

# Three novel species of *Distoseptispora* (Distoseptisporaceae) isolated from bamboo in Jiangxi Province, China

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## Abstract

Decaying bamboo in freshwater is a unique eco-environment for fungi. Three new *Distoseptispora* (Distoseptisporaceae) species, *D. meilingensis*, *D. yongxiuensis* and *D. yunjushanensis* from submerged decaying bamboo culms in Jiangxi Province, China, were discovered, based on phylogenetic analyses and morphological characters. The combined data of ITS-LSU-SSU-*Tef1* sequences were used to infer the phylogenetic relationship between *D. meilingensis*, *D. yongxiuensis*, *D. yunjushanensis* and related species. Both molecular analyses and morphological data supported *D. meilingensis*, *D. yongxiuensis* and *D. yunjushanensis* as three independent taxa.

## Keywords

Hyphomycetes, phylogenetic analysis, *Sordariomycetes*, taxonomy, three new taxa

## Introduction

*Distoseptispora* was established by Su et al. (2016) as the single genus in *Distoseptisporaceae*. This genus morphologically resembles *Ellisembia* and *Sporidesmium* (Subramanian 1992; Shenoy et al. 2006; Yang et al. 2018), while they are not in sister clades in molecular phylogenetic trees (Su et al. 2016; Luo et al. 2019; Hyde et al. 2020, 2021). Multigene analysis showed that *Distoseptispora* formed a stable and well-supported clade within *Distoseptisporales* as a sister clade to *Aquapteridospora* (Luo et al. 2019; Hyde et al. 2020, 2021). *Aquapteridospora* has been raised as a new family *Aquapteridosporaceae* for the divergence time (110 million years ago (mya)) falling within the family-level range (50–130 mya) (Hyde et al. 2021). *Aquapteridospora* and *Distoseptispora* are similar in having macronematous, mononematous, unbranched conidiophores, mono- or polyblastic, holoblastic, conidiogenous cells and acrogenous, solitary conidia. *Distoseptispora* can easily be distinguished from *Aquapteridospora* by its short conidiophores and obclavate or cylindrical, rostrate, euseptate or distoseptate conidia. Additionally, *Distoseptispora* has terminal conidiogenous cells which lack circular scars (Hyde et al. 2021).

*Distoseptispora* was regarded as saprobic lignicolous fungal genus, which has the ability to decompose lignocelluloses in wood (Wong et al. 1998; Hyde et al. 2016). In recent years, the number of new taxa in *Distoseptispora* is steadily increasing and currently comprises 35 species, which have been discovered mostly in freshwater and some in terrestrial habitats (Su et al. 2016; Dong et al. 2021; Hyde et al. 2021; Li et al. 2021). Except for the two species, *D. adscendens* and *D. leonensis*, which were found from Hungary and Malaysia, respectively (Shoemaker and White 1985; McKenzie 1995), 19 of the 33 species has been discovered in Thailand, while the remaining 14 species were introduced from China (Table 2). In China, *Distoseptispora* species are almost exclusively reported in Yunnan Province (Su et al. 2016; Luo et al. 2018; Hyde et al. 2019; Phookamsak et al. 2019; Li et al. 2021). Only three species, *D. martinii*, *D. bambusae* and *D. suoluensis*, have been discovered from Guizhou Province (Xia et al. 2017; Yang et al. 2018; Sun et al. 2020). In this study, we introduce three new species of *Distoseptispora*, including *D. meilingensis*, *D. yongxiensis* and *D. yunjushanensis* from Jiangxi Province in subtropical China. We describe the novel species, based on morphological illustrations and phylogenetic analyses. A synopsis of the morphological characters of *Distoseptispora* species is also provided.

## Materials and methods

### Samples collection, morphological observation and isolation

Dead bamboo samples from different freshwater habitats in Jiangxi Province, China, were taken to the lab for detection of fungi using a Nikon SMZ-1270 microscope (Nikon Corporation, Japan). Micro-morphological characteristics were observed and

captured using a Nikon ECLIPSE Ni-U compound microscope (Nikon Corporation, Japan), equipped with a Nikon DS-Fi3 camera. All measurements were calculated using PhotoRuler Ver. 1.1 software (The Genus Inocybe, Hyogo, Japan) and figures were processed using Adobe Photoshop CS6 Extended version 10.0 software (Adobe Systems, USA). Pure cultures of the fungi were obtained by the single spore isolation method (Chomnunti et al. 2014). The germinating conidia were transferred to potato dextrose agar (PDA) and incubated at 25 °C for two weeks. The fungal cultures were deposited in the Jiangxi Agricultural University Culture Collection (JAUCC) and the holotypic specimens with MycoBank numbers (842065, 842066, 842067) were deposited in the Herbarium of Fungi, Jiangxi Agricultural University (HFJAU).

### DNA extraction, PCR amplification and sequencing

Fungal genomes were extracted from fresh mycelium using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1987). Four deoxyribonucleic acid (DNA) barcodes (ITS, LSU, SSU and *Tef-1 $\alpha$* ) were chosen for polymerase chain reaction (PCR) using the primer pairs ITS1/ITS4 (White et al. 1990), LR0R/LR7 (Hopple and Vilgalys 1999), NS1/NS4 (White et al. 1990) and EF983F/EF2218R (Örstadius et al. 2015), respectively. Amplification reactions were carried out in a volume of 25  $\mu$ l, containing 12.5  $\mu$ l 2  $\times$  Taq PCR MasterMix (Qingke, Changsha, China), 1  $\mu$ l each forward and reverse primer (0.2  $\mu$ M), 1  $\mu$ l template DNA (circa 50–100 ng) and 9.5  $\mu$ l ddH<sub>2</sub>O. Amplifications were conducted under the following conditions: 3 min at 98 °C, 35 cycles of 10 s at 98 °C, 10 s of annealing at 55 °C and extension at 72 °C for 10 s, with a final 2-min extension at 72 °C. Sequencing reactions were conducted with the corresponding forward and reverse primers commercially by QingKe Biotechnology Co. (Changsha, China). All sequences were edited with Sequencher v.4.14 (GeneCodes Corporation, USA) and have been deposited in the NCBI GenBank database (Table 1).

