Article

Morphological Variety in Distoseptispora and Introduction of Six Novel Species

Jing Yang 1,2,3, Ling-Ling Liu 1,4,*, E. B. Gareth Jones 5,6, Wen-Li Li 3, Kevin D. Hyde 2 and Zuo-Yi Liu 4

1 Guizhou Institute of Soil and Fertilizer, Guizhou Academy of Agricultural Sciences, Guiyang 550006, China; yangjing5633@gmail.com
2 Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand; kdhyde3@gmail.com
3 School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu 611731, China; wendy316@tom.com
4 Guizhou Key Laboratory of Agricultural Biotecnology, Guizhou Academy of Agricultural Sciences, Guiyang 550006, China; ggzy8558@gmail.com
5 Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand; torperadgi@gmail.com
6 Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
* Correspondence: liuling1557@gmail.com

Abstract: Distoseptispora is one of the sporidesmium-like taxa with great variation in asexual morphology and delineation of species. Phylogenetic analyses of four gene regions LSU, ITS, TEF1α, and RPB2 revealed the placement of several sporidesmium-like species in Distoseptispora (Distoseptisporaceae, Distoseptisporales, Sordariomycetes), collected on submerged decaying twigs from streams in China and Thailand. Based on morphological examination and molecular DNA data, six new species, Distoseptispora annulata, D. atroviridis, D. effusa, D. fusiformis, D. hyalina, and D. verrucosa, are proposed. Among them, D. hyalina is the first sexual morph confirmed in the genus. A new geographical record is reported for D. lignicola in China. Conidial length proved to be of less taxonomic significance for some Distoseptispora species, whereas the type of conidial septa is informative at species level.

Keywords: new taxa; generic delimitation; molecular phylogeny; multi-gene; sporidesmium-like taxa

1. Introduction

The dematiaceous sporidesmium-like hyphomycetes are common saprobes on decaying wood from terrestrial and freshwater habitats and distributed worldwide. They are characterized by holoblastic phragmoconidia produced on macronematous, proliferating or non-proliferating conidiophores or reduced to conidiogenous cells [1,2]. Considering the taxonomic value of euseptate and distoseptate conidia, absence or presence of conidio- phores, conidiophore proliferation, and the shape of conidiogenous cells, several genera segregated from Sporidesmium sensu lato and allied genera were introduced, e.g., Ellisembia, Imicles, Penzigomyces, Polydesmus, Repetophragma, Sporidesmiella, and Stanjehughesia [1,3–6]. Later, with the incorporation of molecular data in phylogenetic studies, Sporidesmium sensu lato and morphologically similar genera were revealed to be polyphyletic mainly within Dothideomycetes and Sordariomycetes. Many of the morphological characters used to delimit the genera did not appear phylogenetically significant [7–11].

Distoseptispora is one of the sporidesmium-like genera introduced by Su et al. [9] with the type species D. fluminicola and the second species D. aquatica having long, cylindrical, distoseptate conidia. Yang et al. [10] emended the generic concept of Distoseptispora based on the morphological features of D. guttulata and D. suoluoensis, namely taxa with conspicuously longer conidiophores elongating percurrently and obclavate, rostrate, euseptate conidia. The generic circumscription was later expanded since more species with
new morphological traits were described in the genus, such as *D. palmarum* [12] having polyblastic conidiogenous cells, *D. appendiculata* [13] and *D. hydei* [14] characterized by a mucilaginous conidial sheath, and *D. martinii* (*Basionym: Acrodictys martini*) with muriform, transverse conidia [15]. Members in *Distoseptispora* are commonly found in freshwater habitats, and many new species have been discovered from China and Thailand in recent years [10,11,14,16–25]. Currently, more than 30 species are accepted in the genus but without a known sexual morph.

It is challenging to classify *Distoseptispora* species based on morphology alone because of the high morphological similarity to *Sporidesmium* and *Ellisembia* with euseptate and distoseptate conidia, respectively. *Distoseptispora* forms a monophyletic clade that is distinct from other sporidesmium-like taxa [9,10,13]. The genus was the only member belonging to *Distoseptisporaceae* and together with the monotypic *Aquapteridosporaceae* in *Distoseptisporales* [26,27].

During our survey of the taxonomy and diversity of freshwater fungi along a north–south gradient in the Asian/Australasian region [28], several sporidesmium-like taxa were collected from freshwater streams in China and Thailand. Using multi-gene loci of LSU, ITS, TEF1α, and RPB2 gene regions, the systematic placement of these collections revealed several *Distoseptispora* species. Based on the morphology and molecular evidence, we introduce six novel species in *Distoseptispora* including a sexual morph for *D. hyalina* and a new geographical record for *D. lignicola* in China. An updated generic description and backbone tree of *Distoseptispora* is provided and its generic delimitation discussed.

2. Materials and Methods

2.1. Collection and Examination of Specimens

Specimens of submerged decaying twigs were collected from streams in China and Thailand. Samples were taken to the laboratory in zip-lock plastic bags and incubated in plastic boxes lined with moistened tissue at room temperature for one week. Motic SMZ 168 Series (Motic, Xiamen, China) and Nikon SMZ-171 (Nikon, Tokyo, Japan) dissecting microscopes were used to observe the fungal colonies and fruiting bodies. Fungal structures were examined and photographed using a Nikon ECLIPSE 80i (Nikon, Tokyo, Japan) compound microscope fitted with a Canon 600D/70D (Canon, Tokyo, Japan) digital camera. Single spore isolations were made onto freshwater agar (WA) or potato dextrose agar (PDA) and germinated spores were transferred onto malt extract agar (MEA) or PDA following the method in Luo et al. [17]. Tarosoft Image Frame Work (Tarosoft, Nontha Buri, Thailand) was used for measurement and images used for figures were processed with Adobe Photoshop CC 2019 (Adobe Systems, San Jose, CA, USA) software. Herbarium specimens (dry wood with fungal material) 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. Axenic cultures were deposited in Mae Fah Luang University Culture Collection (MFLUCC) and Guizhou Culture Collection (GZCC). Facesoffungi and Index Fungorum numbers were registered as outlined in Jayasiri et al. [29] and Index Fungorum [30].

2.2. DNA Extraction, PCR Amplification, and Sequencing

Germinated spores were grown on MEA/PDA medium at 25 °C for one month. Fungal mycelium was scraped off using a sterilized scalpel and transferred to a 1.5 mL microcentrifuge tube for genomic DNA extraction. A Ezup Column Fungi Genomic DNA Purification Kit (Sangon Biotech, Shanghai, China) was used to extract DNA following the manufacturer’s instructions. DNA amplification was performed by polymerase chain reaction (PCR). LSU, SSU, ITS, TEF1α, and RPB2 gene regions were amplified using the primer pairs LR0R/LR5, NS1/NS4, ITS5/ITS4, 983F/2218R, and fRPB2-5F/fRPB2-7cR [31–34]. The amplifications were performed in a 25 µL reaction volume containing 9.5 µL ddH₂O, 12.5 µL 2 × Taq PCR Master Mix with blue dye (Sangon Biotech, China), 1 µL of DNA template, and 1 µL of each primer (10 µM). The amplification condition for LSU, SSU, ITS,
and TEF1α consisted of initial denaturation at 94 °C for 3 min, followed by 40 cycles of 45 s at 94 °C, 50 s at 56 °C, and 1 min at 72 °C, and a final extension period of 10 min at 72 °C. The amplification condition for RPB2 gene consisted of initial denaturation at 95 °C for 5 min, followed by 37 cycles of 15 s at 95 °C, 50 s at 56 °C, and 2 min at 72 °C, final extension period of 10 min at 72 °C. Purification and sequencing of PCR products were carried out by Shanghai Sangon Biological Engineering Technology and Services Co., Shanghai, China.

2.3. Phylogenetic Analyses

The ex-type and additional strains of Distoseptisporaceae species and related families (Acrodictyaceae, Aquapteridosporaceae, Bullimycetaceae, Cancellidiaceae, Papulosaceae, and Pseudostanjehughesiaceae) were selected in the phylogenetic analyses (Table 1). Four gene regions LSU, ITS, TEF1α, and RPB2 were used for the multi-gene analyses. Sequences were optimized manually to allow maximum alignment and maximum sequence similarity. The sequences were aligned using the online multiple alignment program MAFFT v.7 (Available online: http://mafft.cbrc.jp/alignment/server/ (accessed on 3 August 2021)) [35]. The alignments were checked visually and improved manually where necessary.

