Multi-Gene Phylogenetic Analyses Revealed Five New Species and Two New Records of Distoseptisporales from China

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Abstract: Eight hyphomycetes were collected as part of an investigation into the diversity of hyphomycetes from China. Based on morphology and multi-loci (LSU, ITS, tef1α, and rpb2) phylogenetic analyses, five new taxa, including a new Aquapteridospora species A. hyalina and four novel Distoseptisporales species, viz D. aquisubtropica, D. septata, D. tropica, and D. wuzhishanensis were introduced in Distoseptisporaceae (Sordariomycetes). Two new habitat records, viz Distoseptispora pachyconidia and D. xishuangbannaensis were firstly reported. Also provided in this study are detailed descriptions of eight new collections and a revised phylogenetic tree for the Distoseptisporales.

Keywords: new taxa; Aquapteridospora; Distoseptispora; hyphomycete; submerged wood; taxonomy

1. Introduction

The two genera, Distoseptispora (Distoseptisporaceae) and Aquapteridospora (Aquapteridosporaceae) were recently introduced to Distoseptisporales (Diaporthomycetidae) [1–3]. Yang et al. [4] established Aquapteridospora (Diaporthomycetidae) as a monotypic genus, with A. lignicola as the type species. In later research, Hyde et al. [1] introduced Aquapteridosporaceae to accommodate Aquapteridospora and placed this family in the order Distoseptisporales based on divergence estimates, morphological characteristics, and phylogenetic analysis. This was followed in the “Outline of Fungi and fungus-like taxa” by Wijayawardene et al. [5]. Currently, Aquapteridospora consists of five species, most of which were collected from freshwater habitats. Only, A. bambusinum was discovered on dead bamboo culms [6]. The genus is distinguished by its macronematous, mononematous, solitary, unbranched, cylindrical conidiophores; its polyblastic, pale brown conidiogenous cells; and its fusiform, euseptate, guttulate conidia [1,4]. According to phylogenetic analyses, Aquapteridospora formed a distinct sister clade to all Distoseptisporales species [4,7]. Morphologically, Aquapteridospora has macronematous, mononematous and unbranched conidiophores, mononastic conidiogenous cells, and solitary conidia, similar to Distoseptispora. However, Aquapteridospora differs from Distoseptispora in its terminal conidiogenous cells, longer conidiophores, and fusiform euseptate conidia [2–4,8].

The type species of Distoseptispora was designated as D. fluminicola by Su et al. [9]. In recent years, a number of Distoseptispora (Distoseptisporaceae, Distoseptisporales) species have been accepted. Currently, 55 species including 41 freshwater species and 14 terrestrial species have been accepted in Distoseptispora [2,10–16]. Most Distoseptispora species are located in Asia, primarily in China and Thailand, with 26 species in the former and
27 in the latter [3,17–21], while two species viz D. adscendens and D. leonensis [22,23] were found in Hungary and Malaysia, respectively. Most Distoseptispora species are saprobic on palms, Pandanus, Tectona, bamboo, Clematidais, and Carex including unidentified submerged wood [9,24–28], and only D. caricis has been reported as an endophytic species [29]. In addition, D. hyalina is the only one species known to have a sexual morph [7].

During a survey of hyphomycetes in China, eight hyphomycetous taxa were collected from Hainan and Guizhou Provinces. Based on morphological evidence and phylogenetic analyses of sequence combinations of LSU, ITS, elf1α, and rpb2, five new species in genera Aquapteridospora and Distoseptispora, namely A. hyalina, D. aquisubtropica, D. septata, D. tropica, and D. wuzhishanensis, and two new habitat records of D. pachyconidia and D. xishuangbannaensis, were identified, and their full descriptions and illustrations are provided in the present report.

2. Materials and Methods

2.1. Sample Collection, Specimen Examination, and Isolation

Fresh specimens of decaying wood were randomly collected from freshwater and terrestrial habitats in Hainan and Guizhou Province, China (Figure 1). Samples were brought back to the laboratory in plastic bags with the collection details including localities and dates. Samples were incubated at room temperature in ziplock bags or sterile moist plastic boxes for about two weeks. Colonies on decaying wood surface were examined, observed, and photographed for their appearance with stereomicroscopes (SMZ 745, Nikon, Tokyo, Japan) under a Nikon EOS 90D digital camera attached to ECLIPSE Ni compound microscope from low (0.75 times) to high (5 times) magnification.

Fresh colonies were picked with sterile needles at a stereomicroscope magnification of 5 times and placed on a slide with a small amount of distilled water, and then placed under a Nikon EOS 90D digital camera attached to ECLIPSE Ni compound microscope (Nikon, Tokyo, Japan) for microscopic morphological characteristics. The dimensions of conidiophores, conidiogenous cells, and conidia were measured using Tarosoft (R) Image Frame Workprogram. In the species descriptions, arithmetic means as “$\bar{x}$”, and “n” stand for the number of measured elements. Photoplates were processed with Adobe PhotoShop CC 2019 (Adobe Systems, San Jose, CA, USA).

Single spore isolations were performed on water agar (WA) and germinated conidia were aseptically transferred to fresh potato dextrose agar (PDA) following the method of Senanayake et al. [30]. Cultures were grown on PDA and incubated in an incubator at 25 °C for 5 weeks and morphological characters, including color, shape, and size were recorded.
The dried specimens were deposited at the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (HKAS), Kunming, China, and the Herbarium of Guizhou Academy of Agriculture Sciences (GZAAS), Guiyang, China. Cultures were deposited in Guizhou Culture Collection, China (GZCC). Index Fungorum and Faces of Fungi numbers were acquired by the guideline in Jayasiri et al. [31] and Index Fungorum (2022) [32].

2.2. DNA Extraction, PCR Amplification, and Sequencing

Fresh fungal mycelia were scraped with sterilized toothpicks and transferred to 1.5 mL microcentrifuge tubes. Genomic DNA was extracted using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux, Shanghai, China), following the manufacturer’s protocol. The primer pairs of LR0R/LR5, ITS5/ITS4, 983F/2218R, and frpb2-5f/frpb2-7cr were used to amplify the large subunit ribosomal DNA (LSU) [33], the internal transcribed spacer (ITS) [34], the translation elongation factor 1 alpha (tef1α) [35] and the RNA polymerase II second largest subunit (rpb2) gene regions [36], respectively. The amplification reactions were completed in a 50 µL reaction volume, including 2 µL DNA template, 2 µL of each forward and reverse primers, and 44 µL of 1.1 × T3 Supper PCR Mix (Qingke Biotech, Chongqing, China). Amplification reactions were carried out as follows (Table 1).

Table 1. PCR protocols.

| Locus | Primer            | Initial Denaturation | Denaturation | Annealing | Elongation | Final Extension | Hold |
|-------|-------------------|----------------------|--------------|-----------|------------|----------------|------|
| LSU   | ITS5/ITS4         | 94 °C/3 min          | 94 °C/45 s   | 56 °C/50 s| 72 °C/1 min | 72 °C/10 min   |      |
| ITS   | LR0R/LR5          | 94 °C/3 min          | 94 °C/45 s   | 56 °C/50 s| 72 °C/1 min | 72 °C/10 min   |      |
| tef1α | 983F/2218R        | 94 °C/3 min          | 94 °C/30 s   | 56 °C/50 s| 72 °C/1 min | 72 °C/10 min   |      |
| rpb2  | frpb2-5f/frpb2-7cr| 95 °C/5 min          | 95 °C/15 s   | 56 °C/50 s| 72 °C/1 min | 72 °C/10 min   |      |

The quality of PCR amplification products was verified on 1% agarose electrophoresis gels stained with ethidium bromide. Purification and sequencing of PCR products were completed at Beijing Tsingke Biological Engineering Technology and Services Co., Ltd. (Beijing, China).

