number:* IF558197; *Facesoffungi number:* FoF 09874
**Etymology:** in reference to the dematiaceous conidia  
**Holotype:** HKAS 101710

*Saprobi* on decaying wood submerged in freshwater. Sexual morph: undetermined. Asexual morph: hyphomycetous. Colonies on natural substrate, effuse, gregarious, punctiform, sporodochial, raised, black. *Mycelium* partly immersed in natural substrate, consisting of branched, septate, thin-walled, smooth, pale brown to brown hyphae. *Conidiophores* micronematous, mononematous, vesiculate, consisted of 1–4 subglobose, smooth, hyaline cells (each cell 8.5–12 µm diam.), unbranched, septate, constricted at the septa, smooth, thin-walled. *Conidigenous cells* 8–11 µm diam. (x = 9.7 µm, n = 10), holoblastic, monoblastic, integrated, determinate, terminal, subglobose, hyaline, smooth, thin-walled. *Conidia* 21–36 × 14.5–27 µm (x = 27.5 × 21.5 µm, n = 70), solitary, acrogenous, obovoid to ellipsoidal, subglobose, clearly muriform, olivaceous when young, becoming quite blackish with age and obscuring the septa, with several subhyaline to pale brown basal cells, smooth, thin-walled. *Conidial secession* schizolytic.

**Culture characteristics:** on PDA, colony circular, reaching 50 mm diam. in 30 days at room temperature (25–30 °C), surface rough, with dense mycelia, dry, raised from the side view, edge entire; from above, dark gray at the margin, pale gray to white at the middle; from below, black at the margin, dark olivaceous at the middle; not producing pigmentation in culture.

**Material examined:** CHINA, Yunnan Province, Pingbian District (22°59′13″ N 103°40′30″ E), on decaying wood submerged in an unnamed stream originated from Dawei Mountain Nature Reserve, 20 September 2017, W. Dong, WFL23A (HKAS 101710, holotype), ex-type living culture KUMCC 18-0059; ibid., MFLU 18-1165, isotype.

**Habitat and distribution:** stream is nearby Nature Reserve in Southern Yunnan of Yunnan-Kweichow Plateau, shallow and clear, flowing rapidly from the Dawei Mountain, surrounded by angiosperms.

### 3.2.2. Novel Taxa in Savoryellomycetidae

In this section, one new genus with one new species are introduced and phylogenetically referred to Savoryellomycetidae genera *incertae sedis*.

**Obliquifusoideum** W. Dong, Doilom and K.D. Hyde gen. nov.  
**Index Fungorum number:** IF558198; **Facesoffungi number:** FoF 09875  
**Etymology:** in reference to its neck growing oblique to the host substrate and fusoid ascospores

*Saprobi* on decaying wood submerged in freshwater. Sexual morph: *Ascomata* superficial, ellipsoidal, black, coriaceous, ostiolate, with a lateral neck. *Necks* hyaline to dark, subcylindrical, oblique or horizontal to the host substrate. *Peridium* thin, soft, comprising several layers of brown, thin-walled cells of *textura angularis*. *Paraphyses* tapering towards the apex, dense, hypha-like, septate, unbranched, hyaline. *Asci* 8-spored, uniseriate, fusoid, septate, hyaline, thin-walled. *Ascospores* uniseriate, fusoid, septate, hyaline, thin-walled. Asexual morph: undetermined.