Table 1. Taxa used in the phylogenetic analyses and their GenBank accession numbers. T denotes ex-type strains. Newly generated sequences are in bold.

| Taxon                     | Voucher/Strain Number | GenBank Accession Number |
|---------------------------|-----------------------|--------------------------|
|                           |                       | LSU          | ITS            | TEF1α         | RPB2          |
| Acrodictys bambusicola    | CGMCC 3.18641         | KX033564     | KU999973       | –             | –             |
| Acrodictys elegiicolia    | CGMCC 3.18642         | KX033569     | KU999978       | –             | –             |
| Aquapteridospora aquatica | MFLUCC 17-2371T       | MW287767     | MW286493       | –             | –             |
| Aquapteridospora fusiformis | MFLUCC 18-1606T      | MK849798     | MK828652       | MN194056      | –             |
| Aquapteridospora lignicola| MFLUCC 15-0377T       | KU221018     | MZ868774       | MZ892980      | MZ892986      |
| Bullimyces aurisporus     | AF316-1bT             | JF775590     | –              | –             | –             |
| Bullimyces communis       | AF281-5               | JF775587     | –              | –             | –             |
| Cancellidium applanatum   | CBS 337.76T           | MH872755     | MH860985       | –             | –             |
| Cancellidium cinereum     | MFLUCC 18-0424T       | MT370363     | MT370353       | MT370488      | MT370486      |
| “Distoseptispora adscendens”| HKUCC 10820         | DQ408561     | –              | –             | DQ435092     |
| Distoseptispora amniculi  | MFLUCC 17-2129T       | MZ868761     | MZ868770       | –             | MZ892982      |
| Distoseptispora appendiculata | MFLUCC 18-0259T     | MN163023     | MN163009       | MN174866      | –             |
| Distoseptispora aquatica  | MFLUCC 15-0374T       | KU376268     | MF077552       | –             | –             |
| Distoseptispora aquatica  | MFLUCC 18-0646       | MK849793     | MK828648       | MN194052      | –             |
| Distoseptispora aquatica  | S-965                 | MK849792     | MK828647       | MN194051      | MN124537     |
| Distoseptispora atroviridis | GZCC 20-0511T      | MZ868763     | MZ868772       | MZ892978      | MZ892984      |
| Distoseptispora atroviridis | GZCC 19-0531       | MZ227223     | MW133915       | –             | –             |
Table 1. Cont.

| Taxon                        | Voucher/Strain Number | GenBank Accession Number |
|------------------------------|-----------------------|--------------------------|
|                              |                       | LSU | ITS | TEF1α | RPB2 |
| **Distoseptispora bambusae** | MFLUCC 20-0091^T       | MT232718 | MT232713 | MT232880 | MT232881 |
| **Distoseptispora bambusae** | MFLUCC 14-0583         | MT232717 | MT232712 | –          | MT232882 |
| **Distoseptispora bangkokensis** | MFLUCC 18-0262^T     | MZ518206 | MZ518205 | –          | –          |
| **Distoseptispora cangshanensis** | MFLUCC 16-0970^T      | MG979761 | MG979754 | MG988419   | –          |
| **Distoseptispora caricos**  | CPC 36498^T           | MN567632 | MN562124 | –          | MN556805   |
| **Distoseptispora caricos**  | CPC 36442^T           | –      | MN562125 | –          | MN556806   |
| **Distoseptispora chinensis** | GZCC 21-0665^T        | MZ474867 | MZ474871 | MZ501609   | –          |
| **Distoseptispora clamatidis** | MFLUCC 17-2145^T      | MT214617 | MT310661 | –          | MT394721   |
| **Distoseptispora delongensis** | KUMCC 18-0090^T       | MK079662 | MK085061 | MK087659   | –          |
| **Distoseptispora effusa**   | GZCC 19-0532^T        | MZ227224 | MW133916 | MZ206156   | –          |
| **Distoseptispora euseptata** | MFLUCC 20-0154^T      | MW081544 | MW081539 | –          | MW151860   |
| **Distoseptispora euseptata** | DLUCC S2024           | MW081545 | MW081540 | MW084994   | MW084996   |
| **Distoseptispora fasciculata** | KUMCC 19-0081^T       | MW287775 | MW286501 | MW396656   | –          |
| **Distoseptispora fluminicola** | MFLUCC 15-0417^T      | KU376270 | MF077553 | –          | –          |
| **Distoseptispora fusiformis** | GZCC 20-0512^T        | MZ868764 | MZ868773 | MZ892979   | MZ892985   |
| **Distoseptispora guizhouensis** | GZCC 21-0666^T        | MZ474869 | MZ474868 | MZ501610   | MZ501611   |
| **Distoseptispora guttulata** | MFLUCC 16-0183^T      | MF077554 | MF077543 | MF135651   | –          |
| **Distoseptispora hyalinoid** | MFLUCC 17-2128^T      | MZ868760 | MZ868769 | MZ892976   | MZ892981   |
| **Distoseptispora hyalina**   | MFLUCC 20-0115^T      | MT742830 | MT734661 | –          | MT767128   |
| **Distoseptispora hydei**     | DLUCC 1864^T          | MW879522 | MW723055 | –          | –          |
| **Distoseptispora lancangjiangensis** | HKUCC 10822          | DQ408566 | –      | –          | DQ435089   |
| “**Distoseptispora leonensis**” | MFLUCC 18-0198^T      | MK849797 | MK828651 | –          | –          |
| **Distoseptispora lignicola** | GZCC 19-0529          | MZ227219 | MW133911 | –          | –          |
| **Distoseptispora longispora** | HFJAU 0705^T          | MH553537 | MH553539 | –          | –          |
| **Distoseptispora martini**   | CGMCC 3.18651         | KX033566 | KU999975 | –          | –          |
| **Distoseptispora multisepata** | MFLUCC 15-0609^T      | KX710140 | KX710145 | MF135659   | –          |
| **Distoseptispora multisepata** | MFLUCC 16-1044        | MF077555 | MF077544 | MF135652   | MF135644   |
| **Distoseptispora neoerostrata** | MFLUCC 18-0376^T      | MN163017 | MN163008 | –          | –          |
| Taxon                        | Voucher/Strain Number | GenBank Accession Number | LSU     | ITS      | TEF1α   | RPB2   |
|-----------------------------|-----------------------|--------------------------|---------|----------|---------|--------|
| **Distoseptispora**         |                       |                          |         |          |         |        |
| obclavata                   | MFLUCC 18-0329T       | MN163010                 | MN163012| –        | –       | –      |
| obpyriformis                | MFLUCC 17-1694T       | MG979764                 | –       | MG988422 | MG988415|        |
| obpyriformis                | DLUCC 0867           | MG979765                 | MG979757| MG988423 | MG988416|        |
| palmarum                   | MFLUCC 18-1446T       | MK079663                 | MK085062| MK087660 | MK087670|        |
| **Distoseptispora**         |                       |                          |         |          |         |        |
| phangngaensis               | MFLUCC 18-0415T       | MH457137                 | MH457172| MH463253 | MH463255|        |
| rayongensis                | MFLUCC 16-0969T       | MG979766                 | MG979758| MG988424 | MG988417|        |
| rayongensis                | DLUCC 0885           | MG979767                 | MG979759| MG988425 | –       |        |
| rostrata                   |                       |                          |         |          |         |        |
| saprophytica                | MFLUCC 18-1238T       | MW287780                 | MW286506| MW396651 | MW504069|        |
| songkhlaensis               | MFLUCC 18-1234T       | MW287755                 | MW286482| MW396642 | –       |        |
| suoluoensis                | MFLUCC 17-0224T       | MF077557                 | MF077546| MF135654 | –       |        |
| suoluoensis                |                       |                          |         |          |         |        |
| vulcanensis                |                       |                          |         |          |         |        |
| tectonae                   | MFLUCC 17-1305        | MF077558                 | MF077547| –        | MZ945510|        |
| tectonae                   | MFLUCC 12-0291T       | KX751713                 | KX751711| KX751710| KX751708|        |
| tectonae                   | MFLUCC 15-0981        | MW287763                 | MW286489| MW396641 | –       |        |
| tectonigena                | MFLUCC 16-0946        | MG979768                 | MG979760| MG988426 | MG988418|        |
| thailandica                | MFLUCC 12-0292T       | KX751714                 | KX751712| –        | KX751709|        |
| thysonolaeae               | MFLUCC 16-0270T       | MH260292                 | MH275060| MH412767 | –       |        |
| thaiensis                  | KUMCC 18-0182T        | MK064091                 | MK045851| MK086031 | –       |        |
|                 | HKCC 112710           | MW879524                 | MW723057| MW729783 | –       |        |
| **Distoseptispora**         |                       |                          |         |          |         |        |
| verrucosa                  | GZCC 20-0434T         | MZ868762                 | MZ868771| MZ892977 | MZ892983|        |
| xishuangbannaensis         | KUMCC 17-0290T        | MH260293                 | MH275061| MH412768 | MH412754|        |
| yunnanensis                | MFLUCC 20-0153T       | MW081546                 | MW081541| MW084995 | MW151861|        |
| yunnanensis                | MFLUCC 15-0976T       | MF374367                 | MF374358| MF370956 | MF370954|        |
| banksiae                   | CPC 19852T            | JX069855                 | JX069871| –        | –       | –      |
| schulzeri                  | CBS 100.54            | EU041826                 | EU041769| –        | –       | –      |
| amerospora                 | AFTOL-ID 748          | DQ470950                 | –       | DQ471069 | DQ470901|        |
| bambusinum                 | MFLUCC 12-0850        | KU863149                 | KU940161| KU940213 | –       |        |
| aquitropica                | MFLUCC 16-0569T       | MF077559                 | MF077548| MF135655 | –       |        |
Maximum likelihood (ML), Bayesian inference (BI), and maximum parsimony (MP) analyses were employed to assess phylogenetic relationships. ML and BI analyses were performed through the CIPRES science Gateway V.3.3 [36]. ML analyses were conducted with RAxML-HPC v. 8.2.12 [37] using a GTRGAMMA approximation with rapid bootstrap analysis followed by 1000 bootstrap replicates. For the BI approach, MrModeltest2 v. 2.3 [38] was used to infer the appropriate substitution model that would best fit the model of DNA evolution for the combined dataset. GTR + G + I substitution model was selected for LSU, ITS, TEF1α, and RPB2 partitions. BI analyses were performed in a likelihood framework as implemented in MrBayes 3.2.6 [39]. Six simultaneous Markov chains were run until the average standard deviation of split frequencies was below 0.01, with trees saved every 1000 generations. The first 25% of saved trees, representing the burn-in phase of the analysis, were discarded. The remaining trees were used for calculating posterior probabilities of recovered branches [40]. MP analyses were conducted with PAUP v. 4.0a167 [41]. A heuristic search was performed with the stepwise-addition option with 1000 random taxon addition replicates and tree bisection and reconnection branch swapping. All characters were unordered and of equal weight and gaps were treated as missing data. Maxtrees were unlimited, branches of zero length were collapsed, and all multiple, equally parsimonious trees were saved. Clade stability was assessed using a bootstrap analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa [42].