### Data analyses

Reference sequences of 35 *Distoseptispora* species and three *Aquapteridospora* species, based on recent publications (Luo et al. 2019; Hyde et al. 2020; Monkai et al. 2020; Dong et al. 2021, Li et al. 2021) were downloaded from GenBank. Detailed information on fungal strains used in this paper are provided in Table 1.

All obtained sequences were aligned using the online service of MAFFT (Madeira et al. 2019) and refined manually in MEGA v.7.0 (Kumar et al. 2016). Maximum Likelihood (ML) analysis was conducted with RAxML 8.0 using a GTR-GAMMA model of evolution (Stamatakis 2014). Non-parametric bootstrap analysis was implemented using 1,000 replicates to estimate ML bootstrap (BS) values. Bayesian Inference (BI) analysis was carried out with MrBayes v.3.2 under partitioned models (Ronquist et al. 2012). The best-fit models of nucleotide substitutions were selected according to the Akaike Information Criterion (AIC) implemented in jModelTest2.1.1

**Table 1.** Sequences used in this study.

| Taxa                                       | Voucher           | LSU             | ITS             | SSU             | <i>Tef1α</i>    |
|--------------------------------------------|-------------------|-----------------|-----------------|-----------------|-----------------|
| <i>Aquapteridospora aquatica</i>           | MFLUCC 17-2371    | NG_075413       | NR_172447       | —               | —               |
| <i>Aquapteridospora fusiformis</i>         | MFLU 18-1601      | MK849798        | MK828652        | —               | MN194056        |
| <i>Aquapteridospora lignicola</i>          | MFLU 15-1172      | KU221018        | —               | —               | —               |
| <i>Distoseptispora adscendens</i>          | HKUCC 10820       | DQ408561        | —               | —               | —               |
| <i>Distoseptispora appendiculata</i>       | MFLUCC 18-0259    | MN163023        | MN163009        | —               | MN174866        |
| <i>Distoseptispora aquatica</i>            | GZCC 19-0452      | MZ227216        | MW133908        | MW134689        | —               |
| <i>Distoseptispora aquatica</i>            | MFLUCC 16-0904    | MK849794        | MK828649        | MK828315        | —               |
| <i>Distoseptispora aquatica</i>            | MFLUCC 18-0646    | MK849793        | MK828648        | —               | —               |
| <i>Distoseptispora aquatica</i>            | MFLUCC 16-1357    | MK849796        | MK828650        | MK828317        | —               |
| <i>Distoseptispora aquatica</i>            | S-965             | MK849792        | MK828647        | MK828314        | MN194051        |
| <i>Distoseptispora bambusae</i>            | MFLUCC 20-0091    | NG_074430       | NR_170068       | NG_070348       | —               |
| <i>Distoseptispora bambusae</i>            | MFLU 20-0261      | MT232718        | MT232713        | MT232716        | MT232880        |
| <i>Distoseptispora bambusae</i>            | MFLU 17-1653      | MT232717        | MT232712        | —               | —               |
| <i>Distoseptispora cangshanensis</i>       | MFLUCC 16-0970    | MG979761        | MG979754        | —               | MG988419        |
| <i>Distoseptispora caricis</i>             | CPC 36498         | MN567632        | NR_166325       | —               | —               |
| <i>Distoseptispora clematidis</i>          | MFLUCC 17-2145    | MT214617        | MT310661        | MT226728        | —               |
| <i>Distoseptispora clematidis</i>          | KUN-HKAS 112708   | MW879523        | MW723056        | MW774580        | —               |
| <i>Distoseptispora dehongensis</i>         | KUMCC 18-0090     | MK079662        | MK085061        | —               | MK087659        |
| <i>Distoseptispora euseptata</i>           | MFLUCC 20-0154    | MW081544        | MW081539        | —               | —               |
| <i>Distoseptispora euseptata</i>           | DLUCC S2024       | MW081545        | MW081540        | —               | MW084994        |
| <i>Distoseptispora fasciculata</i>         | KUMCC 19-0081     | NG_075417       | NR_172452       | —               | MW396656        |
| <i>Distoseptispora fluminicola</i>         | DLUCC 0391        | MG979762        | MG979755        | —               | MG988420        |
| <i>Distoseptispora fluminicola</i>         | DLUCC 0999        | MG979763        | MG979756        | —               | MG988421        |
| <i>Distoseptispora guttulata</i>           | MFLU 17-0852      | MF077554        | MF077543        | MF077532        | MF135651        |
| <i>Distoseptispora hydei</i>               | MFLUCC 20-0481    | MT742830        | MT734661        | —               | —               |
| <i>Distoseptispora leonensis</i>           | HKUCC 10822       | DQ408566        | —               | —               | —               |
| <i>Distoseptispora lignicola</i>           | MFLUCC 18-0198    | MK849797        | MK828651        | MK828318        | —               |
| <i>Distoseptispora longispora</i>          | HFJAU 0705        | MH555357        | MH555359        | MH555431        | —               |
| <i>Distoseptispora martinii</i>            | CGMCC 318651      | KX033566        | KU999975        | KX033537        | —               |
| <b><i>Distoseptispora meilingensis</i></b> | <b>JAUCC 4727</b> | <b>OK562396</b> | <b>OK562390</b> | <b>OK562402</b> | <b>OK562408</b> |
| <b><i>Distoseptispora meilingensis</i></b> | <b>JAUCC 4728</b> | <b>OK562397</b> | <b>OK562391</b> | <b>OK562403</b> | <b>OK562409</b> |
| <i>Distoseptispora multiseptata</i>        | MFLUCC 15-0609    | KX710140        | KX710145        | NG_065693       | MF135659        |
| <i>Distoseptispora multiseptata</i>        | MFLU 17-0856      | MF077555        | MF077544        | MF077533        | —               |
| <i>Distoseptispora neostrata</i>           | MFLUCC 18-0376    | MN163017        | MN163008        | —               | —               |
| <i>Distoseptispora obclavata</i>           | MFLUCC 18-0329    | MN163010        | MN163012        | —               | —               |
| <i>Distoseptispora obpyriformis</i>        | DLUCC 0867        | MG979765        | MG979757        | —               | MG988423        |
| <i>Distoseptispora palmarum</i>            | MFLUCC 18-1446    | MK079663        | MK085062        | MK079661        | MK087660        |
| <i>Distoseptispora palmarum</i>            | MFLU 18-0588      | NG_067856       | NR_165897       | —               | MK087660        |
| <i>Distoseptispora phangngaensis</i>       | MFLU 17-0855      | MF077556        | MF077545        | MF077534        | MF135653        |
| <i>Distoseptispora phangngaensis</i>       | MFLUCC 16-0857    | —               | NR_166230       | —               | —               |
| <i>Distoseptispora rayongensis</i>         | MFLUCC 18-0415    | NG_073624       | NR_171938       | NG_073504       | —               |
| <i>Distoseptispora rayongensis</i>         | MFLU 18-1045      | MH457137        | MH457172        | MH457169        | —               |
| <i>Distoseptispora rostrata</i>            | MFLUCC 16-0969    | MG979766        | MG979758        | —               | MG988424        |
| <i>Distoseptispora rostrata</i>            | DLUCC 0885        | MG979767        | MG979759        | —               | MG988425        |
| <i>Distoseptispora rostrata</i>            | MFLU 18-0479      | NG_064513       | NR_157552       | —               | —               |
| <i>Distoseptispora saprophytica</i>        | MFLUCC 18-1238    | NG_075419       | NR_172454       | —               | MW396651        |
| <i>Distoseptispora songkhata</i>           | MFLUCC 18-1234    | MW287755        | MW286482        | —               | MW396642        |
| <i>Distoseptispora submersa</i>            | MFLUCC 16-0946    | MG979768        | MG979760        | —               | MG988426        |
| <i>Distoseptispora suoluensis</i>          | MFLUCC 17-0224    | NG_068552       | NR_168764       | NG_070113       | MF135654        |
| <i>Distoseptispora suoluensis</i>          | MFLU 17-0854      | MF077558        | MF077547        | MF077536        | —               |
| <i>Distoseptispora tectonae</i>            | MFLUCC 15-0981    | MW287763        | MW286489        | —               | MW396641        |
| <i>Distoseptispora tectonae</i>            | MFLUCC 12-0291    | KX751713        | KX751711        | —               | KX751710        |