2.3. Phylogenetic Analyses

Original sequences were checked using BioEdit v 7.0.5.3 [37]. Forward and reverse sequences were assembled using SeqMan v. 7.0.0 (DNASTAR, Madison, WI, USA). The taxa used in this study were selected based on the closest matches from BLASTn search results (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 15 October 2022), and from previous studies (Table 2) [7,12,28]. Sequence alignments for each locus were performed using the online multiple alignment program MAFFT version 7 (https://mafft.cbrc.jp/alignment/server/, accessed on 20 October 2022) and from previous studies (Table 2) [7,12,28]. Sequence alignments for each locus were performed using the online multiple alignment program MAFFT version 7. A phylogenetic tree, which infers a phylogenetic relationship, was reconstructed based on a concatenated LSU, ITS, tef1α, and rpb2 dataset using the online CIPRES Science Gateway (https://www.phylo.org/portal2/home.action, accessed on 29 October 2022) and analyzed using Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian Inference (BI).

The “ALTER” (http://www.sing-group.org/ALTER/, accessed on 29 October 2022) website was used to convert the aligned fasta file to the phylip format for ML analyses [40]. ML analyses were performed through the CIPRES science Gateway V. 3.3 (https://www.phylo.org/portal2/home.action, accessed on 29 October 2022) [41]. The ML analysis was carried out using the RAxML-HPC v.8 on XSEDE (8.2.12) tool using a GTR+GAMMA approximation with rapid bootstrap analysis followed by 1000 bootstrap replicates [42]. Maximum Parsimony (MP) analysis was carried out by using PAUP on XSEDE (4.a168)
online website [43]. The 1000 random taxa were added for a heuristic search to infer MP trees. The value of MaxTrees, which collapsed branches of zero length and saved all multiple parsimonious trees, was set to 5000. Parsimony score values of tree length (TL), consistency index (CI), retention index (RI), and homoplasy index (HI) were calculated for trees generated under different optimum criteria. Clade stability was estimated using a bootstrap analysis with 1000 replicates, and the taxa was added for the random stepwise of each with 10 replicates [44].

The aligned fasta file was converted to the nexus format file for BI analysis by using AliView v. 1.27 [40]. Bayesian Inference (BI) analyses were performed by using MrBayes on XSEDE (3.2.7a) via CIPRES [42]. The best-fit evolutionary model for the individual and combined datasets was determined using MrModeltest v. 2.3. 10 [45]. GTR + G + I substitution model was selected for LSU, ITS, _tef1_, and _rpb2_. The posterior probabilities (PP) were determined based on Bayesian Markov chain Monte Carlo (BMCMC) sampling [46]. Four simultaneous Markov chains were run for 10,000,000 generations, and trees were sampled every 1000th generation (resulting in 10,000 trees). The first 2500 trees, which represented the burn-in phase of the analysis, were discarded. The posterior probabilities (PP) in the majority rule consensus tree were calculated by the remaining 7500 trees.

Phylogenetic trees were visualized with FigTree v. 1.4.4. Adobe Photoshop CC 2019 (Adobe Systems, San Jose, CA, USA) and Adobe Illustrator CC 2019v. 23.1.0 (Adobe Systems, San Jose, CA, USA) were used to edit trees and figures layout. Sequences generated in this study were deposited in GenBank (Table 2).

Table 2. Taxa used in this study and the GenBank accession numbers of DNA sequences.