The resulting trees were printed with FigTree v. 1.4.4 and the layout was created in Adobe Illustrator 2019 (Adobe Systems, San Jose, CA, USA). Sequences generated in this study were deposited in GenBank (Table 1).

### 3. Phylogenetic Results

Phylogenetic relationships of seven *Distoseptispora* species were assessed in the combined analysis using four gene regions of 76 strains representing 61 species in *Distoseptisporaceae* and related families (*Acrodicycaceae*, *Aquapteridosporaceae*, *Bullimycetaceae*, *Cancellidiaceae*, *Papulosaceae*, and *Pseudostanjehuglesiaceae*). The analyzed alignment consisted of combined LSU (1–858 bp), ITS (859–1522 bp), TEF1α (1523–2432 bp), and RPB2 (2433–3490 bp) sequence data including gaps.

**Table 1.** Cont.

| Taxon                      | Voucher/Strain Number | GenBank Accession Number |
|----------------------------|-----------------------|--------------------------|
|                            |                       | LSU | ITS | TEF1α | RPB2 |
| *Pseudostanjehuglesia*     | MFLUCC 15-0352T       | MK849787 | MK828643 | MN194047 | MN124534 |
| lignicola                  |                       |     |     |       |      |
| *Wongia* griffinii         | DAR 80512T            | KU850471 | KU850473 | –      | –      |

*Myrmecridium schulzeri* (CBS 100.54) and *Myrmecridium banksiae* (CPC 19852) served as outgroup taxa. The best scoring RAxML tree is shown in Figure 1. The analyzed ML, MP, and Bayesian trees were similar in topology and did not conflict significantly. *Distoseptispora* was resolved as a monophyletic clade. Our eight strains nested within the genus representing seven species. *Distoseptispora amniculi* (MFLUCC 17-2129) clustered as sister taxon to *D. bangkokensis* (MFLUCC 18-0262) but with weak support. *Distoseptispora effusa* (GZCC 19-0532) formed a distinct clade sister to the clade containing *D. hydei* (MFLUCC 20-0115), *D. rostrata* (MFLUCC 16-0969 and DLUCC 0885), and *D. obpyriformis* (MFLUCC 17-1694 and DLUCC 0867) with strong statistical support (100% ML BS/1.0 PP/100% MP BS). *Distoseptispora atroviridis* (GZCC 20-0511 and GZCC 19-0531), based on two strains, grouped with “*D. leonensis*” (HKUCC 10822) and was close to *D. fusiformis* (GZCC 20-0512). *Distoseptispora verrucosa* (GZCC 20-0434) grouped with *D. suoluoensis* (MFLUCC 17-1305 and MFLUCC 17-0224), *D. lancangjiangensis* (DLUCC 1864), and *D. bambusae* (MFLUCC 20-0091 and MFLUCC 14-0583), in a sister group of *D. euseptata* (MFLUCC 20-0154 and DLUCC S2024), *D. hyalina* (MFLUCC 17-2128), and *D. yunnanensis* (MFLUCC 20-0153), forming a statistically well supported clade by ML and
BI analyses. Members of this clade have similar conidial morphology except *D. hyalina* in sexual stage. The strain GZCC 19-0529 positioned sister to the ex-type strain (MFLUCC 18-0198) of *D. lignicola* with identical LSU sequences and three base pair differences in ITS sequences, and therefore is identified as *D. lignicola* based on the morphology and molecular evidence.

Figure 1. Cont.
Figure 1. Maximum likelihood majority rule consensus tree for Distoseptisporaceae and related families using LSU, ITS, TEF1α, and RPB2 sequence data. Bootstrap support values for maximum likelihood (ML) and maximum parsimony (MP) greater than 70% and Bayesian posterior probabilities greater than 0.90 are indicated above branches as ML BS/PP/MP BS. The scale bar represents the expected number of changes per site. The tree is rooted with Myrmecridium schulzeri (CBS 100.54) and Myrmecridium banksiae (CPC 19852). Ex-type strains are indicated with T. The new collections are in bold with new taxa in blue. Branches with 100% ML BS, 1.0 PP, and 100% MP BS are thickened. Families are indicated as colored blocks.

4. Taxonomy

Distoseptispora K.D. Hyde, McKenzie, and Maharachch., Fungal Diversity 80: 402 (2016).

Sexual morph: Ascomata solitary or gregarious, immersed to semi-immersed, perithecial, subglobose to ellipsoidal, dark brown, ostiolate, with a short neck. Ostiole periphysate. Ascomatal wall cariosaceous, 2-layered, outer layer consisting of multi-rows of brown, thick-walled, polyhedral cells of textura angularis, inner layer comprising multi-rows of pale brown to hyaline, thin-walled, elongated cells of textura angularis or textura prismatica. Paraphyses sparse, persistent, septate, hyaline, tapering towards the apex, constricted at the septa. Asci unitunicate, 8-spored, cylindrical, pedicellate, obtuse at the apex, apex with a non-amyloid apical annulus. Ascospores overlapping, uniseriate, fusiform, hyaline, 0–3-septate, smooth-walled, guttulate, thin-walled, with a mucilaginous sheath. Asexual morph: Hyphomycetous. Colonies effuse, hairy, velvety, olivaceous to dark brown. Mycelium mostly immersed, composed of branched, septate, smooth, pale brown hyphae. Conidiophores macronematous, mononematous, erect, single or in groups or fasciculate, septate, unbranched, straight or slightly flexuous, smooth, olivaceous to brown, cylindrical, rounded or truncate at the apex, sometimes elongating percurrently, rarely reduced to conidiogenous cells. Conidiogenous cells mostly monoblastic, sometimes polyblastic, integrated, determinate, terminal, cylindrical or clavate with flared apex. Conidia acrogenous, solitary, cylindrical or obclavate, rostrate, ellipsoidal, obvoid to fusiform, subhyaline, olivaceous, dark green, brown or yellowish-brown to reddish-brown, euseptate or distosep-
tate, rarely muriform, truncate at base, sometimes indeterminate in length or producing a secondary conidium, septal pore and mucilaginous sheath present or absent.

Type species—*Distoseptispora fluminicola* McKenzie, H.Y. Su, Z.L. Luo, and K.D. Hyde

Notes: Hyde et al. [11] provided the family description for the monotypic *Distoseptisporaceae*. The diagnosis of the sexual morph in the family was based on *Miyoshiella triseptata*, which was associated with “*Distoseptispora adscendens*” (as *Ellisembia adscendens*) in the same collection [7,43]. However, neither the cultural study nor molecular data has proved their connection. We prefer to treat *Miyoshiella triseptata* as a possible sexual morph of sporidesmium-like taxa. The sexual description here is based on *Distoseptispora hyalína*. *Distoseptispora martinii* is unique in the genus by transverse ellipsoid or subglobose, muriform conidia [15]. Additional collections and further molecular evidence are needed to confirm its taxonomy.