| Taxa                                         | Voucher           | LSU             | ITS             | SSU             | <i>Tef-1α</i>   |
|----------------------------------------------|-------------------|-----------------|-----------------|-----------------|-----------------|
| <i>Distoseptispora tectonae</i>              | S-2023            | MW081543        | MW081538        | —               | —               |
| <i>Distoseptispora tectonae</i>              | GZ 25             | MH555358        | MH555361        | —               | —               |
| <i>Distoseptispora tectonigena</i>           | MFLUCC 12-0292    | KX751714        | NR_154018       | —               | —               |
| <i>Distoseptispora thailandica</i>           | MFLUCC 16-0270    | MH260292        | MH275060        | MH260334        | MH412767        |
| <i>Distoseptispora thysanolaenae</i>         | KUN-HKAS 112710   | MW879524        | MW723057        | —               | —               |
| <i>Distoseptispora thysanolaenae</i>         | KUN-HKAS 102247   | MK064091        | MK045851        | —               | MK086031        |
| <i>Distoseptispora xishuangbannaensis</i>    | KUMCC 17-0290     | MH260293        | MH275061        | MH260335        | MH412768        |
| <b><i>Distoseptispora yongxiuensis</i></b>   | <b>JAUCC 4725</b> | <b>OK562394</b> | <b>OK562388</b> | <b>OK562400</b> | <b>OK562406</b> |
| <b><i>Distoseptispora yongxiuensis</i></b>   | <b>JAUCC 4726</b> | <b>OK562395</b> | <b>OK562389</b> | <b>OK562401</b> | <b>OK562407</b> |
| <b><i>Distoseptispora yunjushanensis</i></b> | <b>JAUCC 4723</b> | <b>OK562398</b> | <b>OK562392</b> | <b>OK562404</b> | <b>OK562410</b> |
| <b><i>Distoseptispora yunjushanensis</i></b> | <b>JAUCC 4724</b> | <b>OK562399</b> | <b>OK562393</b> | <b>OK562405</b> | <b>OK562411</b> |
| <i>Distoseptispora yunnansis</i>             | MFLUCC 20-0153    | MW081546        | MW081541        | —               | MW084995        |