| Taxon | Strain | GenBank Accessions | Reference |
|-------|--------|--------------------|-----------|
| *Aquapteridospora fusiformis* | MFLU 18-1601<sup>T</sup> | MK849798 MK828652 MN194056 – | Luo et al. (2019) |
| *A. aquatica* | MFLUCC 17-2371<sup>T</sup> | MW287767 MW286493 – – | Dong et al. (2021) |
| *A. bambusinum* | MFLUCC 12-0850<sup>T</sup> | KU863149 KU940161 KU940213 | Dai et al. (2016) |
| *A. bambusinum* | MFLUCC 21-0027 | MZ412536 MZ412514 MZ442688 | Bao et al. (2021) |
| *A. hyalina* | GZCC 22-0072<sup>T</sup> | ON527945 ON527937 ON533681 ON533691 | This study |
| *A. jiangxiensis* | JAUCC 3008<sup>T</sup> | MZ871501 MZ871502 MZ855767 MZ855768 | Peng et al. (2022) |
| *A. lignicola* | MFLU 15-1172<sup>T</sup> | KU221018 MZ868774 MZ892980 MZ892986 | Yang et al. (2015) |
| *Distoseptispora adscendens* | HKUCC 10820 | DQ408561 – – | DQ435092 Sherry et al. (2006) |
| *D. amniculi* | MFLU 21-0138<sup>T</sup> | MZ868761 MZ868770 – | MZ892982 Yang et al. (2021) |
| *D. appendiculata* | MFLUCC 18-0259<sup>T</sup> | MN163023 MN163009 MN174866 – | Luo et al. (2019) |
| *D. aquatigicola* | KUNC 21-10729<sup>T</sup> | ON400845 OK341186 OP413480 OP413474 | Zhang et al. (2022) |
| *D. aquamyces* | KUNC 21-10731<sup>T</sup> | OK341199 OK341187 OP413482 OP413476 | Zhang et al. (2022) |
| *D. aquatica* | MFLUCC 15-0374<sup>T</sup> | KU376268 MF077552 – – | Su et al. (2016) |
| *D. aquisubtropica* | MFLUCC 18-0646 | MK849793 MK828648 – – | Luo et al. (2019) |
| *D. aquisubtropica* | GZCC 22-0075<sup>T</sup> | ON527941 ON527933 ON533677 ON533685 | This study |
| *D. atroviridis* | HKAS 112616<sup>T</sup> | MZ868763 MZ868772 MZ892978 MZ892984 | Yang et al. (2021) |
| *D. bambusae* | MFLUCC 20-0091<sup>T</sup> | MT232718 MT232713 MT232880 MT232881 | Sun et al. (2020) |
| Taxon               | Strain       | GenBank Accessions | Reference                           |
|---------------------|--------------|--------------------|-------------------------------------|
|                     |              | LSU    | ITS    | tef1a  | rpb2    |                     |
| D. bambusae         | MFLUCC 14-0583 | MT232717 | MT232712 | –       | MT232882 | Sun et al. (2020)   |
| D. bambusicola      | GZCC 21-0667T | MZ747872 | MZ747873 | –       | –        | Sheng et al. (2021) |
| D. bangkokensis      | MFLUCC 21-0110T | MZ518206 | MZ518205 | –       | –        | Crous et al. (2019)  |
| D. cangshanensis    | MFLUCC 16-0970T | MG979761 | MG979754 | MG988419 | –        | Luo et al. (2018)  |
| D. caricis          | CPC 36498T     | MN567632 | MN562124 | –       | MN556805 | Crous et al. (2019)  |
| D. caricis          | CPC 36442      | –        | MN562125 | –       | MN556806 | Crous et al. (2019)  |
| D. chinesis         | GZCC 21-0665T | MZ747867 | MZ747871 | MZ501609 | –        | Hyde et al. (2021)  |
| D. clematidis       | MFLUCC 17-2145T | MT214617 | MT310661 | –       | MT394721 | Phukhamsakda et al. (2020) |
| D. crassispora      | KUMCC 21-10725 | OK341196 | OK310698 | OP413479 | OP413473 | Zhang et al. (2022) |
| D. cylindricospora  | HKAS 115796T  | OK513523 | OK491122 | OK524220 | –        | Phukhamsakda et al. (2022) |
| D. dehongensis      | KUMCC 18-0090T | MK079662 | MK085061 | MK087659 | –        | Hyde et al. (2019)  |
| D. effusa           | GZCC 19-0532T | MZ227224 | MW139916 | –       | –        | Yang et al. (2021)  |
| D. eusiptata        | MFLUCC 20-0154T | MW081544 | MW081539 | –       | MW151860 | Li et al. (2021)    |
| D. eusiptata        | MFLUCC 20-0568 | MW081545 | MW081540 | MW084994 | MW084996 | Li et al. (2021)    |
| D. fasciculata      | KUMCC 19-0081T | MW287775 | MW286501 | MW396565 | –        | Dong et al. (2021)  |
| D. fluminicola      | MFLUCC 15-0417T | KU376270 | MF077553 | –       | –        | Su et al. (2016)    |
| D. fusiformis       | HKAS 112617T  | MZ868764 | MZ868773 | MZ892979 | MZ892985 | Yang et al. (2021)  |
| D. guizhouensis     | GZCC 21-0666T | MZ747869 | MZ747868 | MZ501610 | MZ501611 | Hyde et al. (2021)  |
| D. guttulata        | MFLUCC 16-0183T | MF077554 | MF077543 | MF135651 | –        | Yang et al. (2018)  |
| D. guttulata        | DLUCC B43     | MN163016 | MN163011 | –       | –        | Luo et al. (2019)   |
| D. hyalina          | MFLUCC 21-0137T | MZ868760 | MZ868769 | MZ892976 | MZ892981 | Yang et al. (2021)  |
| D. hydei            | MFLUCC 20-0481T | MT742830 | MT734661 | –       | MT767128 | Monkai et al. (2020) |
| D. lancangjiangensis| KUN-HKAS 112712T | MW879522 | MW723055 | –       | MW882260 | Shen et al. (2021)  |
| D. leonensis        | HKUCC 10822  | DQ408566 | –       | –       | DQ435089 | Shenoy et al. (2006) |
| D. lignicola        | MFLUCC 18-0198T | MK849797 | MK826851 | –       | –        | Luo et al. (2019)   |
| D. longispora       | HFJAU 0705T   | MH555357 | MH555359 | –       | –        | Yang et al. (2021)  |
| D. martini          | CGMCC 318651T | KX033566 | KU999975 | –       | –        | Xia et al. (2017)   |
| D. meilingensis     | JAUCC 4272T   | OK562396 | OK562390 | OK562408 | –        | Zhai et al. (2022)  |
| D. multiseptata     | MFLUCC 16-1044 | MF077555 | MF077544 | MF135652 | MF135644 | Yang et al. (2018)  |
| D. multiseptata     | MFLUCC 15-0609T | KX710140 | KX710145 | MF135659 | –        | Hyde et al. (2016)  |
| D. nonrostrata      | KUNCC 21-10730T | OK341198 | OK310699 | OP413481 | OP413475 | Zhang et al. (2022) |
| D. obclavata        | MFLUCC 18-0329T | MN163010 | MN163012 | –       | –        | Luo et al. (2019)   |
| Taxon                     | Strain       | GenBank Accessions                      | Reference                          |
|--------------------------|--------------|-----------------------------------------|------------------------------------|
|                          |              | LSU | ITS    | tef1a | rpb2   |                                |
| *D. obpyriformis*        | MFLUCC 17-01694<sup>T</sup> | MG979764 | – | MG988422 | MG988415 | Luo et al. (2018) |
| *D. obpyriformis*        | DLUCC 0867               | OK341194 | OK310696 | OP413477 | OP413471 | Zhang et al. (2022) |
| *D. pachyconidia*       | MFLUCC 16-0857<sup>T</sup> | MF077556 | MF077545 | MF135653 | –         | Yang et al. (2018) |
| *D. pachyconidia*       | MFLUCC 18-0415<sup>T</sup> | MH457138 | MH457172 | MH463253 | MH463255 | Hyde et al. (2020) |
| *D. pachyconidia*       | MFLUCC 18-0417<sup>T</sup> | MH457138 | MH457173 | MH463254 | MH463256 | Hyde et al. (2020) |
| *D. palmarum*           | MFLUCC 18-1446<sup>T</sup> | MK079663 | MK085062 | MK087660 | MK087670 | Hyde et al. (2019) |
| *D. phangngaensis*       | MFLUCC 16-0857<sup>T</sup> | MG979766 | MG979758 | MG988424 | MG988417 | Luo et al. (2018) |
| *D. rayongensis*         | MFLUCC 18-1238<sup>T</sup> | MW287780 | MW286506 | MW396651 | MW504069 | Dong et al. (2021) |
| *D. saprophytica*        | MFLUCC 18-1238<sup>T</sup> | MW287755 | MW286482 | MW396642 | –         | Dong et al. (2021) |
| *D. septata*             | MFLUCC 18-1234<sup>T</sup> | MW287755 | MW286482 | MW396642 | –         | Dong et al. (2021) |
| *D. suoluoensis*         | MFLUCC 18-0415<sup>T</sup> | MG979768 | MG979760 | MG988426 | MG988418 | Luo et al. (2018) |
| *D. suoluoensis*         | MFLUCC 17-0224<sup>T</sup> | MF077557 | MF077546 | MF135654 | –         | Yang et al. (2018) |
| *D. suoluoensis*         | MFLUCC 17-1305<sup>T</sup> | MF077558 | MF077547 | –         | –         | Yang et al. (2018) |
| *D. tectonae*            | MFLUCC 15-0981<sup>T</sup> | KX751713 | KX751711 | KX751710 | KX751708 | Hyde et al. (2016) |
| *D. tectonae*            | MFLUCC 12-0292<sup>T</sup> | MT232719 | MT232714 | –         | –         | Sun et al. (2020) |
| *D. tectonae*            | MFLUCC 15-0262<sup>T</sup> | MW287763 | MW286489 | MW396641 | –         | Dong et al. (2021) |
| *D. tectonigena*         | MFLUCC 15-0981<sup>T</sup> | KX751714 | KX751712 | –         | KX751709 | Hyde et al. (2016) |
| *D. thailandica*         | KUMCC 18-0182<sup>T</sup> | MH260292 | MH275060 | MH412767 | –         | Tibpromma et al. (2018) |
| *D. thysanolaena*        | KUMCC 18-0182<sup>T</sup> | MK064091 | MK045851 | MK086031 | –         | Phukhamsak et al. (2019) |
| *D. tropica*             | GZCC 22-0076<sup>T</sup> | ON527943 | ON527935 | ON533679 | ON533687 | This study |
| *D. verrucosa*           | HKAS 112652<sup>T</sup> | MZ868762 | MZ868771 | MZ892977 | MZ892983 | Yang et al. (2021) |
| *D. wuzhishanensis*      | GZCC 22-0077<sup>T</sup> | ON527946 | ON527938 | ON533682 | –         | This study |
| *D. xishuangbananensis*  | GZCC 22-0077<sup>T</sup> | MH260293 | MH275061 | MH412768 | MH412754 | Tibpromma et al. (2018) |
| *D. xishuangbananensis*  | GZCC 22-0079<sup>T</sup> | ON527944 | ON527936 | ON533680 | ON533688 | This study |
| *D. yongxiuensis*        | JAUCC 4725<sup>T</sup> | OK562394 | OK562388 | OK562406 | –         | Zhai et al. (2022) |
| *D. yunnanensis*         | JAUCC 4727<sup>T</sup> | OK562398 | OK562392 | OK562410 | –         | Zhai et al. (2022) |
| *Myrmecridium aquaticum* | MFLUCC 15-0366<sup>T</sup> | MK349804 | –         | –         | –         | Luo et al. (2019) |
Table 2. Cont.