**Distoseptispora amniculi** J. Yang and K.D. Hyde, sp. nov., Figure 2.

Index Fungorum number: IF558670; Facesoffungi number: FoF10250.

Etymology: referring to the collecting site of a small stream.

Holotype: MFLU 21-0138.

Saprobic on submerged decaying wood in a freshwater habitat. **Asexual morph:** Colonies on wood effuse, hairy, dark brown, scattered or in small groups, glistening, usually retiform. Mycelium partly immersed, partly superficial, composed of septate, smooth-walled, pale brown to hyaline hyphae. Conidiophores macronematous, mononeumatous, erect, solitary or caespitose, straight or flexuous, cylindrical, rounded at the apex, smooth-walled, septate, unbranched, grayish brown, 90–180 × 3–4.5 µm (X = 125 × 4 µm, n = 20). Conidiogenous cells monoblastic, integrated, terminal, determinate, cylindrical, pale brown, rounded and darkened at the apex, sometimes elongating percurrently. Conidia acrogenous, obclavate, rostrate, olivaceous brown, grayish brown or mid brown, paler towards the apex, (7–)12–24-septate, 85–167 × 9–11.8 µm (X = 120 × 10 µm, n = 20), smooth-walled, truncate and darkened at the base, sometimes with a secondary conidium. **Sexual morph:** undetermined.

Culture characteristics: conidia germinating on PDA within 24 h and germ tubes produced from both ends. Colonies growing on PDA slow growing, reaching 10–15 mm in a month at 25 °C in natural light, circular, with dense, gray mycelium in the middle, darker of the inner ring, with sparser, white mycelium of the outer ring on the surface, in reverse dark brown to black with smooth margin.

Material examined: Thailand, Trat Province, Amphoe Ko Chang, 12°7.98′ N, 102°37.98′ E, on decaying wood submerged in a freshwater stream, 27 April 2017, YZ Lu, YJT 26-2 (MFLU 21-0138, holotype); ex-type living culture MFLUCC 17-2129; additional sequence, SSU: MZ868766.

Notes: *Distoseptispora amniculi* is similar to *D. neorostrata* and *D. rostrata* in the relative long conidiophores (more than 80 µm long) and obclavate, rostrate, distoseptate conidia. *Distoseptispora neorostrata* [13] can be distinguished from the present species in having truncate apex to the conidiophores and dark and wider conidia (109–155 × 9–11 µm). Conidiophores of *D. amniculi* are longer than that in *D. neorostrata* (90–180 µm vs. 93–117 µm) and *D. rostrata* (90–180 µm vs. 82–126 µm). In the multi-gene phylogenetic tree (Figure 1), *D. amniculi* clustered with *D. bangkokensis*. They have distoseptate conidia and rounded apex of conidiophores. *Distoseptispora amniculi* is well distinguishable from *D. bangkokensis* [44] by longer conidiophores (90–180 µm vs. 37–55 µm) and shorter conidia (85–167 µm vs. 400–568 µm). Comparison of the LSU, ITS, and SSU sequences of *D. amniculi* and *D. bangkokensis* showed 99.75% (798/800bp), 92.91% (485/522bp including 11bp of gaps), and 99.44% (893/898bp) sequence identity, respectively.
bangkokensis showed 99.75% (798/800bp), 92.91% (485/522bp including 11bp of gaps), and 99.44% (893/898bp) sequence identity, respectively.

Figure 2. Distoseptispora amniculi (MFLU 21-0138, holotype). (a) Colonies on wood. (b, c) Conidiophores. (d) Conidiogenous cell. (e–j) Conidia. (k) Germinated conidium. (l, m) Colony on PDA, l from above, m from below. Scale bars: (a) 200 µm, (b, c, e–k) 30 µm, (d) 20 µm.

Distoseptispora atroviridis J. Yang and K.D. Hyde, sp. nov., Figure 3. Index Fungorum number: IF558671; Facesoffungi number: FoF10252. Etymology: referring to the dark green conidia. Holotype: HKAS 112616.
Conidiophores macronematous, fasciculate, loosely compact, erect, straight or slightly flexuous, cylindrical, wider and truncate at the apex, smooth-walled, septate, unbranched, pale grayish brown, slightly paler at the apical cell, 100–167 × 2.7–4 μm (x = 124.5 × 3.5 μm, n = 30), 4.7–6.9 μm wide at the apex. Conidiogenous cells monoblastic, integrated, terminal, determinate, sometimes elongating percurrently, flared, pale grayish brown.

Saprobic on submerged decaying wood in a freshwater habitat. Asexual morph: Colonies on wood effuse, dark brown to black, scattered or in small groups, glistening. Mycelium mostly immersed, composed of septate, smooth-walled, brown to hyaline hyphae. Conidiophores macronematous, fasciculate, loosely compact, erect, straight or slightly flexuous, cylindrical, wider and truncate at the apex, smooth-walled, septate, unbranched, pale grayish brown, slightly paler at the apical cell, 100–167 × 2.7–4 μm (x = 124.5 × 3.5 μm, n = 30), 4.7–6.9 μm wide at the apex. Conidiogenous cells monoblastic, integrated, terminal, determinate, sometimes elongating percurrently, flared, pale grayish brown.
brown. Conidia acrogenous, solitary, ellipsoidal to obovoid, dark green, subhyaline at the basal cell, 6-septate, 31–43 × 13–20 µm (x = 39 × 18 µm, n = 40), smooth-walled, guttulate, truncate at the base, sometimes released with part of conidiogenous cells. **Sexual morph:** Undetermined.

Culture characteristics: conidia germinating on PDA within 24 h and swollen germ tubes produced from both ends. Colonies growing on PDA reaching 5–10 mm in two weeks at 25 °C in the dark, with dense, velvety, dark green mycelium on the surface; in reverse dark green with entire margin.

Material examined: China, Guizhou Province, Chishui City, Sidonggou Waterfall, 25°27.38′ N, 107°39.85′ E, on submerged decaying twig in a freshwater stream, 11 July 2019, J Yang, CS 40-1 (HKAS 112616, **holotype**); ex-type living culture GZCC 20-0511; ibid, near 28°25′ N, 106°0′ E, at an altitude of 500 m, on submerged decaying wood in a freshwater stream, 16 July 2019, LL Liu, CS 1-4-2 (GZAAS 20-0426, **paratype**); living culture GZCC 19-0531 (additional sequence, SSU: MW134695).

Notes: *Distoseptispora atroviridis* is well distinguishable among other species of the genus by fasciculate, synnematous-like conidiophores, flared conidiogenous cells and ellipsoidal to obovoid, dark green, 6-septate conidia with paler to subhyaline basal cell. *Distoseptispora atroviridis* resembles some *Phragmocephala* species, such as *P. atra*, *P. elliptica*, *P. hughesii*, and *P. garethjonesii*, in having loosely to compactly fasciculate conidiophores aggregated at the base, clavate, flared conidiogenous cells sometimes elongating percurrently and ellipsoidal to obovoid conidia that secedes rheolytically [6,45]. However, *Phragmocephala* species differ from *D. atroviridis* by brown conidia with thickened and darkened bands. Molecular analyses revealed the placement of *Phragmocephala* in Melanommataceae (Pleosporales, Dothideomycetes) distinct from *D. atroviridis* [45,46]. In our phylogenetic tree, *D. atroviridis* (GZCC 20-0511 and GZCC 19-0531) was sister to “*Distoseptispora leonensis*” HKUCC10822 (99% ML BS/1.0 PP/100% MP BS, Figure 1), but they are distinguishable in morphology. “*Distoseptispora leonensis*” was characterized by solitary, mid to dark brown conidiophores with up to three successive proliferations [47] while *D. atroviridis* has fasciculate, loosely compact, longer but narrower conidiophores (100–167 × 2.7–4 µm vs. 70–120 × 4.5–8 µm) rarely percurrently proliferating. Conidiogenous cells are cylindrical and slightly narrower at the apex in “*D. leonensis*” [47] while those are flared with wider apex in *D. atroviridis*. The former species has fusiform or rostrate, mid to dark brown, 9–17-septate conidia with tapering apical cells [47] while the latter has ellipsoidal to obovoid, dark green, 6-septate, smaller conidia (31–43 × 13–20 µm vs. 45–90 × 15–18 µm).

*Distoseptispora effusa* L.L. Liu and Z.Y. Liu, sp. nov., Figure 4.