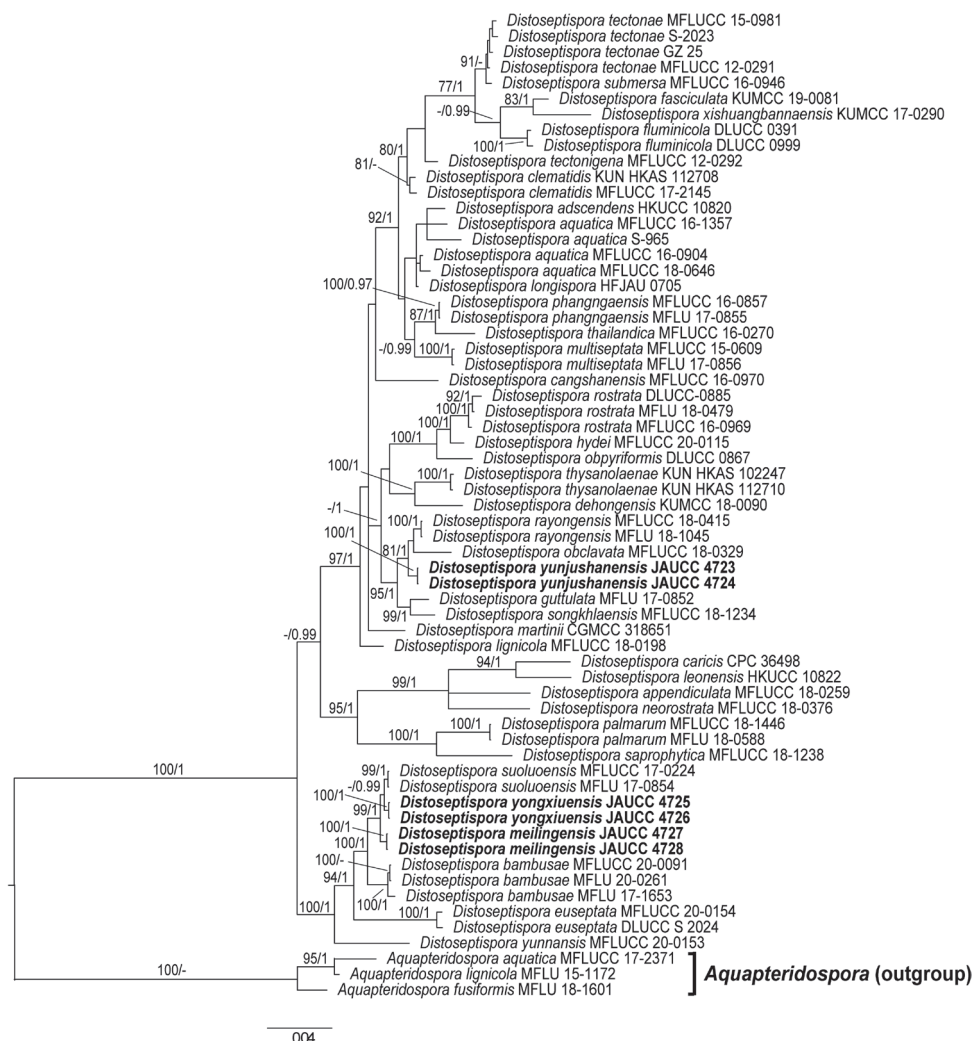
“—”, sequence is unavailable.

(Darriba et al. 2012) on XSEDE in the CIPRES web portal (Miller et al. 2010). The models for ITS, LSU, SSU and *Tef-1α* datasets used for phylogenetic analysis are GTR+I+G model (-lnL = 4965.1122), GTR+I+G model (-lnL = 2716.7536), TIM2+G (-lnL = 4344.2295) and TrN+I+G (-lnL = 4479.4914), respectively. The datasets were run for 10,000,000 generations, with four chains and trees sampled every 1,000 generations. The first 10% trees were discarded as burn-in. We used three *Aquapteridospora* species as outgroups. The Bayesian consensus tree with posterior probabilities (PP) was visualised with FigTree v.1.4.4 (Rambaut 2018) and was edited in Adobe Illustrator CS6. Our aligned matrices and trees can be obtained from TreeBASE (<http://purl.org/phylo/treebase/phyloids/study/TB2:S29465>).

Results

Molecular phylogenetic results

According to the results of BLAST analysis and sequence alignment, the ITS sequence of *D. meilingensis* has 11 different loci from those of *D. yongxiuensis*, the ITS sequence of which shares 99% similarity (five different loci) with that of *D. suoluoensis*. The ITS sequence of *D. yunjushanensis* is 97% similar (22 different loci) to that of *D. obclavata*. The aligned matrix for the combined analysis, ITS + LSU + SSU + *Tef-1α*, had 4015 bp, including ITS 596 bp, LSU 799 bp, SSU 1715 bp and *Tef-1α* 905 bp. The topologies of trees generated by ML and BI analyses are highly similar. The Bayesian tree with BS and PP is shown in Fig. 1. All species of *Distoseptispora* form a monophyletic group (BS/PP = 100/1.00). *D. yongxiuensis* groups together with *D. suoluoensis* (BS/PP = 60/0.99). These two species and collections of *D. meilingensis* form a strong-supported clade (BS/PP = 99/1.00), which is strongly linked with sequences of *D. bambusae* (BS/PP = 100/1.00). Collections of *D. yunjushanensis* form a moderate-support clade (BS/PP = 81/1.00) with the lineage consisting of *D. obclavata* and *D. rayongensis*.



**Figure 1.** Phylogenetic tree of *Distoseptispora*, inferred from the combined regions (ITS-LSU-SSU-*Tef 1α*) using Bayesian Inference (BI) analysis. The *Aquapteridospora* clade was used as the outgroup. The lineages with new species were shown in bold. PP  $\geq 0.95$  and BS  $\geq 75\%$  were indicated around the branches. The new sequences generated in this study are given in bold.

## Taxonomy

### *Distoseptispora meilingensis* Z. J. Zhai & D. M. Hu, sp. nov.

Mycobank No: 842067

Fig. 2

**Etymology.** Referring to the collecting site of the Meiling Mountain in Jiangxi Province, China.

**Holotype.** HFJAU 10009.



**Figure 2.** *Distoseptispora meilingensis* (HFJAU10009, holotype) **a, b** colonies on bamboo culms **c–e** conidiophores with conidia **f** conidiogenous cells **g, n** conidiogenous cells with conidia **h–m** conidia **o** germinating conidium **p** culture on PDA from above and reverse. Scale bars: 100  $\mu$ m (**a, b**), 20  $\mu$ m (**c–e, o**), 5  $\mu$ m (**f–n**).