**Type species:** Obliquifusoideum guttulatum W. Dong, Doilom and K.D. Hyde  
**Notes:** The BlastN search of NCBI’s GenBank using LSU sequence shows *Obliquifusoideum guttulatum* has the closest hits with several genera in Pleurotheciaceae, but with low percentage similarity, i.e., *Melanotrigonum ovale* (CBS 138743, similarity = 92.06%), *Pleurothecia saprophytica* (MFLUCC 16-1251, similarity = 92.03%), *Phaeoisaria annesophiae* (MFLUCC 19-0531, similarity = 92.03%) and *Sterigmatobotrys rudis* (DAOM 229838, similarity = 93.83%). The closest hits using SSU sequence are several genera in Pleurotheciaceae, i.e., *Pseudostrigospora aquilaria* (3-11-1, similarity = 98.13%), *Phaeoisaria clematidis* (MFLUCC 18-1017, similarity = 97.99%) and *P. fasciculata* (DAOM 230055, similarity = 97.92%). The closest hits using ITS sequence are however several genera in Conioscyphales and Pleurotheciaceae, i.e., *Pleurothecium recurvatum* (DAOM 230069, similarity = 98.75%), *Conioscypha varia* (CBS 604.70, similarity = 94.89%) and *Neomonodictys muriformis* (MFLUCC 16-1136, similarity = 93.19%).
The placement of *Obliquifusoideum guttulatum* is different in multi-locus and individual LSU and ITS phylogenetic trees and lacks significant support in all trees. *Obliquifusoideum guttulatum* is revealed as a sister taxon of Pleurotheciales in the multi-locus analysis of concatenated LSU-SSU-ITS-rpb2 matrix (Figure 1), while it clusters with Savoryellales in the individual LSU phylogeny (Supplementary Figure S1); Savoryellales and Pleurothecium species in the individual ITS phylogeny (Supplementary Figure S2). *Obliquifusoideum* is similar to *Helicoascotaiwania* in Pleurotheciales, and *Ascotaiwania*, *Neoascotaiwania* and *Savoryella* in Savoryellales. They generally have dark ascomata with a lateral neck, which is oblique or horizontal to the host substrate, and septate ascospores. However, the ascospores of the four genera are mostly ellipsoidal and versicolorous with dark middle cells and hyaline polar cells [2–4,46,47]. In contrast, *Obliquifusoideum* has fusoid and evenly hyaline ascospores. The morphological differences and the independent lineage in the multi-locus and individual phylogenetic trees therefore support *Obliquifusoideum* as a new genus.

Although the relationships of *Obliquifusoideum* were weak with four orders in Savoryellomycetidae (Figure 1), it was shown to be a genus in Savoryellomycetidae with strong bootstrap support (Figure 2). We consider it is wise to refer *Obliquifusoideum* to Savoryellomycetidae genera incertae sedis for now, until its phylogeny is better resolved with additional taxon sampling followed by divergence time estimates studies.

**Obliquifusoideum guttulatum** W. Dong, Doilom and K.D. Hyde sp. nov. (Figure 6)

*Index Fungorum number* : IF558199; *Facesoffungi number* : FoF 09876

*Etymology*: in reference to the guttulate ascospores of the fungus

*Holotype*: MFLU 18-1575

*Saprobic* on decaying wood submerged in freshwater. Sexual morph: *Ascomata* 100–120 µm high, 155–170 µm diam., scattered, superficial, ellipsoidal, black, coriaceous, ostiolate, with a lateral neck, ejecting ascii and ascospores soon during incubation and becoming empty. *Necks* 160–180 µm long, 17–30 µm wide, hyaline to black, subcylindrical, oblique or horizontal to the host substrate. *Peridium* thin, 8–20 µm thick, soft, comprising several layers of pale brown, thin-walled cells of *textura angularis*, dark brown outwards. *Paraphyses* 3.5–5 µm wide, tapering towards the apex, dense, hypha-like, septate, unbranched, hyaline, embedded in a gelatinous matrix. *Asci* 97–110 × 7.3–7.7 µm (̄x = 105 × 7.5 µm, n = 10), 8-spored, unitunicate, cylindrical, slightly narrower and truncate at the apex, short pedicellate, with a small, distinct, refractive, barrel- or jar-shaped, apical ring, 2 × 2.7 µm, persistent. *Ascospores* 14–17.5 × 4.3–5 µm (̄x = 15.5 × 4.6 µm, n = 10), overlapping uniseriate, fusoid, straight or slightly curved, one median septate, with two additional obscure septate at two sides, guttulate, hyaline, thin and smooth-walled, without a gelatinous sheath. Asexual morph: undetermined.

*Culture characteristics*: on PDA, colony circular, reaching 8 mm diam. in 48 days at room temperature (25–30 °C), surface rough, with dense mycelia, dry, rigid, umbonal from the side view, edge entire; from above, creamy at the margin, dark grey to dark brown at the middle, brown at the center; dark brown from below; not producing pigmentation in culture.

*Material examined*: THAILAND, Songkhla Province, Rattaphum District, Khao Phra Sub-District (7°00'03" N 100°08'33" E), on decaying wood submerged in a stream originated from Borriphat Waterfall, 10 May 2018, W. Dong, hat138 (MFLU 18-1575, holotype), ex-type living culture MFLUC 18-1233; *ibid.*, HKAS 105007, isotype, ex-isotype living culture KUMCC 19-0023.