**Index Fungorum number:** IF558406; **Facesoffungi number:** FoF09863.

**Etymology:** referring to the effuse colonies.

**Holotype:** GZAAS 20-0427.

Saprobic on decaying wood in a freshwater habitat. **Asexual morph:** Colonies on natural substrates superficial, effuse, dark brown to black, hairy, velvety. Mycelium mostly immersed, consisting of branched, hyaline to pale brown, smooth, septate hyphae. Conidiophores macronematos, mononematos, erect, solitary or in small groups, cylindrical, dark brown, 5–14-septate, straight or slightly curved, smooth, 72–171 × 5–6.5 µm (x = 104.5 × 5.5 µm, n = 15), rounded at the apex. Conidiogenous cells monoblastic, integrated, terminal, determinate, cylindrical, brown, sometimes elongating percurrently, darkened at the rounded apex and percurrent loci. Conidia acrogenous, solitary, obclavate, rostrate, smooth-walled, olivaceous brown to dark brown, sometimes slightly paler at the apex, straight or slightly curved, 4–9-distoseptate, 35.5–113 × 7–12.5 µm (x = 71 × 10 µm, n = 20), truncate and darkened at the base. **Sexual morph:** Undetermined.
Notes: Phylogenetically, *Distoseptispora effusa* (GZCC 19-0532) nested within *Distoseptispora* and formed a distinct clade sister to the clade containing *D. rostrata*, *D. hydei*, and *D. obpyriformis* (Figure 1). Morphologically, *D. effusa* is similar to *D. rostrata* [17] with percurrently elongate conidiophores and obclavate, distoseptate conidia but differs by longer conidiophores (72–171 μm vs. 82–126 μm) and shorter conidia (35.5–113 μm vs. 115–155 μm). *Distoseptispora hydei* [14] is distinguished by fusiform to obpyriform, light olivaceous to brown conidia. *Distoseptispora obpyriformis* [17] has obclavate to obpyriform, olivaceous to dark brown conidia that are shorter but wider than *D. effusa* (53–71 × 12–16 μm vs. 35.5–113 × 7–12.5 μm).

**Figure 4.** *Distoseptispora effusa* (GZAAS 20-0427, holotype). (a) Colonies on natural substrate. (b,c) Conidiophores. (d–i) Conidia. (j) Conidiogenous cell with a conidium. (k) Germinated conidium. (l,m) Culture, l from above, m from below. Scale bars: (b–k) 50 μm.

Cultural characteristics: conidia germinated on WA within 24 h and germ tube produced from the apex. Colonies on PDA reaching 15–20 mm diam. after 2 weeks at 25 °C in dark, circular, with fluffy, dense, dark olivaceous brown aerial mycelium on the surface; in reverse dark brown with entire margin.

Material examined: China, Guizhou Province, Chishui City, Chishui river basin, near 28°25′ N, 106°0′ E, at an altitude of 500 m, on submerged decaying wood in a freshwater stream, July 2019, LL Liu, CS 3-7 (GZAAS 20-0427, holotype); ex-type living culture GZCC 19-0532; additional sequences, SSU: MW134696.
Notes: Phylogenetically, *Distoseptispora effusa* (GZCC 19-0532) nested within *Distoseptispora* and formed a distinct clade sister to the clade containing *D. rostrata*, *D. hydei*, and *D. obpyriformis* (Figure 1). Morphologically, *D. effusa* is similar to *D. rostrata* [17] with percurrently elongate conidiophores and obclavate, distoseptate conidia but differs by longer conidiophores (72–171 µm vs. 82–126 µm) and shorter conidia (35.5–113 µm vs. 115–155 µm). *Distoseptispora hydei* [14] is distinguished by fusiform to obpyriform, light olivaceous to brown conidia. *Distoseptispora obpyriformis* [17] has obclavate to obpyriform, olivaceous to dark brown conidia that are shorter but wider than *D. effusa* (53–71 × 12–16 µm vs. 35.5–113 × 7–12.5 µm).

*Distoseptispora fusiformis* J. Yang and K.D. Hyde, sp. nov., Figure 5.
Index Fungorum number: IF558672; Facesoffungi number: FoF10253.
Etymology: referring to the fusiform conidia.
Holotype: HKAS 112617.

Saprobic on submerged decaying wood in a freshwater habitat. **Asexual morph:** Colonies on wood effuse, hairy, dark brown to black, scattered or in small groups, glistening. Mycelium mostly immersed, composed of septate, smooth-walled, brown to hyaline hyphae. Conidiophores macronematous, mononematous, erect, straight or slightly flexuous, cylindrical, slightly tapering towards the apex, smooth-walled, septate, unbranched, pale to dark brown, slightly paler at the apical cells, 40–110 × 3.5–5.8 µm (x = 86 × 4.6 µm, n = 20). Conidiogenous cells monoblastic, integrated, terminal, determinate, sometimes elongating percurrently, cylindrical, brown. Conidia acrogenous, solitary, ellipsoidal to fusiform, dark olivaceous brown to dark brown, pale brown at both ends, 6–8-septate, 35–52 × 13.5–22 µm (x = 42 × 18.5 µm, n = 30), smooth-walled, guttulate, truncate at the base, with an obconical basal cell.

**Sexual morph:** Undetermined.

Culture characteristics: conidia germinating on PDA within 24 h and swollen germ tubes produced from both ends. Colonies growing on PDA reaching 5–10 mm in two weeks at 25 °C in dark, circular, with velvety, dark olivaceous brown mycelium on the surface; in reverse dark brown with filiform margin.

Material examined: China, Guizhou Province, Chishui City, Sidonggou Waterfall, 25°27.38′ N, 107°39.85′ E, on submerged decaying twig in a freshwater stream, 11 July 2019, J Yang, CS 40-2 (HKAS 112617, holotype); ex-type living culture GZCC 20-0512; additional sequence, SSU: MZ868768.

Notes: *Distoseptispora fusiformis* can be distinguished from other species in the genus by relatively long conidiophores with truncate apex and ellipsoidal to broadly fusiform, 6–8-septate, dark olivaceous brown to brown conidia with paler polar cells. *Distoseptispora fusiformis* was collected on the same material as *D. atroviridis* but they are distinct in morphology and phylogeny. *Distoseptispora atroviridis* can be distinguished from *D. fusiformis* by fasciculate or loosely compact conidiophores, trapezoidal conidiogenous cells and ellipsoidal to obovoid, dark green conidia. Conidiophores and conidia of *D. atroviridis* are longer and slightly smaller than those in *D. fusiformis* (100–167 µm vs. 40–110 µm; 31–43 × 13–20 µm vs. 35–52 × 13.5–22 µm), respectively. Comparing the LSU, ITS, TEF1α, and RPB2 sequences of *D. atroviridis* and *D. fusiformis* showed 96.07% (32 bp differences), 90.21% (55 bp differences), 93.77% (56 bp differences), and 89.56% (114 bp differences) sequence similarity, respectively.

*Distoseptispora hyalina* J. Yang and K.D. Hyde, sp. nov., Figure 6.
Index Fungorum number: IF558673; Facesoffungi number: FoF10249.
Etymology: referring to the hyaline conidia.
Holotype: MFLU 21-0137.

Saprobic on submerged decaying wood in a freshwater habitat. **Asexual morph:** Ascomata solitary or gregarious, immersed to semi-immersed, perithecial, 150–200 µm high, 120–190 µm diam., subglobose to ellipsoidal, dark brown, ostiolate, with a short neck erumpent through host surface. Ostiole periphysate. Ascomatal wall coriaceous, 20–31 µm thick, 2-layered; outer layer consisting of multi-rows of brown, thick-walled, polyhedral cells of textura angularis, inner layer comprising multi-rows of pale brown to hyaline,
thin-walled, elongated cells of textura angularis or textura prismatica. Paraphyses sparse, persistent, septate, hyaline, tapering towards the apex, c. 4–7 µm wide near the base, constricted at the septa. Asci 145–190 × 8–11 µm (x = 165 × 9.8 µm, n = 20), cylindrical, with a short pedicel, obtuse at the apex, 8-spored, apex with a non-amyloid apical annulus. Ascospores (19.5–)23–26(–28.5) × 4.5–7 µm (x = 25 × 6 µm, n = 30), overlapping, uniseriate, fusiform, straight, rarely slightly curved, hyaline, 0–3-septate, smooth-walled, guttulate, thin-walled, with a mucilaginous sheath. **Asexual morph:** Undetermined.

**Figure 5.** Distoseptispora fusiformis (HKAS 112617, holotype). (a,b) Colonies on woody substrates. (c–h) Conidiophores with conidia. (i,j) Conidiogenous cells. (k–n) Conidia. (o) Germinated conidium. (p,q) Culture, p from above, q from below. Scale bars: (c–h) 40 µm, (i,j,o) 30 µm, (k–n) 20 µm.
Figure 6. Distoseptispora hyalina (MFLU 21-0137, holotype). (a) Ascomata on woody substrate. (b) Vertical section of an ascoma. (c) Section of the bottom wall. (d) Section of the lateral wall near the beak. (e) Paraphyses. (f–i) Asci. (j–o) Ascospores, n and o using Indian ink. (p) Apical ring. (q) Germinated spore. (r,s) Culture, r from above, s from below. Scale bars: (a) 200 µm, (b) 50 µm, (f–i,q) 30 µm, (c,e,n,p) 20 µm, (j–m,o) 15 µm, (d) 10 µm.