**Description.** Saprobic on culms of bamboo. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. **Colonies** effuse, brown to dark brown, hairy. **Mycelium** mostly immersed, composed of pale to dark brown, septate, branched, smooth, hyaline to subhyaline hyphae. **Conidiophores** 69–192 × 4–7 µm ( $\bar{x}$  = 120.6 × 5.5 µm, n = 25), macronematous, mononematous, erect, cylindrical, straight or slightly flexuous, 5–12-septate, yellowish-brown or brown, robust at the base. **Conidiogenous cells** holoblastic, mono- to polyblastic, integrated, terminal, cylindrical, yellowish-brown or brown. **Conidia** 32–64.5 × (7–)9–12.5 µm ( $\bar{x}$  = 43.7 × 9.8 µm, n = 30), acrogenous, solitary, straight or slightly curved, obclavate, 5–7-distoseptate, thick-walled, rounded at the apex, truncate at the base, tapering towards apex, bud scars disjunctors at base, mostly brown when mature.

**Cultural characteristics.** Conidia germinating on PDA within 24 h and germ tubes produced from both ends. Colonies on PDA reaching 17–23 mm diam. at two weeks at 25 °C, in natural light, circular, with dense, light olivaceous mycelium on the surface with entire margin; reverse brown to dark brown.

**Material examine.** CHINA, Jiangxi Province, Nanchang City, Meiling Mountain, alt. 305 m, near 28.79°N, 115.72°E, on decaying bamboo culms submerged in a fresh-water stream, 16 Aug 2021, Z. J. Zhai, SLT-3 (HFJAU10009, **holotype**), ex-type living culture, JAUCC 4727 = JAUCC 4728.

**Notes.** *Distoseptispora meilingensis* clusters with the clade including *D. suoluoensis* and *D. yongxiuensis* with high support in the phylogenetic tree (Fig. 1). *Distoseptispora meilingensis* is distinct from *D. suoluoensis* (Yang et al. 2018) and *D. yongxiuensis* by its conidial colour (mostly brown, yellowish-brown to dark olivaceous and yellowish-brown or brown, respectively). Furthermore, *D. meilingensis* has shorter conidia (32–64.5 µm vs. (65–)80–125(–145) µm) than those of *D. suoluoensis* (Yang et al. 2018) and slightly shorter conidiophores (69–192 µm vs. 112–253 µm) than those of *D. yongxiuensis*. *Distoseptispora meilingensis* resembles *D. bambusae* in similar habitats and polyblastic conidiogenous cells (Sun et al. 2020). However, *D. meilingensis* can be distinguished from *D. bambusae* in its longer conidiophores (69–192 µm vs. 40–96 µm), slightly wider (up to 12.5 µm vs. up to 9.5 µm) and brighter (light brown vs. brown) conidia (Sun et al. 2020). A comparison of morphological features of *Distoseptispora* species is provided in Table 2.

***Distoseptispora yongxiuensis* Z. J. Zhai & D. M. Hu, sp. nov.**

MycoBank No: 842066

Fig. 3

**Etymology.** With reference to Yongxiu, from where the holotype was collected.

**Holotype.** HFJAU10007

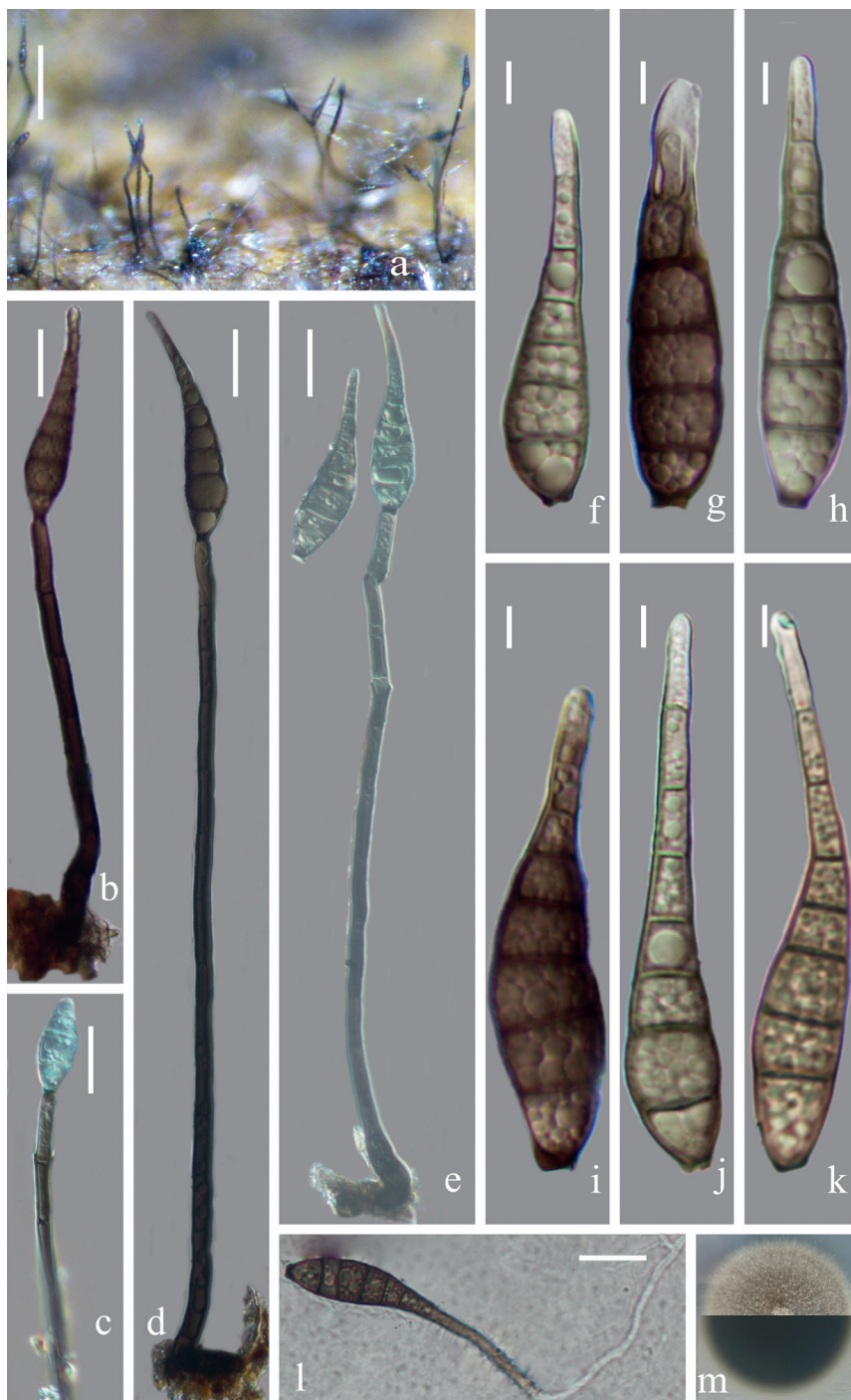
**Description.** Saprobic on decaying bamboo culms. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. **Colonies** effuse, brown, hairy, glistening, often inconspicuous. **Mycelium** partly superficial, partly immersed in the substra-