| Taxon                              | Strain      | GenBank Accessions                  | Reference                          |
|------------------------------------|-------------|-------------------------------------|------------------------------------|
|                                    |             | LSU - ITS - tef1a - rpb2            |                                    |
| *M. aquaticum*                     | S 1158      | MK849803 - MK828656 - MN194061 - MN124540 | Luo et al. (2019)                  |
| *M. banksiae*                      | CBS 132536T | JX069855 - JX069871 - – - –         | Crous et al. (2012)                |
| *M. montsegurinum*                 | JF 13180T   | KT991664 - KT991674 - – - KT991654  | Rebllová et al. (2016)             |
| *M. schulzeri*                     | CBS 100.54  | EU041826 - EU041769 - – - –         | Arzanlou et al. (2007)             |
| *Pseudostanjehughesia aquitropica* | MFLUCC 16-0569T | MF077559 - MF077548 - MF135655 - – | Yang et al. (2018)                 |
| *Ps. lignicola*                    | MFLUCC 15-0352T | MK849787 - MK828643 - MN194047 - MN124534 | Luo et al. (2019)                  |
| *Sporidesmium bambusicola*         | HKUCC 3578T | DQ408562 - - - - -                   | Shenoy et al. (2006)               |
| *S. dulongense*                    | MFLUCC 17-0116T | MH795817 - MH795812 - MH801191 - MH801190 | Luo et al. (2019)                  |
| *S. lageniforme*                   | DLUCC 0880T | MK849782 - MK828640 - MN194044 - MN124533 | Luo et al. (2019)                  |
| *S. pyriformatum*                  | MFLUCC 15-0620T | KX710141 - KX710146 - MF135662 - MF135649 | Hyde et al. (2016)                |
| *S. thailandense*                  | MFLUCC 15-0617 | MF077561 - MF077550 - MF135657 - – | Yang et al. (2018)                 |
| *S. thailandense*                  | MFLUCC 15-0964T | MF374370 - MF374361 - MF370957 - MF370955 | Zhang et al. (2017)               |

Note: T denote ex-type strain. Newly generated sequences are indicated in bold. “–” means no data available in GenBank.

3. Phylogenetic Results

Using partial nucleotide sequences from four genes, the phylogenetic placements of our new collections were determined. The concatenated sequence matrix comprised LSU (1–855 bp), ITS (856–1448 bp), tef1a (1449–2367 bp), and rpb2 (2368–3420 bp) with a total of 3420 characters for 93 taxa and two outgroups (*Pseudostanjehughesia aquitropica* MFLUCC 16-0569 and *Ps. lignicola* MFLUCC 15-0352) [7,12,16,47–50]. Four gene analyses were conducted to compare the respective topologies and clade stabilities. There were 1733 distinct alignment patterns in the matrix, along with 31.09% undetermined characters or gaps. The ML, MP, and BI analyses of the concatenated LSU, ITS, tef1a, and rpb2 dataset yielded similar tree topologies, and Figure 2 depicts the final likelihood value of the ML analysis.

The phylogenetic tree (Figure 2) demonstrates that our eight collections represent seven species within *Distoseptisporales*. Two isolates (GZCC 22-0072 and GZCC 22-0073) represent the new species *Aquapteridospora hyalina* which forms a distinct clade from other taxa of *Aquapteridospora* with strong support. *Distoseptispora aquisubtropica* and *D. martini* are distinguished by their distinct conidial characteristics. *Distoseptispora septata* forms a sister lineage to *D. guizhouensis* with well support (96% ML/90% MP/1 PP). *Distoseptispora tropica* (GZCC 22-0076) is a significantly distinct lineage from other taxa (Figure 2). *Distoseptispora wuzhishanensis* forms a sister lineage to *D. fasciculata* with well support (97% ML/1 PP). Our isolate GZCC 22-0079 is recognized as *D. xishuangbannaensis* and GZCC 22-0074 as *D. pachyconidia*, and their molecular data are provided, respectively.
Figure 2. Phylogenetic tree generated from maximum likelihood (ML) analysis based on a combined of LSU, ITS, tef1α, and rpb2 sequence data. Bootstrap support values of maximum likelihood (ML) and Maximum Parsimony (MP) equal to or greater than 75%, and Bayesian posterior probabilities (PP) equal to or greater than 0.95 are given near the nodes as ML/MP/PP. T. Pseudostanjehughesia aquitropica (MFLUCC 16-0569) and P. lignicola (MFLUCC 15-0352) were used as outgroup taxa. The newly generated sequences are indicated in red bold.
4. Taxonomy

*Aquapteridospora hyalina* J. Ma and Y.Z. Lu., sp. nov., Figure 3.

Index Fungorum number: IF559932; Facesoffungi number: FoF11002.

Etymology: The epithet 'hyalina' referring to the colourless conidia.

Holotype: HKAS 123764.

*Saprobic* on decaying wood in a freshwater habitat. **Sexual morph:** Undetermined. **Asexual morph:** Colonies on the natural substrate, effuse, solitary, hyaline. *Mycelium* mostly superficial, consisting of branched, septate, smooth, pale brown to brown hyphae. *Conidiophores* 68–130 × 4.5–6.5 μm (μ = 91 × 5.5 μm, n = 30), macronematous, mononematous, solitary, erect, simple, straight, or slightly flexuous, unbranched, smooth, cylindrical, 3–7-septate, thick-walled, smooth-walled, brown at the base, subhyaline to pale brown towards apex. *Conidiogenous cells* 25–62 × 4–6.5 μm (μ = 44 × 5 μm, n = 30), polyblastic, monoblastic, smooth-walled, terminal, sub-cylindrical or gradually tapering towards tip, sub-hyaline to pale brown, forming conidia sympodially on conspicuous denticles, bearing tiny, protuberant, circular scars. *Conidia* 17–28 × 4–6 μm (μ = 20 × 5.5 μm, n = 50) acropleurogenous, solitary, fusiform, 1–3-septate, truncate obtuse at septum, hyaline when young, sub-hyaline to pale brown when mature, tapering and pointed at both ends, smooth-walled, often with single guttulate in each cell when young.

Culture characteristics: Conidia were germinated on water agar and produced germ tubes within 10 h. Colonies grown on PDA, circular, flat, reaching 18 mm diam. after 25 days of incubation at 25 °C, lightly gray at the entire margin, brown in the center, reverse-side pale brown or brown at the entire margin, pale grey in the center.

Material examined: China, Hainan Province, Baoting County, Sandao Town, Yanoda Rainforest cultural tourism area, 18°46′N, 109°65′E, on rotting wood in a freshwater stream, 23 October 2021, Jian Ma, Y6 (HKAS 123764, holotype; GZAAS 22-0077, isotype), ex-type living culture GZCC 22-0072; idem, Y10-1 (HKAS 123763), living culture GZCC 22-0073.

Notes: The proposed new species *Aquapteridospora hyalina* shares similar characteristics with other species of the genus *Aquapteridospora* in having macronematous, mononematous, unbranched, polyblastic conidiophores, sympodial proliferations conidiogenous cells, and fusiform and acropleurogenous conidia [4]. However, *A. hyalina* differs from all species of *Aquapteridospora* in having hyaline to pale brown conidia with distinguished guttulae in each cell when young. The phylogenetic analyses confirmed that the two strains (GZCC 22-0072 and GZCC 22-0073) of *A. hyalina* form a distinct clade sister to *A. fusiformis* within *Aquapteridosporaceae* (Figure 2). Therefore, based on the morphological evidence with multi-gene phylogenetic results, a new species *A. hyalina* is introduced.
Figure 3. *Aquapteridospora hyalina* (HKAS 123764, holotype). (a,b) Colonies on the host surface. (c–e) Conidiophores, conidiogenous cells with attached conidia. (f–h) Conidiogenous cells bearing conidia. (i–k) Conidia. (l) Germinated conidium. (m,n) Colonies on PDA, m from above, n from below. Scale bars: (e–l) 20 μm, (c,d) 10 μm.
Distoseptispora aquisubtropica J. Ma and Y.Z. Lu, sp. nov., Figure 4.

Index Fungorum number: IF559686; Facesoffungi number: FoF11334.

Etymology: ‘aquisubtropica’ is derived from subtropical, which means climate type and ‘aqui’ refers to its presence in aquatic habitat.

Holotype: HKAS 124023.