Culture characteristics: conidia germinating on PDA within 24 h. Germ tubes produced from both ends. Colonies on PDA reaching 10–15 mm diam. after 2 weeks at 25 ºC in natural light, with dense mycelium on the surface, gray in the middle, dark grayish green of the inner ring, and grayish green of the outer ring; in reverse dark olivaceous green with entire margin.

Material examined: Thailand, Trat Province, Amphoe Ko Chang, 12°7.98′ N, 102°37.98′ E, on decaying wood submerged in a freshwater stream, 27 April 2017, YZ Lu, YJT 26-1.
Distoseptispora hyalina is the first sexual morph reported in the genus based on molecular DNA data. Distoseptispora hyalina resembles Sporidesmium thailandense in possessing cylindrical, pedicellate asci with a non-amyloid apical annulus and obliquely uniseriate, fusiform, hyaline ascospores with a mucilaginous sheath. However, D. hyalina possesses immersed to semi-immersed, erumpent ascomata, larger brown cells of ascomatal wall and 3-septate ascospores when mature while S. thailandense has immersed ascomata with compact layers of cells of the peridium that is undifferentiated from host tissue and 3–4-septate ascospores [10,48]. In addition, D. hyalina differs from S. thailandense by the smaller asci (145–190 × 8–11 µm vs. 160–220 × 11–14 µm) and narrower ascospores but similar in length (4.5–7 µm wide vs. 8–10 µm wide). Distoseptispora hyalina is similar to Miyoshiella trisepata in having a non-amyloid apical annulus of asci and fusiform, 3-septate ascospores with comparable dimension (20–25 × 5–7 µm) [43], but Miyoshiella trisepata is distinguished by carbonaceous, papillate ascomata, shorter and broader asci (90–110 × 12–17 µm) and hyaline to light yellowish brown ascospores lacking a sheath [43].

In the phylogenetic analyses, Distoseptispora hyalina was sister to D. yunnanensis with good support (97% ML BS/1.0 PP/98% MP BS, Figure 1). The LSU, ITS, TEF1α, and RPB2 sequence identity of D. hyalina and D. yunnanensis was 95.05% (807/849bp including 4bp of gaps), 94.59% (525/555bp including 9bp of gaps), 96.66% (898/929bp), and 93.41% (1035/1108bp), respectively.

Distoseptispora hyalina were colonized close to D. amniculi on the same twig in sexual and asexual stages, respectively. However, they are separate taxa based on molecular evidence.

Distoseptispora lignicola D.F. Bao, Z.L. Luo, H.Y. Su, and K.D. Hyde, Fungal Diversity 99: 487 (2019) Figure 7.

Index Fungorum number: IF555641, Facesoffungi number: FoF05413.

Etymology: referring to the verrucose conidia.

Holotype: HKAS 112652.

Saprobic on submerged decaying wood in a freshwater habitat. Asexual morph: Colonies on wood effuse, hairy, dark brown to black, scattered or in small groups, glistening. Mycelium partly immersed, partly superficial, composed of septate, smooth-walled, pale brown to hyaline hyphae. Conidiophores macronematous, mononematous, erect, solitary or caespitose, straight or flexuous, cylindrical, rounded and usually darkened at the apex, smooth-walled, septate, unbranched, dark brown, slightly paler at the upper part, 92–250 × 4.7–6.3 µm (X = 162 × 5.7 µm, n = 20). Conidiogenous cells monoblastic, integrated, terminal, determinate, sometimes percurrently proliferating, cylindrical, brown. Conidia acrogenous, solitary, obclavate, rostrate, upper part tapering towards the apex, olivaceous brown, becoming paler at the apex, 6–8-septate, 41–63 × 8.8–12.6 µm (X = 51.5 × 10.8 µm, n = 30), verrucose, guttulate, truncate with a darkened scar at the base. Sexual morph: Undetermined.
Material examined: China, Guizhou Province, Chishui City, Chishui river basin, near 28°25′ N, 106°0′ E, at an altitude of 500 m, on submerged decaying wood in a freshwater stream, July 2019, LL Liu, CS 1-5-1 (GZAAS 20-0424), living culture GZCC 19-0529.

Notes: Our collection GZAAS 20-0424 matches the original diagnosis of the holotype of Distoseptispora lignicola (MFLU 18-1458) [13]. Comparison of their LSU and ITS sequences showed 100% and 99.43% (526/529bp) similarity, respectively. We therefore identify our two collections as D. lignicola and report a new geographical record of this species in China.

Figure 7. Distoseptispora lignicola (GZAAS20-0424). (a,b) Colonies on natural substrate. (c-f) Conidia. (g) Conidiophores. (h) Conidiophore and a conidium. (i) Germinated conidium. (j,k) Culture, j from above, k from below. Scale bars: (c-i) 30 μm.

Cultural characteristics: conidia germinating on PDA within 24 h and germ tubes produced from both ends. Colonies on PDA reaching 5–10 mm diam. after 14 days at 25 °C, in natural light, circular, with velvety, dense, grayish brown mycelium on the surface with entire margin; in reverse dark brown.

Material examined: China, Guizhou Province, Dushan District, 25°57.9′ N, 107°39′ E, on decaying wood submerged in a freshwater stream, 26 Aug 2017, J Yang, SG 75-1 (HKAS 112652, holotype), ex-type living culture GZCC 20-0434; additional sequence, SSU: MZ868767.

Notes: In the phylogenetic analyses, Distoseptispora verrucosa clustered with D. lancangjiangensis, D. suoluoensis, and D. bambusae. Their systematic placement is correlated to the highly similar morphological characters in having dark brown conidiophores with rounded apex and narrowly obclavate, rostrate, euseptate, verrucose conidia with a dark scar at the base. They differ in conidial color and dimensions: D. verrucosa has the smallest, olivaceous brown conidia 41–63 × 8.8–12.6 μm; D. suoluoensis [10] has the largest, dark yellowish brown to dark olivaceous brown conidia (65–)80–125(–145) × 8–13 μm usually with a secondary conidium; D. bambusae [23] and D. lancangjiangensis [44] possess brown or olivaceous conidia measuring 45–74 × 5.5–9.5 μm and 64–84 × 9–10 μm, respectively. The length of conidiophores of D. verrucosa (92–250 μm) and D. suoluoensis (80–250 μm) are comparable and longer than that in D. bambusae (40–96 μm) and D. lancangjiangensis...
Phylogenetically, *D. verrucosa* was sister to *D. suoluoensis*, showing 99.88% (838/839 bp), 98.65% (513/520 bp), 98.44% (884/898 bp), and 98.25% (1015/1033 bp) sequence identity of LSU, ITS, TEF1α, and RPB2 sequences, respectively. *Distoseptispora verrucosa* resembles *Sporidesmium tengii* and *S. tunicatum* with long conidiophores and obclavate, rostrate, euseptate conidia. However, conidiophores in the latter two species are truncate at the apex and conidia lack a basal darkened scar. *Sporidesmium tengii* [6] differs by shorter conidiophores (60–100 µm) and smooth-walled, smaller conidia (45–50 × 7–8.5 µm). *Sporidesmium tunicatum* [49] has shorter conidiophores (110–180 µm), verrucose but slightly longer conidia (43–75 × 9–13 µm) with an apical sheath. Molecular DNA data are not available for *S. tengii* and *S. tunicatum*.

**Figure 8.** *Distoseptispora verrucosa* (HKAS 112652, holotype). (a) Conidiophore with a conidium. (b,c) Conidiophores. (d) Germinated conidium. (e–g) Conidia. (h,i) Culture, h from above, i from below. Scale bars: (a) 50 µm, (b–d) 30 µm, (e–g) 20 µm.

**5. Discussion**

The establishment of *Distoseptispora* [9] was based on morphology and molecular DNA data. More than 30 species in the genus are supported by sequence data. The genus forms monophyletic clade (Figure 1) distinct from other sporidesmium-like taxa. Members in the genus mainly occur in the asexual morph, forming effuse, hairy colonies (144–204 µm).
on decaying wood, bamboo culms, plant stems, rachis, and fallen leaves from terrestrial and freshwater habitats. The morphological concept of *Distoseptispora* is macronematous, mononematous, solitary or fasciculate conidiophores, sometimes elongating percurrently and rarely reduced to conidiogenous cells; monoblastic or polyblastic, cylindrical or clavate conidiogenous cells; conidia are cylindrical, obclavate, rostrate, ellipsoidal, obvoid or fusiform, subhyaline, olivaceous, dark green, brown or yellowish-brown to reddish-brown, euseptate or distoseptate, rarely muriform, sometimes born a secondary conidium, with or without septal pore and mucilaginous sheath. The characters delineating the genus *Distoseptispora* also cover the criteria of *Distoseptispora* among sporidesmium-like species, Su et al. [9] recognized the distoseptate *Ellisembia* as a synonym of the redefined *Sporidesmium sensu stricto* which forms a robust monoclade and accommodates species with distoseptate/euseptate, obclavate or subcylindrical conidia, and conidiophores with or without percurrent extensions. In this study, we follow Hyde et al. [12] in treating them as separate taxa because of their unclear relationship due to the absence of molecular DNA data from their type species.