**Table 2.** Synopsis of morphological characteristics, habitats, hosts and district compared across *Distoseptispora* species.

| Species                           | Conidiophores (µm) | Conidia (µm)                       | Conidia septation  | Conidia characteristics                                                                                                        | Habitat     | Host                                         | District                | References                                 |
|-----------------------------------|--------------------|------------------------------------|--------------------|--------------------------------------------------------------------------------------------------------------------------------|-------------|----------------------------------------------|-------------------------|--------------------------------------------|
| <i>Distoseptispora mellingsii</i> | 69–192 × 4–7       | 32–64.5 × (7–)9–12.5               | 5–7-distoseptate   | Obclavate, mostly bright brown when mature                                                                                     | Freshwater  | Dead bamboo culms                            | China, Jiangxi          | This study                                 |
| <i>D. youngxiensis</i>            | 112–253 × 4–9      | 46–74(–86) × 10–13(–16)            | 6–9-euseptate      | Obclavate or obspathulate, olivaceous to yellowish-brown or brown, guttulate                                                   | Freshwater  | Dead bamboo culms                            | China, Jiangxi          | This study                                 |
| <i>D. yunjushanensis</i>          | 100–175 × 5.5–10   | 39–67.5(–77) × (7–)9.5–13.5(–16.5) | 7–13-distoseptate  | Obpyriform or obclavate, olivaceous when young, dark brown when mature                                                         | Freshwater  | Dead bamboo culms                            | China, Jiangxi          | This study                                 |
| <i>D. adscendens</i>              | 28–46 × 8–10       | (80–)350–500 × 15–18               | 80-distoseptate    | Cylindrical, hemispherical apex, hyaline                                                                                       | Terrestrial | Decaying wood of <i>Fagus sylvatica</i>      | Hungary                 | Shoemaker and White (1985), Rěhlová (1999) |
| <i>D. appendiculata</i>           | 62–86 × 4.5–5.5    | 67–89 × 10–16                      | 13–17-distoseptate | Obpyriform or obclavate, olivaceous or dark brown, with gelatinous sheath around tip                                           | Freshwater  | Unidentified submerged wood                  | Thailand, Kh-waeng Phra | Luo et al. (2019)                          |
| <i>D. aquatica</i>                | 29–41 × 7–9        | 110–157 × 13.5–16.5                | 15–28-distoseptate | Obclavate, dark brown with bluish-green to malachite green tinge                                                               | Freshwater  | Unidentified submerged wood                  | China, Yunnan           | Su et al. (2016)                           |
| <i>D. bambusae</i>                | 40–96 × 4–5.5      | 45–74 × 5.5–9.5                    | 5–10-distoseptate  | Obclavate, olivaceous or brown                                                                                                 | Terrestrial | Dead bamboo culms                            | China and Thailand      | Sun et al. (2020), Monkai et al. (2020)    |
| <i>D. cangshanensis</i>           | 44–68 × 4–8        | 58–166(–287) × 10–14               | Multi-distoseptate | Obclavate or lanceolate, rostrate, olivaceous or brown                                                                         | Freshwater  | Unidentified submerged wood                  | China, Yunnan           | Luo et al. (2018)                          |
| <i>D. carici</i>                  | 35–90 × 6–7        | (55–)65–85(–100) × 15–16(–17)      | 5–10-distoseptate  | Obclavate, brown, septa with central pore, basal cell pale brown, with truncate hilum                                          | Terrestrial | Leaves of <i>Carex</i> sp.                   | Thailand, Chiang Mai    | Crous et al. (2019)                        |
| <i>D. clematidis</i>              | 22–40 × 4–10       | 120–210 × 12–20                    | 28–35-distoseptate | Oblong, obclavate, cylindrical or rostrate, brown with green tinge, bud scars or disjunctors present at the site of attachment | Terrestrial | Dried branches of <i>Clematis sikimensis</i> | Thailand, Chiang Rai    | Phukhamsakda et al. (2020)                 |
| <i>D. dehongensis</i>             | 45–80 × 4–5        | 17–30 × 7.5–10                     | 3–5-distoseptate   | Obpyriform to obclavate, broad cylindrical or irregular, olivaceous                                                            | Freshwater  | Unidentified submerged wood                  | China, Yunnan           | Hyde et al. (2019)                         |
| <i>D. euseptata</i>               | 19–28 × 4–5        | 37–54 × 8–9                        | 4–7-euseptate      | Obpyriform to obclavate, often constricted at septa, olivaceous                                                                | Freshwater  | Unidentified submerged wood                  | China, Yunnan           | Li et al. (2021)                           |