Saprobic on decaying wood submerged in a freshwater habitat. Sexual morph: Undetermined. Asexual morph: hyphomycetous. Colonies on natural substrate superficial, effuse, gregarious, smooth, septate, hairy, brown, or dark brown. Mycelium mostly immersed, composed of branched, septate, smooth, pale brown to brown hyphae. Conidiophores 16–83 × 5–11 µm (x = 51 × 8 µm, n = 25), macronematous, mononematous, cylindrical, erect, simple, straight, or slightly flexuous, unbranched, smooth, thick-walled, solitary, brown at the base, pale brown or sub-hyaline towards the apex, 2–5-septate. Conidiogenous cells 3–11 × 3–7 µm (x = 6 × 5.5 µm, n = 25), holoblastic, monoblastic, terminal, integrated, cylindrical, pale brown or brown, smooth. Conidia 43–278 × 11–19 µm (x = 141 × 14.5 µm, n = 38), acrogenous, solitary, multi-distoseptate, obclavate or lanceolate, rostrate, straight or slightly curved, verrucose, guttulate, thick-walled, smooth-walled, pale brown or dark brown, olivaceous, 16–31-distoseptate, usually paler towards apex, sometimes have conspicuous hyphae attached to the conidium, rounded at apex, with a truncate base.

Culture characteristics: Conidia were germinated on water agar and produced germ tubes within 10 h. Colonies grown on PDA, circular, flat, dense, dark gray, fluffy, reaching 34 mm diam. after 25 days of incubation at 25 °C, break in the center, from below olivaceous to brown at the center, pale yellow at the entire margin.

Material examined: China, Guizhou Province, Zhenyuan County, 27°05′ N, 108°41′ E, on decaying wood submerged from a freshwater stream, 1 May 2021, Jian Ma, XXJ11-3 (HKAS 124023, holotype; GZAAS 22-0080, isotype), ex-type living culture GZCC 22-0075.

Notes: According to a BLASTn search on NCBI GenBank, the ITS and LSU sequences of the new isolate (Distoseptispora aquisubtropica) share 92.95% similarity across 85% of the query sequence coverage and 99.6% similarity across 86% of the query sequence coverage with D. martini, respectively. The phylogenetic results indicate that D. aquisubtropica forms a sister clade to D. martini with well-supported values (100% ML/75% MP/1 PP). The morphological features of D. aquisubtropica are compatible with the Distoseptispora generic concept, while D. martini resembles Acrodictys rather than Distoseptospora [13]. Distoseptispora aquisubtropica can be distinguished from D. martini by its obclavate or lanceolate conidia, while the latter is oblate or subglobose [13]. Based on a pairwise comparison of ITS and LSU nucleotides, D. aquisubtropica differs from D. martini in 24/453 bp (5.3%) for ITS and 5/515 bp (0.97%) for LSU, respectively. Therefore, we identified D. aquisubtropica as a novel species, according to the species delimitation guidelines proposed by Chethana et al. and Maharachchikumbura et al. [51,52].
Figure 4. *Distoseptispora aquisubtropica* (GZAAS 22-0080, holotype). (a,b) Colonies on the host surface. (c–g) Conidiophores and conidiogenous cells bearing conidia. (h–k) Conidia. (l) Germinating conidium. (m,n) Colonies on PDA, m from above, n from below. Scale bars: (c–l) 20 μm.
Distoseptispora pachyconidia R. Zhu and H. Zhang. Journal of Fungi 30: 22 (2022). Figure 5.

Index Fungorum Number: IF559924; Facesoffungi number: FoF12581.

Saprobic on decaying wood in terrestrial habitats. Sexual morph: Undetermined.

Asexual morph: Colonies effuse, gregarious, brown, or dark brown, hairy. Mycelium immersed and partly superficial, consisting of branched, septate, smooth, pale brown to brown hyphae. Conidiophores 14–44 × 4–7 μm (x = 26 × 5 μm, n = 20), macronematous, mononematous, brown to dark brown, solitary, 2–4-septate, erect, straight, or flexuous, unbranched, smooth, cylindrical, singly or in groups, truncate at the apex, slightly constricted at septa. Conidiogenous cells 4–9 × 4–5.5 μm (x = 6 × 4.5 μm, n = 20), holoblastic, monoblastic, integrated, terminal, determinate, pale brown to brown, smooth, cylindrical. Conidia 50–242 × 11–20 μm (x = 111 × 13 μm, n = 30) acrogenous, solitary, obclavate, rostrate, smooth-walled, straight or slightly curved, up to 38-distoseptate, guttulate, olivaceous to dark brown, mostly slightly constricted at septa, tapering towards the rounded apex, truncate at the base.

Culture characteristics: Conidia were germinated on water agar and produced germ tubes within 10 h. Colonies grown on PDA, circular, mycelium flat, dense, reaching 40 mm diam. after 30 days of incubation at 25 °C, gray or brown, reverse-side dark brown.

Material examined: China, Hainan Province, Haikou City, Xiuying District, Ecological leisure trail, on decaying wood on the ground, 20°01′ N, 110°25′ E, 10 August 2021, Jian Ma, HK2 (HKAS 123754), living culture GZCC 22-0074.

Notes: Zhang et al. [16] introduced Distoseptispora pachyconidia from decaying wood in Yunnan Province, China. Analyses of multi-gene revealed that the new isolate GZCC 22-0074 clustered with D. pachyconidia. The morphological characteristics of this isolate are similar to the protologue of D. pachyconidia. However, GZCC 22-0074 differs from D. pachyconidia in having different colors of conidia (olivaceous to dark brown vs. pale-brown with a green tinge) and the number of conidial septa (up to 38 vs. 8–21-distoseptate) [16]. According to a pairwise nucleotide comparison of ITS, LSU, tef1α and rpb2, our isolate differs from the type strain of D. pachyconidia (HKAS 122179) in 1/520 bp (0.2%) for ITS, 1/852 bp (0.1%) for LSU, 1/913 bp (0.1%) for tef1α and 0/1052 bp (0%) for rpb2, respectively. Thus, the phylogenetic evidence did not show significant differences between them (Figure 2). We therefore identified the new isolate as D. pachyconidia.
Figure 5. *Distoseptispora pachyconidia* (HKAS 123754). (a,b) Colonies on the host surface. (c–f) Conidiophores and conidiogenous cells bearing conidia. (g–l) Conidia. (m) Germinating conidium. (n,o) Colonies on PDA, n from above, o from reverse. Scale bars: (c,e–h,j–m) 20 μm, (d,i) 10 μm.

*Distoseptispora septata* J. Ma and Y.Z. Lu, sp. nov., Figure 6. Index Fungorum number: IF559688; Facesoffungi number: FoF11337. Etymology: 'septata' referring to 'septate' conidia.
Distoseptispora septata J. Ma and Y.Z. Lu, sp. nov., Figure 6.

Index Fungorum number: IF559688; Facesoffungi number: FoF11337.

Etymology: ‘septata’ referring to ‘septate’ conidia.

Holotype: HKAS 123759.

Saprobic on decaying wood submerged in a freshwater habitat. Sexual morph: Undetermined. Asexual morph: hyphomycetous. Colonies on natural substrate superficial, effuse, gregarious, smooth, septate, hairy, brown, or dark brown. Mycelium mostly immersed, composed of branched, septate, smooth, pale brown to brown hyphae. Conidiophores 23–86 × 3–7 µm (± 44 × 5.5 µm, n = 30), macronematous, mononematous, cylindrical, erect, simple, mostly flexuous, unbranched, smooth, thick-walled, solitary, brown, 1–6-septate. Conidiogenous cells 5–18 × 4–6 µm (± 9 × 5 µm, n = 30), holoblastic, monoblastic, terminal, integrated, cylindrical, pale brown or brown, smooth. Conidia 22–179 × 10–16 µm (± 89 × 13 µm, n = 35), acrogenous, solitary, multi-distoseptate and up to 25-septate, obclavate, rostrate, straight or slightly curved, verrucose, guttulate, thick-walled, smooth-walled, pale brown or dark brown, olivaceous-green, usually paler towards apex, rounded at apex, with a truncate base.

Culture characteristics: Conidia were germinated on water agar and produced germ tubes within 10 h. Colonies grown on PDA, circular, flat, dense, fluffy, reaching 35 mm diam. after 25 days of incubation at 25 °C, olivaceous-green, paler brown and dark brown, reverse-side dark brown.