Several sexual morphs have been linked to *Ellisembia* and *Sporidesmium* through cultural and/or molecular studies. *Ellisembia folliculata* (sexual morph: *Lecythothecium duriligum*) [50] and *E. aurea* [12] differ from the sexual morph *D. hyalina* by versicolorous ascospores and position within *Chaetosphaeriaceae* (*Chaetosphaeriales, Sordariomycetes*). *Sporidesmium thailandense* [10,48] and *S. lignicola* [13] can be distinguished by brown ascomata with a hyaline neck and compact, elongated cells of the ascomatal wall, outer layer undifferentiated from host tissue, and their systematic placement in *Sporidesmiaceae* (*Sporidesmiales, Sordariomycetes*). The morphology of sexual morphs of sporidesmium-like genera along with molecular DNA data characterizes their identification although they have similar asexual morphs.

It is challenging to identify some *Distoseptispora* species with highly similar morphology, such as those of *D. multiseptata*, *D. phangngaensis*, and *D. xishuangbannaensis*. Still, they can be well separated by molecular DNA data [10,16,18]. Some *Distoseptispora* species have a wide range of conidial length, for example, conidia of *D. multiseptata* are 95–290 μm long of the holotype but 300–700 μm long in the additional collection; those in *D. phangngaensis* 165–350 μm long and *D. tectonigena* 83–360 μm long [9,10,16]. The indeterminate conidial length may depend on the incubation period. Thus, conidial length is less taxonomically informative in separating some *Distoseptispora* species. The type of septa, however, is proven to have no taxonomic significance for generic delineation of sporidesmium-like taxa, but it is informative at the species level [10,14].

Su et al. [9] accepted two former *Ellisembia* species *E. adscendens* and *E. leonensis* in *Distoseptispora* that were not validly published, based on the non-type strains “*Distoseptispora adscendens*” HKUCC 10820 and “*D. leonensis*” HKUCC 10822 lacking associated morphology. *Ellisembia adscendens* was initially introduced as *Sporidesmium adscendens* forming elongated black patches on the pileus of *Polyporus versicolor* Fr. (No. 1345) which was collected on the underside of timber in the Falkland Islands [51]. *Ellisembia adscendens* is a widespread species. It is similar to *E. vaga* [52] but differs by wider conidia. *Ellisembia adscendens* and *E. vaga* highly resemble a group of morphologically indistinguishable species in *Distoseptispora*, e.g., *D. multiseptata* and *D. phangngaensis*. They are probably members of *Distoseptispora* because of the typical *Distoseptispora* morphology in having short conidiophores and subcylindrical long conidia. On the other hand, the large number of specimens of *Ellisembia adscendens* may be a complex comprising several separate taxa. *Ellisembia leonensis* [47] is characterized by relatively long conidiophores with percurrent extensions and fusiform to rostrate, distoseptate conidia. It matches the morphological concept of both *Distoseptispora* and *Sporidesmium sensu stricto*. At present, we avoid reassigning *E. adscendens* and *E. leonensis* in *Distoseptispora* until their systematic placement is confirmed by molecular data from type materials or resolved by epitypifications.
Author Contributions: Conceptualization: J.Y., K.D.H. and Z.-Y.L.; methodology: J.Y. and L.-L.L.; formal analysis and investigation: J.Y., L.-L.L. and W.-L.L.; resources: K.D.H., Z.-Y.L. and E.B.G.J.; writing—original draft preparation, J.Y.; writing—review and editing, J.Y., E.B.G.J., K.D.H., L.-L.L., Z.-Y.L. and W.-L.L.; supervision, E.B.G.J., K.D.H. and Z.-Y.L.; funding acquisition, L.-L.L. and Z.-Y.L. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Foundation of Key Laboratory of Microbial Resources Collection and Preservation, Ministry of Agriculture and Rural Affairs (Project no. KLMRC2021-08).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The datasets generated for this study can be found in the NCBI database.

Acknowledgments: E.B.G.J. is supported under the Distinguished Scientist Fellowship Program (DSFP), King Saud University, Saudi Arabia. J.Y. is grateful to Shaun Pennycook for corrections on the Latin names of the novel taxa. Jian-Kui Liu is thanked for the corrections on the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Subramanian, C.V. A reassessment of Sporidesmium (hyphomycetes) and some related taxa. Proc. Indian Nat. Sci. Acad. 1992, 58, 179–190.
2. Seifert, K.A.; Morgan-Jones, G.; Gams, W.; Kendrick, B. The Genera of Hyphomycetes; CBS-KNAW Fungal Biodiversity Centre: Utrecht, The Netherlands, 2011; pp. 1–997.
3. Kirk, P. New or interesting microfungi VI. Sporidesmiella gen. nov. (Hyphomycetes). Trans. Br. Mycol. Soc. 1982, 79, 479–489. [CrossRef]
4. Hernández-Gutiérrez, A.; Sutton, B.C. Imimyces and Linkosia, two new genera segregated from Sporidesmium sensu lato, and redescription of Polydesmus. Mycol. Res. 1997, 101, 201–209. [CrossRef]
5. Shoemaker, R.A.; Hambleton, S. “Helminthosporium” aurinum, Polydesmus elegans, Imimyces, and allies. Can. J. Bot. 2001, 79, 592–599. [CrossRef]
6. Wu, W.P.; Zhuang, W.Y. Sporidesmium, Endophagmiella and related genera from China. Fungal Divers. Res. Ser. 2005, 15, 1–351.
7. Rěbllová, M. Studies in Chaetosphaeria sensu lato III. Umbrinosphaeria gen. nov. and Miyoshiella with Sporidesmium anamorphs. Mycotaxon 1999, 71, 13–43.
8. 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][PubMed]
9. Su, H.Y.; Hyde, K.D.; Maharachchikumbura, S.S.N.; Ariyawansa, H.A.; Luo, Z.L.; Promputtha, I.; Tian, Q.; Lin, C.G.; Shang, Q.J.; Zhao, Y.C.; et al. The families Distoseptisporaceae fam. nov., Kirschsteinitiotheliaceae, Sporormiaceae and Torulaceae, with new species from freshwater in Yunnan Province, China. Fungal Divers. 2016, 80, 375–409. [CrossRef]
10. Yang, J.; Maharachchikumbura, S.S.N.; Liu, J.-K.; Hyde, K.D.; Jones, E.B.G.; Al-Sadi, A.M.; Liu, Z.-Y. Pseudostanjeighuesia aquitropica gen. et sp. nov. and Sporidesmium sensu lato species from freshwater habitats. Mycol. Prog. 2017, 17, 591–616. [CrossRef]
11. Hyde, K.D.; Norphanphoun, C.; Maharachchikumbura, S.S.N.; Bhat, D.J.; Jones, E.B.G.; Bundhun, D.; Chen, Y.J.; Bao, D.F.; Boonmee, S.; Calabon, M.S.; et al. Refined families of Sordariomycetes. Mycosphere 2020, 11, 305–1099. [CrossRef]
12. Hyde, K.D.; Tennakoon, D.S.; Jeewon, R.; Bhat, D.J.; Maharachchikumbura, S.; Rossi, W.; Leonardi, M.; Lee, H.B.; Mun, H.Y.; Houbraken, J.; et al. Fungal diversity notes 1036–1150: Taxonomic and phylogenetic contributions on genera and species of fungal taxa. Fungal Divers. 2019, 96, 1–242. [CrossRef]
13. Luo, Z.-L.; Hyde, K.D.; Liu, J.-K.; Maharachchikumbura, S.S.N.; Jeewon, R.; Bao, D.-F.; Bhat, D.J.; Lin, C.-G.; Li, W.-L.; Yang, J.; et al. Freshwater Sordariomycetes. Fungal Divers. 2019, 99, 451–660. [CrossRef]
14. Monkaï, J.; Boonmee, S.; Ren, G.-C.; Wei, D.-P.; Phookamsak, R.; Mortimer, P.E. Distoseptispora hydei sp. nov. (Distoseptisporaceae), a novel lignicolous fungus on decaying bamboo in Thailand. Mycotaxa 2020, 459, 93–107. [CrossRef]
15. Xia, J.W.; Ma, Y.R.; Li, Z.; Zhang, X.G. Acrodictys-like wood decay fungi from southern China, with two new families Acrodictyaceae and Junuwangiacae. Sci. Rep. 2017, 7, 1–21. [CrossRef]
16. Hyde, K.D.; Hongsanan, S.; Jeewon, R.; Bhat, D.J.; McKenzie, E.H.C.; Jones, E.B.G.; Phookamsak, R.; Ariyawansa, H.; Boonmee, S.; Zhao, Q.; et al. Fungal diversity notes 367–490: Taxonomic and phylogenetic contributions to fungal taxa. Fungal Divers. 2016, 80, 1–270. [CrossRef]
17. Luo, Z.L.; Hyde, K.D.; Liu, J.K.; Bhat, D.J.; Bao, D.F.; Li, W.L.; Su, H.Y. Lignicolous freshwater fungi from China II: Novel Distoseptispora (Distoseptisporaceae) species from northwestern Yunnan Province and a suggested unified method for studying lignicolous freshwater fungi. Mycosphere 2018, 9, 444–461. [CrossRef]
18. Tibpromma, S.; Hyde, K.D.; McKenzie, E.H.C.; Bhat, D.J.; Phillips, A.; Wanasinghe, D.N.; Samarakoon, M.C.; Jayawardena, R.; Dissanayake, A.J.; Tennakoon, D.S.; et al. Fungal diversity notes 840–928: Micro-fungi associated with Pandanaceae. Fungal Divers. 2018, 93, 1–160. [CrossRef]
19. Crous, P.; Wingfield, M.; Lombard, L.; Roets, F.; Swart, W.; Alvarado, P.; Carnegie, A.; Moreno, G.; Luangsaa-Ard, J.; Thangavel, R.; et al. Fungal Plant description sheets: 951–1041. Pers. Mol. Phylogeny Evol. Fungi 2019, 43, 223–425. [CrossRef]