| Species                | Conidiophores (µm)    | Conidia (µm)            | Conidia septation    | Conidia characteristics                                                                            | Habitat     | Host                                | District                            | References           |
|------------------------|-----------------------|-------------------------|----------------------|----------------------------------------------------------------------------------------------------|-------------|-------------------------------------|-------------------------------------|----------------------|
| <i>D. fasciculata</i>  | 12–16 × 5–6           | 46–200 × 10–16.5        | 10–40-distoseptate   | Subcylindrical to obclavate, olivaceous when young, dark brown when mature                         | Freshwater  | Unidentified submerged wood         | Thailand, Nakhon Si Thammarat       | Dong et al. (2021)   |
| <i>D. fluminicola</i>  | 21–33 × 5.5–6.5       | 125–250 × 13–15         | 17–34-distoseptate   | Oblong, obclavate, cylindrical or rostrate, brown with green tinge                                 | Freshwater  | Unidentified submerged wood         | China, Yunnan                       | Su et al. (2016)     |
| <i>D. guttulata</i>    | 55–90(–145) × 3.5–5.5 | 75–130(–165) × 7–11     | 11–14(–20)-euseptate | Obclavate or lanceolate, rostrate, mid to dark brown or olivaceous                                 | Freshwater  | Unidentified submerged wood         | Thailand, Prachuap Khiri Khan       | Yang et al. (2018)   |
| <i>D. hydei</i>        | 87–145 × 3–7          | 32–58 × 10–15           | 7–9-distoseptate     | Obpyriform to fusiform, olivaceous to brown, with a hyaline, globose, gelatinous sheath around tip | Terrestrial | Dead bamboo culms                   | Thailand, Phitsanulok               | Monkai et al. (2020) |
| <i>D. leonensis</i>    | Up to 175 × 6–7       | (38–)50–75(–85) × 11–15 | 7–12-distoseptate    | Obclavate, rostrate, brown                                                                         | Terrestrial | Dead culms of <i>Fryxinetia</i> sp. | Malaysia                            | McKenzie (1995)      |
| <i>D. lignicola</i>    | 84–124 × 4–5          | 60–108 × 7–9            | 5–9-euseptate        | Obclavate, curved, brown                                                                           | Freshwater  | Unidentified submerged wood         | Thailand, Sai-Khu Waterfall         | Luo et al. (2019)    |
| <i>D. longispora</i>   | 17–37 × 6–10          | 189–297 × 16–23         | 31–56-distoseptate   | Obclavate, elongated, brown to yellowish-brown                                                     | Freshwater  | Unidentified submerged wood         | China, Yunnan                       | Song et al. (2020)   |
| <i>D. martinii</i>     | 50–110 × 3.5–4.5      | 15–20 × 11–16           | Transversal septa    | Transversal ellipsoid, oblate or subglobose, muriform, pale brown to brown                         | Terrestrial | Unidentified dead branches          | China, Guizhou                      | Xia et al. (2017)    |
| <i>D. multisepiata</i> | 29–47 × 4–6           | 147–185 × 12–14         | Multi-distoseptate   | Obclavate, rostrate, dark olivaceous green                                                         | Freshwater  | Unidentified submerged wood         | Thailand, Prachuap Khiri Khan       | Hyde et al. (2016)   |
| <i>D. neostrata</i>    | 93–117 × 5.5–6.5      | 109–147 × 13–15         | Multi-distoseptate   | Obclavate, rostrate, dark olivaceous to mid or dark brown                                          | Freshwater  | Unidentified submerged wood         | Thailand, Khwaeng Phra Khanong Nuea | Luo et al. (2019)    |
| <i>D. obclavata</i>    | 117.5–162.5 × 5–7     | 46–66 × 9–11            | 9–11-distoseptate    | Obclavate, olivaceous to pale or dark brown, guttulate                                             | Freshwater  | Unidentified submerged wood         | Thailand, Khwaeng Phra Khanong Nuea | Luo et al. (2019)    |
| <i>D. obpyriformis</i> | 97–119 × 5–7          | 53–71 × 12–16           | 9–11-distoseptate    | Obpyriform, olivaceous to pale or dark brown, guttulate                                            | Freshwater  | Unidentified submerged wood         | China, Yunnan                       | Luo et al. (2018)    |
| <i>D. palmarum</i>     | 90–165 × 4–7          | 35–180 × 7–11           | 7–27-distoseptate    | Oblong, obclavate, greenish-black to brown                                                         | Terrestrial | Rachis of <i>Cocos nucifera</i>     | Thailand, Trat                      | Hyde et al. (2019)   |
| <i>D. phanangensis</i> | 18–30(–40) × 4.3–6.5  | 165–350 × 14–19         | Multi-distoseptate   | Elongate, obclavate, rostrate, dark olivaceous to mid or dark brown                                | Freshwater  | Unidentified submerged wood         | Thailand, Phang Nga                 | Yang et al. (2018)   |