Material examined: China, Hainan Province, Wuzhishan City, Shui Man Town, Wuzhis- han National Nature Reserve, 18°92′ N, 109°63′ E, on dead wood in a freshwater stream, 26 December 2021, Xia Tang, W17 (HKAS 123759, holotype; GZAAS 22-0083, isotype), ex-type living culture GZCC 22-0078.

Notes: Distoseptispora septata shares a sister relationship to D. guizhouensis with strong support (96% ML/90% MP/1 PP). However, D. septata differs from D. guizhouensis in having smaller conidia (22–179 × 10–16 µm vs. 90–273 × 15–21 µm). Particularly, D. guizhouensis is distinguished from D. septata by distinctly flexuous conidiophores. Based on a pairwise comparison of ITS nucleotides, D. septata differs from D. guizhouensis by 13/535 bp (2.4%). Following the guidelines for defining species boundaries of Chethana et al. and Pem et al. [51,53], we therefore introduce GZCC 22-0078 as a new species.
Figure 6. *Distoseptispora septata* (HKAS 123759, holotype). (a,b) Colonies on the host surface. (c–g) Conidiophores and conidiogenous cells bearing conidia. (h–j) Conidia. (k,l) Conidiophores and conidiogenous cells. (m) Germinating conidium. (n,o) Colonies on PDA, n from above, o from reverse. Scale bars: (c–m) 20 μm.
Distoseptispora tropica J. Ma and Y.Z. Lu, sp. nov., Figure 7.
Index Fungorum number: IF559689; Facesoffungi number: FoF11335.
Etymology: ‘tropica’ derived from the climate in which the species was discovered.
Holotype: HKAS 123761.
Saprobic on dead wood, in terrestrial habitat. Sexual morph: Undetermined. Asexual morph: hyphomycetous. Colonies on dead wood, effuse, scattered or in small groups, gregarious, hairy, smooth, brown, or dark brown. Mycelium mostly immersed, composed of branched, septate, smooth, brown to dark brown. Conidiophores 60–151 × 3.5–7 µm (µ = 94 × 5 µm, n = 20), macronematous, mononematous, cylindrical, erect, straight, or slightly flexuous, unbranched, smooth, thick-walled, dark brown, solitary or caespitose, dark brown and rounded at the apex, paler brown at the upper part, 5–7 septate. Conidigenous cells 10–21 × 3.5–6 µm (µ = 159 × 5 µm, n = 20), holoblastic, monoblastic, terminal, integrated, cylindrical, pale brown or brown, smooth. Conidia 39–75 × 7.5–10.5 µm (µ = 47 × 8 µm, n = 30), acrogenous, verrucose, solitary, multi-distoseptate, obclavate, rostrate, upper part tapering towards the apex, guttulate, thick-walled, smooth, olivaceous brown or dark brown, 5–7 distoseptate, with conspicuous hyphae attachment conidium, with a truncate base.
Culture characteristics: Conidia were germinated on water agar and produced germ tubes within 10 h. Colonies grown on PDA, circular, flat, dense, fluffy, reaching 40 mm diam. after 40 days of incubation at 25 °C, grayish brown mycelium on the surface, reverse-side dark brown.
Material examined: China, Hainan Province, Haikou City, Xiuying District, Ecological leisure trail, 20°01′ N, 110°25′ E, on decaying wood in terrestrial habitat, 10 August 2021, Jian Ma, HK9 (HKAS 123761, holotype; GZAAS 22-0081, isotype), ex-type living culture GZCC 22-0076.
Notes: Distoseptispora tropica shares an extremely similar morphology with the species of Distoseptispora, Ellisembia, and Sporidesmium [7,9,54]. However, phylogenetic analysis (Figure 2) indicates that D. tropica belongs to Distoseptispora. Distoseptispora tropica forms a distinct lineage basal to Clade 1 with strong support (100% ML/100% MP/1 PP). However, D. tropica differs from other taxa in Clade 1 in having longer conidiophores and smaller conidia.
Figure 7. Distoseptispora tropica (HKAS 123761, holotype). (a,b) Colonies on the host surface. (c) Conidiophores and conidiogenous cells. (d,e) Conidiophores with attached conidia. (f–h) Conidia. (i) Conidiogenous cell. (j) Germinating conidium. (k,l) Colonies on PDA, k from above, l from reverse. Scale bars: (c–e) 20 μm, (f–j) 10 μm.
Distoseptispora wuzhishanensis J. Ma and Y.Z. Lu, sp. nov., Figure 8.

Index Fungorum number: IF559706; Facesoffungi number: FoF11336.

Etymology: ‘wuzhishanensis’ derived from the city where the species was discovered.

Holotype: HKAS 123762.

Saprobic on decaying wood in a freshwater habitat. **Sexual morph**: Undetermined. **Asexual morph**: hyphomycetous. Colonies on substrate superficial, effuse, solitary, smooth, septate, hairy, brown, or dark brown. Mycelium mostly immersed, composed of branched, septate, smooth, pale brown to brown hyphae. Conidiophores 16–56 × 5–7 µm (μ = 35 × 6 µm, n = 25), macronematous, mononematous, cylindrical, erect, scattered or in small groups, straight or slightly flexuous, unbranched, smooth, thick-walled, brown at the base, pale brown towards the apex, 1–4 septate. Conidiogenous cells 6.5–10.5 × 4.5–6 µm (μ = 8 × 5 µm, n = 25), holoblastic, monoblastic, terminal, integrated, cylindrical, pale brown or brown, smooth. Conidia 76–143 × 11–17 µm (μ = 108 × 13.5 µm, n = 40), acrogenous, solitary, multi-distoseptate, obclavate, rostrate, straight, or slightly curved, verrucose, guttulate, thick-walled, smooth-walled, pale brown or dark brown, olivaceous-green and yellow, usually up to 22-distoseptate, strongly constricted at septa, usually paler towards apex, rounded at apex, with a truncate base.

Culture characteristics: Conidia were germinated on water agar and produced germ tubes within 10 h. Colonies grown on PDA, circular, flat, dense, fluffy, reaching 24 mm diam. after 25 days of incubation at 25 °C, olivaceous-green or brown mycelium on the surface, reverse side partly paler brown to dark brown.

Material examined: China, Hainan Province, Wuzhishan City, Shui Man Town, Wuzhishan National Nature Reserve, 18°92’ N, 109°63’ E, on dead wood in a freshwater stream, 26 December 2021, Xia Tang, W21 (HKAS 123762, holotype; GZAAS 22-0082, isotype), ex-type living culture GZCC 22-0077.

Notes: According to phylogenetic tree (Figure 2), Distoseptispora wuzhishanensis forms a close lineage to D. fasciculata with high bootstrap support (99% ML/0.99 PP). The morphological characteristics of the new taxon match well with the species concept of Distoseptispora in conidiophores and conidia. However, D. wuzhishanensis differs from D. fasciculata in having longer conidiophores (16–56 × 5–7 µm vs. 12–16 × 5–6 µm) [3].
Figure 8. *Distoseptispora wuzhishanensis* (HKAS 123762, holotype). (a,b) Colonies on the host surface. (c–e) Conidiophores and conidia. (f–i) Conidia. (j–m) Conidiophores and conidiogenous cells. (n) Germinating conidium. (o,p) Colonies on PDA, o from above, p from reverse. Scale bars: (c–j,n) 20 μm, (k–m) 10 μm.
Distoseptispora xishuangbannaensis Tibpromma and K.D. Hyde. Fungal Diversity 93: 82 (2018). Figure 9.

Index Fungorum Number: IF554554; Facesoffungi number: FoF04563.