*Habitat and distribution*: stream is located in tropical rainforest in Southern Thailand with hot and humid climate conditions, shallow and clear, flowing slowly from the Borriphat Waterfall, surrounded by angiosperms.
Figure 6. *Obliquifusoideum guttulatum* (MFLU 18-1575, holotype). (a,b) ascomata lying on submerged wood; (c) section of ascoma; (d) peridium; (e) section of neck; (f) paraphyses; (g,h) unitunicate asci; (i,j) apical rings; (k–m) ascospores (m ascospore in Indian Ink); (n,o) colony on PDA (up-front, down-reverse). Scale bars, (c) 50 μm; (d,f) 20 μm; (e) 30 μm; (g,h) 15 μm; (i,j) 5 μm; (k–m) 10 μm.

4. Discussion

Doilom et al. [29] established a novel genus *Pseudocoleodictyospora* to accommodate three hyphomycetous species collected from the bark of living *Tectona grandis* (teak) and distinguished them from *Coleodictyospora* by the absence of a hyaline sheath. This establishment, however, lacks the support from the DNA sequence data of *Coleodictyospora*. The presence of conidial sheath as a criterion for delimiting two genera is interesting as this...
is often used for species delimitation in classification, such as species in *Astrosphaeriella, Dictyosporium, Kirschsteiniothelia* and *Natipusilla* [48]. In this study, we collected a freshwater hyphomycetous species which has very similar morphs to *Pseudocoleodictyospora*, but is characterized by a hyaline sheath. This peculiar phenotype further confirms it as a novel species in *Coleodictyospora*, namely *C. muriformis* (see notes of *C. muriformis*). On the basis of DNA-based phylogeny, *Coleodictyospora muriformis* is phylogenetically distant from *Pseudocoleodictyospora* (Pseudocoleodictyosporaceae, Pleosporales) and clusters as a member of the Pleurotheciales (Figure 2). This study further confirms the Doilom et al. [29] taxonomic assumption of establishing a novel genus based on the presence of conidial sheath with the support from the DNA sequence data. Amazingly, *Coleodictyospora* and *Pseudocoleodictyospora* are positioned in two different classes Sordariomycetes and Dothideomycetes, respectively (Figure 2), though they are quite similar except in terms of the conidial sheath. Nevertheless, it is not advisable to use conidial sheath as a criterion segregating species at a higher taxonomic level as it is often an unstable characteristic, especially among freshwater species such as *Caryospora submersa* and *Pseudoastrosphaeriella bambusae* [48].

In our multi-locus phylogenetic tree (Figure 1), *Coleodictyospora* is affiliated to *Neomondictys muriformis* and *Pleurothecium obovoideum*. *Coleodictyospora* is similar to *Neomondictys* in having muriform conidia, but they are entirely different genera. The conidia of *Neomondictys* are subglobose to globose, comprising several subglobose cells, which are irregularly arranged in the conidia, pale brown when immature, producing black pigmentation and obscuring the conidial septa, with a protruding basal cell which attaches to the conidiophore [8]. In contrast, *Coleodictyospora* has cylindro-ellipsoidal conidia, with (7–8)–9 transverse and (2–3) longitudinal septa, deeply constricted and with dark brown bands at the transverse septa, generally produced in the middle position and are perpendicular on the conidiophore. *Pleurothecium obovoideum* was proposed based on a known species, *Ramichloridium obovoideum* [49]. In the phylogenetic tree of Arzanlou et al. [49], they showed that the strain CBS 209.95 of *R. obovoideum* clustered with the sexual morph of *Pleurothecium recurvatum* (type species) and its morphological characteristics fit well with *Pleurothecium* and *R. obovoideum* was therefore transferred to *Pleurothecium*, namely *P. obovoideum*. However, with more species in *Pleurothecium*, *P. obovoideum* was reported to be distant from *P. recurvatum* and clustered with *Neomondictys* in a well-supported clade [6,8]. The reassessment of *P. obovoideum* is pending, however, its pleurothecium-like morphological characteristics [49] warrant it cannot be congeneric with *Coleodictyospora*.

Interestingly, we found that the conidia of *Coleodictyospora* are quite similar to the ascospores of a sexual species *Boerlagiomyces websteri*. *Boerlagiomyces* were recognized in *Tubufiaeaceae* [11,50,51] and confirmed with DNA sequence data derived from a reference specimen of *Boerlagiomyces macrospora* [29]. However, *B. websteri* represented by a putative strain BCC 3834 clustered with several apothecial taxa in *Pezizomyctina* [52], and Boonmee et al. [50] had some doubts on this species because of its perithecial characteristic. Therefore, the accurate phylogenetic position of *B. websteri* is still questionable. Although the soft, membranous, setose ascomata and large dictyosporous ascospores of *B. websteri* fit with the features of *Boerlagiomyces* [50], the two-spored asci are unusual compared with the eight-spored asci of the type species *B. velutinus* [50]. Whether *B. websteri* has close phylogenetic relationships with *Coleodictyospora* in Savoryellomycetidae is pending and has to be resolved.