20. Phookamsak, R.; Hyde, K.D.; Jeewon, R.; Bhat, D.J.; Jones, E.B.G.; Maharachchikumbura, S.; Raspe, O.; Karunarathna, S.C.; Wasingshe, D.; Hongsanan, S.; et al. Fungal diversity notes 929–1035: Taxonomic and phylogenetic contributions on genera and species of fungi. Fungal Divers. 2019, 95, 1–273. [CrossRef]

21. Phookamsakda, C.; McKenzie, E.H.C.; Phillips, A.; Jones, E.B.G.; Bhat, D.J.; Stadler, M.; Bhunjun, C.S.; Wasingshe, D.N.; Thongbai, B.; Camporesi, E.; et al. Microfungi associated with Cladophora (Ranunculaceae) with an integrated approach to delimiting species boundaries. Fungal Divers. 2020, 102, 1–203. [CrossRef]

22. Song, H.-Y.; El Sheikha, A.F.; Zhai, Z.-J.; Zhou, J.-P.; Chen, M.-H.; Huo, G.-H.; Huang, X.-G.; Huo, D.-M. Distoseptispora longispora sp. nov. from freshwater habitats in China. Mycotaxon 2020, 135, 513–523. [CrossRef]

23. Sun, Y.; Goonasekara, I.D.; Thambugala, K.M.; Jayawardena, R.S.; Wang, Y.; Hyde, K.D. Distoseptispora bambusa sp. nov. (Distoseptisporaceae) on bamboo from China and Thailand. Biodivers. Data J. 2020, 8, e53678. [CrossRef]

24. Dong, W.; Hyde, K.D.; Jeewon, R.; Doilom, M.; Yu, X.D.; Wang, G.N.; Liu, N.G.; Hu, D.M.; Nalumpang, S.; Zhang, H. Towards a natural classification of annulatascaceae-like taxa II: Introducing five new genera and eighteen new species from freshwater. Mycosphere 2021, 12, 1–88. [CrossRef]

25. Li, W.-L.; Liu, Z.-P.; Zhang, T.; Dissanayake, A.J.; Luo, Z.-L.; Su, H.-Y.; Liu, J.-K. Additions to Distoseptispora (Distoseptisporaceae) associated with submerged decaying wood in China. Phytotaxa 2021, 520, 75–86. [CrossRef]

26. Wijayawardene, N.N.; Hyde, K.D.; Al-Ani, L.K.T.; Tedersoo, L.; Haelweaters, D.; Rajeshkumar, K.C.; Zhao, R.L.; Aptroot, A.; Leontyev, D.V.; Saxena, R.K.; et al. Outline of Fungi and fungus-like taxa. Mycosphere 2020, 11, 1060–1456. [CrossRef]

27. Hyde, K.D.; Bao, D.-F.; Hongsanan, S.; Chethana, K.W.T.; Yang, J.; Suwannarach, N.; et al. Distoseptispora bambusa sp. nov. (Distoseptisporaceae) provides evidence for five new orders and six new families. Fungal Divers. 2021, 107, 1–105. [CrossRef]

28. Hydie, K.D.; Fryar, S.; Tian, Q.; Bahkali, A.H.; Xu, J. Lignicolous freshwater fungi along a north–south latitudinal gradient in China and Thailand. MycoKeys 2020, 8, 1–203. [CrossRef]

29. Jayasiri, S.C.; Hyde, K.D.; Ariyawansa, H.; Bhat, J.; Buyck, B.; Cai, L.; Dai, Y.-C.; Abd-Elsalam, K.A.; Ertz, D.; Hidayat, I.; et al. The Fungal diversity notes 929–1035: Taxonomic and phylogenetic contributions on genera and species. Fungal Divers. 2020, 102, 1–203. [CrossRef]

30. Index Fungorum. Available online: http://www.indexfungorum.org/names/names.asp (accessed on 3 August 2021).

31. 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]

32. 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.

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

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

35. Katoh, K.; Standley, D.M. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Mol. Biol. Evol. 2013, 30, 772–780. [CrossRef] [PubMed]

36. 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]

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

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

39. Katoh, K.; Standley, D.M. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Mol. Biol. Evol. 2013, 30, 772–780. [CrossRef] [PubMed]

40. 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]

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

42. Nylander, J. MrModeltest2 v. 2.3 (Program for Selecting DNA Substitution Models Using PAUP*); Evolutionary Biology Centre: Uppsala, Sweden, 2008.

43. Huelsenbeck, J.P.; Ronquist, F. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 2001, 17, 754–755. [CrossRef] [PubMed]

44. Larget, B.; Simon, D.L. Markov Chain Monte Carlo Algorithms for the Bayesian Analysis of Phylogenetic Trees. Mol. Biol. Evol. 1999, 16, 750–759. [CrossRef]

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

46. 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]

47. Shoemaker, R.A.; White, G.P. Lasiosphaeria caesaritroides with Sporidesmium hormiscoioides and L. iriseptata with Sporidesmium adscendens. Sydowia 1985, 38, 278–283.

48. Shen, H.W.; Bao, D.F.; Hyde, K.D.; Su, H.Y.; Bhat, D.J.; Luo, Z.L. New species and records of Distoseptispora (Distoseptisporaceae) from freshwater habitats in China and Thailand. MycoKeys 2021, in press. [CrossRef]
45. Su, H.-Y.; Udayanga, D.; Luo, Z.-L.; Manamgoda, D.; Zhao, Y.-C.; Yang, J.; Liu, X.-Y.; McKenzie, E.H.C.; Zhou, D.-Q.; Hyde, K.D. Hyphomycetes from aquatic habitats in Southern China: Species of Curvularia (Pleosporaceae) and Phragmocephala (Melannomataceae). *Phytotaxa* 2015, 226, 201–216. [CrossRef]

46. Hongsanan, S.; Hyde, K.D.; Phookamsak, R.; Wanasinghe, D.N.; McKenzie, E.H.C.; Sarma, V.V.; Boonmee, S.; Lücking, R.; Bhat, D.J.; Liu, N.G.; et al. Refined families of Dothideomycetes: Dothideomycetidae and Pleosporomycetidae. *Mycosphere* 2020, 11, 1553–2107. [CrossRef]

47. Ellis, M.B. Clasterosporium and some allied Dematiaceae-Phragmosporeae: I. Mycol. Pap. 1958, 70, 1–89.

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

49. Mena-Portales, J.; Hernández-Restrepo, M.; Guerrero, J.; Minter, D.W.; Gené, J. New species of Penzigomyces, Sporidesmium and Stanjehughesia from plant debris in Spain. *Nova Hedwig.* 2016, 103, 359–371. [CrossRef]

50. Reblová, M.; Winka, K. Generic concepts and correlations in ascomycetes based on molecular and morphological data: Lecythothecium duriligni gen. et sp. nov. with a Sporidesmium anamorph, and Ascolacicola austriaca sp. nov. *Mycologia* 2001, 93, 478–493. [CrossRef]

51. Berkeley, M. XXXII.—Notice of some Fungi collected by C. Darwin, Esq., during the expedition of H. M. Ship Beagle. *J. Nat. Hist.* 1840, 4, 291–293. [CrossRef]

52. Ellis, M.B. *Dematiaceous Hyphomycetes*; Kew, Commonwealth Mycological Institute: Surrey, UK, 1971; pp. 117–118.