Saprobic on decaying wood, submerged in freshwater habitats. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. Colonies effuse, hairy, gregarious, olivaceous, or brown. Mycelium mostly immersed, composed of branched, smooth, septate, pale brown to brown hyphae. Conidiophores 19–52 × 5–7 µm (\( \bar{x} = 29 \times 6 \mu m \), \( n = 20 \)), macronematous, mononematous, unbranched, solitary, straight or slightly flexuous, brown at the base, pale brown towards the apex, slightly tapering distally, truncate at the apex, 1–4 septate. Conidiogenous cells 2–8.5 × 4.0–7 µm (\( \bar{x} = 6 \times 5 \mu m \), \( n = 20 \)), blastic, holoblastic, monoblastic, terminal, integrated, cylindrical, pale brown. Conidia 88–269 × 11–15 µm (\( \bar{x} = 165 \times 13 \mu m \), \( n = 30 \)), solitary, acrogenous, cylindrical-obclavate, rostrate, straight, or slightly curved, guttulate, thick-walled, smooth-walled, green-brown or brown, up to 42-distoseptate, usually paler olivaceous towards apex, rounded at apex, with a truncate base.

Culture characteristics: Conidia were germinated on water agar and produced germ tubes within 10 h. Colonies grown on PDA, circular, fluffy, reaching 36 mm diam. after 28 days of incubation at 25 °C, lightly grey in the center, olivaceous at the entire margin, reverse side pale brown and brown mycelium.

Material examined: China, Hainan Province, Lingshui Lizu Autonomous County, Diolouoshan National Nature Reserve, 18°43′N, 109°43′E, on rotting wood in a freshwater habitat, 24 August 2021, Jian Ma, DL40 (HKAS 123760), living culture GZCC 22-0079.

Notes: Tibpromma et al. [20] introduced *Distoseptispora xishuangbannaensis* from dead leaf sheaths of *Pandanus utilis* in China. Analyses of multiple genes revealed that our new isolate (GZCC 22-0079) is closely related to *D. xishuangbannaensis*. *Distoseptispora xishuangbannaensis* possesses macronematous, straight or slightly flexuous conidiophores, holoblastic, monoblastic, brown conidiogenous cells, and acrogenous, cylindrical-obclavate conidia. The morphological characteristics of our isolate share similar characters with the holotype of *D. xishuangbannaensis* (HKAS 101809), but our isolate has longer and wider conidiophores (19–52 × 5–7 µm vs. 12–17 × 2–5 µm) and smaller conidia (88–269 × 11–15 µm vs. 160–305 × 8–15 µm) [20]. According to a pairwise nucleotide comparison of ITS, tef1α and rpb2, our new isolate differs from *D. xishuangbannaensis* in 5/498 bp (1%) for ITS, 3/898 bp (0.3%) for tef1α and 2/1052 bp (0.2%) for rpb2, respectively, and the phylogenetic result did not show significant differences between the new isolate and *D. xishuangbannaensis* (Figure 2). We therefore identified the new isolate as *D. xishuangbannaensis*. 
Figure 9. Distoseptispora xishuangbannaensis (HKAS 123760). (a,b) Colonies on the host surface. (c–h) Conidiophores, conidiogenous cells with attached conidia. (i) Germinating conidium. (j,k) Conidiophores and conidiogenous cells. (l,m) Colonies on PDA, l from above, m from below. Scale bars: (c,k,e–i) 20 μm, (d,j) 10 μm.
5. Discussion

In recent years, studies on the number of saprotrophic fungi have received extensive attention [55,56]. For example, freshwater fungi occur in streams and other aquatic bodies [2,57], Calabon et al. [51] listed 3870 species, and numerous taxa are still being discovered [1]. Calabon et al. [51] listed 22 Distoseptispora species from freshwater and this study brings the total to 40 species. Analyses of morphological characteristics and molecular data indicate that eight collections, including five new species, namely A. hyalina, D. aquisubtropica, D. septata, D. tropica, and D. wuzhishanensis are introduced. Five Aquapteridospora epithets (A. lignicola, A. fusiformis, A. bambusinum, A. jiangxiensis, and A. aquatica) are listed in Index Fungorum [32,58]. Morphologically, A. hyalina corresponds well with the generic concept of Aquapteridospora [4,6,58]. However, the new isolate differs from other new species of genus Aquapteridospora by having conidia that have a truncate obtuse at their septum. Multi-gene analyses indicated that A. hyalina, a species phylogenetically distinct in the genus Aquapteridospora, was most closely related to A. fusiformis with weak support.

Morphologically, the asexual morph of Distoseptispora resembles Sporidesmium taxa [9]. Yang et al. [7] reported the existence of the sexual morph of Distoseptispora. Combining the morphological characteristics and the phylogenetic evidence of all species in genus Distoseptispora, we found that some Distoseptispora species form sister clades in phylogeny but have different morphologies. For example, D. martini and D. aquisubtropica have a close phylogenetic relationship. However, D. martini has distinctive oblate or subglobose conidia, while D. aquisubtropica has obclavate or lanceolate conidia. In addition, there are some species with similar morphologies but are genetically unrelated. For example, D. tropica and D. verrocosa have an identical morphology of conidiophores and conidia, but the two species have a distant phylogenetic relationship. Considering this phenomenon, additional molecular data and morphological characteristics are required for verification and expansion.

Previous studies of Distoseptispora have primarily been conducted in China (Guizhou, Yunnan, Sichuan, and Jiangxi Provinces) and in Thailand, which are subtropical and tropical regions. In this paper, eight collections were discovered in freshwater and terrestrial habitats in the Provinces of Hainan and Guizhou, China. Thus, it is unclear whether it has a close relationship with the climate. It may be a result of the limited geographical regions sampled.

Author Contributions: The specimens in this study were collected by J.M., X.-J.X. and Y.-Z.L. Morphological data were collected by J.M. and X.-J.X. Molecular data and phylogenetic analyses were performed by J.M. and X.-J.X. Writing the original draft, and review and editing were completed by J.M., J.-Y.Z., X.-J.X., Y.-P.X., X.T., S.B., J.-C.K. and Y.-Z.L. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the Science and Technology Foundation of Guizhou Province ([2020]1Y058), the National Natural Science Foundation of China (NSFC 31900020), China Post-doctoral Science Foundation (2020M683657XB), Youth Science and Technology Talent Development Project from Guizhou Provincial Department of Education (QJHKYZ[2021]263) and Guizhou Province high-level talent innovation and entrepreneurship merit funding project (No. 202104).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All sequences generated in this study were submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank/, accessed on 16 May 2022).

Acknowledgments: We would like to thank Shaun Pennycook (Manaaki Whenua Landcare Research, New Zealand) for advising on fungal nomenclature. Jian Ma would like to thanks Na Wu, Xing-Juan Xiao, Meng-Lan Chen, Xue-Mei Chen, Chuan-Gen Lin, Yao Feng, Ya-ya Chen, Hong-zhi Du their valuable suggestions and help. We thank Abhaya Balasuriya under the Reinventing Visiting Professor Program 2022, Mae Fah Luang University for his valuable correction and suggestion.

Conflicts of Interest: The authors declare no conflict of interest.
26. Phukhamsakda, C.; McKenzie, E.H.C.; Phillips, A.; Jones, E.B.G.; Bhat, D.J.; stadler, M.; Bjunjun, C.S.; Wanasinghe, D.N.; Thongbai, B.; Camporesi, E.; et al. Microfungi associated with Cematis (Ranunculaceae) with an integrated approach to delimiting species boundaries. *Fungal Divers. 2020, 102*, 1–203. [CrossRef]

27. Phukhamsakda, C.; Nilsson, R.H.; Bjunjun, C.S.; Farias, A.R.G.D.; Sun, Y.; Wijesinghe, S.N.; Raza, M.; Bao, D.F.; Lu, L.; Tibpromma, S.; et al. The numbers of fungi: Contributions from traditional taxonomic studies and challenges of metabarcoding. *Fungal Divers. 2022, 114*, 327–386. [CrossRef]

28. Liu, Y.J.; Whelen, S.; Hall, B.D. Phylogenetic relationships among ascomycetes: Evidence from an RNA polymerse II subunit. *MycoKeys 2022*, 88, 35–54. [CrossRef]