On the basis of morphology, we initially considered *Obliquifusoideum* as a member of Annulatascales due to its black ascomata with a lateral neck which is oblique or horizontal to the host substrate, hypha-like paraphyses with tapering apex, cylindrical asci with a distinct, refractive apical ring, and fusoid, hyaline ascospores. It is of interest, however, that *Obliquifusoideum* clusters in Savoryellomycetidae with relationships to Pleurotheciales and Savoryellales, which is distant from Annulatascales and annulatascales-like taxa in *Diaporthomycetidae* (Figure 2). It is not unexpected that *Obliquifusoideum* with annulatascales-like morphology can be discovered in another subclass Savoryellomycetidae as Annulatascales is commonly recognized to be polyphyletic and the species are
often encountered from freshwater habitats [53,54]. It is reasonable that *Obliquifusoideum* is placed in Savoryellomycetidae due to its dark ascomata with an oblique or horizontal neck, and phragmoseptate conidia which are the sexual features of the other two members Pleurotheciales and Savoryellales.

DNA-based phylogeny has helped to provide better insights into the taxonomy of Pleurotheciales and a recommendation of species boundaries was established [55], leading to recent classification updates. *Rhexoacrodictys*, which was treated as a genus in Savoryellales by Xia et al. [56], was accepted in Pleurotheciales in a later phylogenetic study [6]. Our phylogenetic results corroborate those of Luo et al. [6], and our new genus *Saprodesmium* forms a well-supported lineage basal to *Rhexoacrodictys* in Pleurotheciales (Figure 1). The BlastN search of *Saprodesmium* using SSU sequence in NCBI’s GenBank reveals a high similarity (99.49%) with a hyphomycetous species *Dematipyriforma aquilaria*. *Dematipyriforma*, typified by *D. australia*, was isolated as an endophyte from the trunk of *Aquilaria crassna*, producing dark muriform conidia [43]. *Dematipyriforma* was placed in Savoryellales in the phylogenetic tree of Sun et al. [43], however, they did not include other related orders (Conioscyphales, Fuscosporellales and Pleurotheciales) in Savoryellomycetidae. Our multi-locus phylogeny places *Dematipyriforma* as a sister genus to *Rhexoacrodictys* and *Saprodesmium* with good bootstrap support in Pleurotheciales (99% ML BS/1.00 BI PP, Figure 1). On the other hand, the muriform conidia of *Dematipyriforma* are similar to *Neomonodictys* in Pleurotheciales. According to this morphological trait and phylogenetic result, we accept *Dematipyriforma* in Pleurotheciales. Besides saprobes and few opportunistic human pathogens, *Dematipyriforma* is the only presently known endophytic genus in the order, which increases our understanding of different life modes of Pleurotheciales.

Freshwater fungi are a unique group of organisms with a special ability to survive and grow on submerged wood in water by producing soft rot cavities [57,58]. There is very little overlap between the fungi growing on wood submerged in freshwater and those on adjacent stream sides [59,60]. Thus, we are continually finding novel taxa from this unique habitat and since streams are often disparate elements, we are likely to discover many more which will improve our understanding of fungal classification [61]. Freshwater appears to be an ecologically important niche for species in Pleurotheciales [62]. Almost all *Pleurotheciella* species were collected from freshwater [3,4,6,9,10,13], as well as some species from *Helicoscotaiwania*, *Phaeoisaria*, *Pleurothecium* and *Sterigmatobotrys* [6,10,12,15,63].

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/jof7090711/s1, Figure S1: Phylogenetic tree generated from maximum likelihood analysis (RAxML) based on LSU sequence data, Figure S2: Phylogenetic tree generated from maximum likelihood analysis (RAxML) based on ITS sequence data.

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**Data Availability Statement:** The data generated from this study can be found in the Index Fungorum (http://www.indexfungorum.org/names/names.asp, accessed on 1 August 2021) and GenBank (https://www.ncbi.nlm.nih.gov/nuccore, accessed on 1 August 2021).

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