29. Crous, P.; Wingfield, M.; Lombard, L.; Roets, F.; Swart, W.; Alvarado, P.; Carnegie, A.; Moreno, G.; Luangsa-Ard, J.; Thangavel, R.; et al. Fungal Plant description sheets: 951–1041. *Pers. Mol. Phylogeny Evol. Fungi.* 2019, *43*, 223–245. [CrossRef]

30. Senanayake, I.C.; Rathnayaka, A.R.; Marasinghe, D.S.; Calabon, M.S.; Gentekaki, E.; Lee, H.B.; Hurdeal, V.G.; Pem, D.; Dissanayake, L.S.; Wijesinghe, S.N.; et al. Morphological approaches in studying fungi: Collection, examination, isolation, sporulation and preservation. *Mycosphere 2020, 11*, 2678–2754. [CrossRef]

31. Jayasiri, S.C.; Hyde, K.D.; Ariyawansa, H.A.; Bhat, J.; Buyck, B.; Cai, L.; Dai, Y.C.; Abd-Elsalam, K.A.; Ertz, D.; Hidayat, I.; et al. The Faces of Fungi database: Fungal names linked with morphology, phylogeny and human impacts. *Fungal Divers. 2015, 74*, 3–18. [CrossRef]

32. Index Fungorum. Available online: http://www.indexfungorum.org/Names/Names.asp. (accessed on 5 May 2021).

33. Vilgalys, R.; Hester, M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several CRYPTOCoccus species. *J. Bacteriol., 1990, 172*, 4238–4246. [CrossRef] [PubMed]

34. White, T.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR Protocols—A Guide to Methods and Applications; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds.; Academic Press: San Diego, CA, USA, 1990; pp. 315–322.

35. Rehner, S.A.; Samuels, G.J. Taxonomy and phylogeny of Glicoladium analysed from nuclear large subunit ribosomal DNA sequences. *Mycol. Res. 1994, 98*, 625–634. [CrossRef]

36. Liu, Y.J.; Whelen, S.; Hall, B.D. Phylogenetic relationships among ascomycetes: Evidence from an RNA polymerase II subunit. *Biol. Evol. 1999, 16*, 1799–1808. [CrossRef] [PubMed]

37. Hall, T.A. BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symp. Ser. 1999, 41*, 95–98.

38. Kato, K.; Standley, D.M. Evolution. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Biol. Ecol. 2013, 30*, 772–780. [CrossRef]

39. Capella-Gutiérrez, S.; Silla-Martínez, J.M.; Gabaldón, T. trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics 2009, 25*, 1972–1973. [CrossRef]

40. Daniel, G.P.; Daniel, G.B.; Miguel, R.J.; Florentino, F.R.; David, P. ALTER: Program-oriented conversion of DNA and protein alignments. *Nucleic Acids Res. 2010, 38*, W14–W18. [CrossRef]

41. Miller, M.A.; Pfeiffer, W.; Schwartz, T. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In Proceedings of the 2010 Gateway Computing Environments Workshop (GCE), New Orleans, LA, USA, 14 November 2010; pp. 1–8. [CrossRef]

42. Stamatakis, A. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics 2014, 30*, 1312–1313. [CrossRef]

43. Swofford, D.L. PAUP*: *Phylogenetic Analysis Using Parsimony (and Other Methods)*; Version 4; Sinauer Associates: Sunderland, UK, 2003.

44. Hillis, D.M.; Bull, J.J. An Empirical Test of Bootstrapping as a Method for Assessing Confidence in Phylogenetic Analysis. *Syst. Biol. 1993, 42*, 182–192. [CrossRef]

45. Nylander, J.A.A.; Zoology, S.; Posada, D.; Mrmodtest, R.; Os, F. *MrModeltest2 v. 2.3 (Program for Selecting DNA Substitution Models Using PAUP*)*; Evolutionary Biology Centre: Uppsala, Sweden, 2008.

46. Huelsenbeck, J.P.; Ronquist, F.J.B. *MRBAYES: Bayesian inference of phylogenetic trees.* [CrossRef] [PubMed]

47. Zhang, H.; Dong, W.; Hyde, K.D.; Maharachchikumbura, S.S.; Hongsanan, S.; Bhat, D.J.; Al-Sadi, A.M.; Zhang, D. Towards a natural classification of Atractosporales ord. nov. and six new families. *Fungal Divers. 2017, 85*, 75–110. [CrossRef]

48. Crous, P.W.; Summerell, B.A.; Shivas, R.G.; Burgess, T.I.; Decock, C.A.; Dreyer, L.L.; Granke, L.L.; Guest, D.J.; Hardy, G.; Hausbeck, M.; et al. Fungal Planet description sheets: 107–127. *Persoonia 2012*, 28, 138–182. [CrossRef] [PubMed]

49. Arzanlou, M.; Groenewald, J.Z.; Gams, W.; Braun, U.; Shin, H.D.; Crous, P.W. Phylogenetic and morphotaxonomic revision of Ramichloridium and allied genera. *Stud. Mycol. 2007, 58*, 57–93. [CrossRef] [PubMed]

50. Chethana, K.W.T.; Manawasinghe, I.S.; Hurdeal, V.G.; Bjunjun, C.S.; Appadoo, M.A.; Gentekaki, E.; Raspé, O.; Promputtha, I.; Hyde, K.D. What are fungal species and how to delineate them? *Fungal Divers. 2021, 109*, 1–25. [CrossRef]
52. Maharachchikumbura, S.S.N.; Chen, Y.; Ariyawansa, H.A.; Hyde, K.D.; Haelewaters, D.; Perera, R.H.; Samara-koon, M.C.; Wanasinghe, D.N.; Bustamante, D.E.; Liu, J.K.; et al. Integrative approaches for species delimitation in Ascomycota. Fungal Divers. 2021, 109, 155–179. [CrossRef]

53. Pem, D.; Jeewon, R.; Chethana, K.W.T.; Hongsanan, S.; Doilom, M.; Suwannarach, N.; Hyde, K.D. Species concepts of Dothideomycetes: Classification, phylogenetic inconsistencies and taxonomic standardization. Fungal Divers. 2021, 109, 283–319. [CrossRef]

54. Shenoy, B.D.; Jeewon, R.; Wu, W.P.; Bhat, D.J.; Hyde, K.D. Ribosomal and RPB2 DNA sequence analyses suggest that Sporidesmium and morphologically similar genera are polyphyletic. Mycol. Res. 2006, 110, 916–928. [CrossRef]

55. Bhunjun, C.S.; Niskanen, T.; Suwannarach, N.; Wannathes, N.; Chen, Y.-J.; McKenzie, E.H.C.; Maharachchikumbura, S.S.N.; Buyck, B.; Zhao, C.-L.; Fan, Y.-G.; et al. The numbers of fungi: Are the most speciose genera truly diverse? Fungal Divers. 2022, 114, 387–462. [CrossRef]

56. Hyde, K.D.; Jeewon, R.; Chen, Y.J.; Bhunjun, C.S.; Calabon, M.S.; Jiang, H.B.; Lin, C.G.; Norphanphoun, C.; Sysouphanthong, P.; Pem, D.; et al. The numbers of fungi: Is the descriptive curve flattening? Fungal Divers. 2020, 103, 219–271. [CrossRef]

57. Dong, W.; Wang, B.; Hyde, K.D.; McKenzie, E.H.C.; Raja, H.A.; Tanaka, K.; Abdel-Wahab, M.A.; Abdel-Aziz, F.A.; Doilom, M.; Phookamsak, R.; et al. Freshwater Dothideomycetes. Fungal Divers. 2020, 105, 319–575. [CrossRef]

58. Peng, S.Q.; Liu, Y.L.; Huang, J.E.; Li, X.H.; Yan, X.Y.; Song, H.Y.; Gao, Y.; Zhai, Z.J.; Liu, Y.Q.; Hu, D.M. Aquapteridospora jiangxiensis, a new aquatic hyphomycetous fungus from a freshwater habitat in China. Arch. Microbiol. 2022, 204, 378. [CrossRef] [PubMed]