| Species                      | Conidiophores (µm) | Conidia (µm)                         | Conidia septation                    | Conidia characteristics                                                                                          | Habitat                | Host                                              | District                      | References               |
|------------------------------|--------------------|--------------------------------------|--------------------------------------|------------------------------------------------------------------------------------------------------------------|------------------------|---------------------------------------------------|-------------------------------|--------------------------|
| <i>D. mayongensis</i>        | 75–125 × 3.5–5.5   | (36–)60–106(–120) × 9–14.5           | 9–13-euseptate, rarely 14–15-septate | Obclavate or obspathulate, rostrate, pale brown or pale olivaceous, with percurrent proliferation                | Freshwater             | Unidentified submerged wood                       | Thailand, Rayong              | Hyde et al. (2020)       |
| <i>D. rostrata</i>           | 82–126 × 5–7       | 115–155 × 9–11                       | (15–)18–23-distoseptate              | Obclavate or lanceolate, rostrate, olivaceous to pale brown                                                      | Freshwater             | Unidentified submerged wood                       | China, Yunnan                 | Luo et al. (2018)        |
| <i>D. saprophytica</i>       | 50–140 × 3.2–4.2   | 14.5–30 × 4.5–7.5                    | 2–6-distoseptate                     | Subcylindrical to obclavate, olivaceous to brown                                                                 | Freshwater             | Unidentified submerged wood                       | Thailand, Songkhla            | Dong et al. (2021)       |
| <i>D. songkhlaensis</i>      | 70–90 × 4–5.5      | 44–125 × 9–14.5                      | 9–16-distoseptate                    | Obclavate, constricted at septa, olivaceous to brown                                                             | Freshwater             | Unidentified submerged wood                       | Thailand, Songkhla            | Dong et al. (2021)       |
| <i>D. submersa</i>           | 55–73 × 7–9        | 95–123 × 15–19                       | 17–23(–28)-distoseptate              | Obclavate, brown to dark brown or olivaceous                                                                     | Freshwater             | Unidentified submerged wood                       | China, Yunnan                 | Luo et al. (2018)        |
| <i>D. sudluensis</i>         | 80–250 × 4.5–5.8   | (65–)80–125(–145) × 8–13             | 8–10-euseptate                       | Narrowly obclavate or obspathulate, yellowish-brown or dark olivaceous, verrucose, with percurrent proliferation | Freshwater             | Unidentified submerged wood                       | China, Guizhou                | Yang et al. (2018)       |
| <i>D. tectonae</i>           | 19.5–95 × 4.5–9    | 45–270 × 11–16                       | 10–40-distoseptate                   | Obclavate, brown to dark brown or olivaceous                                                                     | Terrestrial/Freshwater | Dead twig of <i>tectonia grandis</i> (Lamiaceae)  | Thailand, Prachuap Khiri Khan | Hyde et al. (2016)       |
| <i>D. tectonigena</i>        | Up to 110 × 5–11   | (83–)148–225(360–) × (10–)11–12(–13) | 20–46-distoseptate                   | Flexuous, cylindrical-obclavate, elongated, verruculose, dark reddish-brown                                      | Terrestrial            | Dead culms of <i>Tectonia grandis</i> (Lamiaceae) | Thailand, Chiang Rai          | Hyde et al. (2016)       |
| <i>D. thailandica</i>        | 15–26 × 3–6        | 130–230 × 13.5–17                    | 35–52-distoseptate                   | Oblong, obclavate, cylindrical or rostrate, reddish-brown to brown                                               | Terrestrial            | Dead leaves of <i>Pandanus</i> sp.                | Thailand, Prachuap Khiri Khan | Tibpromma et al. (2018)  |
| <i>D. thysanolaenae</i>      | 30–80 × 3.5–5.5    | 21.5–80 × 6.5–12.8                   | 8–14-distoseptate                    | Elongated obclavate, light to dark brown, flat apex, with conspicuous spore attachment loci                      | Terrestrial            | Dead culms of <i>Thysanotena maxima</i>           | China, Yunnan                 | Phookamsak et al. (2019) |
| <i>D. xishuangbannaensis</i> | 12–17 × 2–5        | 160–305 × 8–15                       | Up to 40-distoseptate                | Cylindrical-obclavate, green-brown to brown, tapering towards apex                                               | Terrestrial            | Dead leaf sheaths of <i>Pandanus utilis</i>       | China, Yunnan                 | Tibpromma et al. (2018)  |
| <i>D. yunnanensis</i>        | 131–175 × 6–7      | 58–108 × 8–10                        | 6–10-euseptate                       | Obclavate, rostrate, mid-olivaceous to brown                                                                     | Freshwater             | Unidentified submerged wood                       | China, Yunnan                 | Li et al. (2021)         |



**Figure 3.** *Distoseptispora yongxiuensis* HFJAU 10007, holotype) **a** Colonies on bamboo culm **b, d** conidiophores with conidia **c** conidiogenous cell bearing conidium **e** conidiogenous cells with young conidia **f-k** conidia **l** germinating conidium **m** culture on PDA from above and reverse. Scale bars: 100  $\mu\text{m}$  (**a**), 20  $\mu\text{m}$  (**b-e, l**), 5  $\mu\text{m}$  (**f-k**).

tum, composed of hyaline to pale brown, septate, branched hyphae. **Conidiophores**  $112\text{--}253 \times 4\text{--}9 \mu\text{m}$  ( $\bar{x} = 198 \times 6.9 \mu\text{m}$ ,  $n = 15$ ), macronematous, mononematous, solitary or aggregated at the base, cylindrical, straight or slightly flexuous, 8–13-septate, olivaceous to dark brown, sharply curving near the base, paler at the apical part, rounded at the apex. **Conidiogenous cells** integrated, terminal, monoblastic, rarely polyblastic, cylindrical, olivaceous to dark brown. **Conidia**  $46\text{--}74(-86) \times 10\text{--}13(-16) \mu\text{m}$  ( $\bar{x} = 65.6 \times 12.6 \mu\text{m}$ ,  $n = 30$ ), acrogenous, solitary, obclavate or obspathulate, straight or flexuous, rostrate, 6–9-euseptate, olivaceous to yellowish-brown or brown, becoming paler or hyaline towards the apex, guttulate,  $2.5\text{--}4 \mu\text{m}$  wide at the base and  $2.5\text{--}5 \mu\text{m}$  wide at the apex, with a darkened scar at the base.

**Cultural characteristics.** Conidia germinating on PDA within 24 h and germ tubes produced from both ends. Colonies on PDA reaching 24–32 mm diam. at two weeks at 25 °C, in natural light, circular, with dense, light olivaceous mycelium on the surface with entire margin; reverse dark brown to black.

**Material examined.** CHINA, Jiangxi Province, Jiujiang City, Yongxiu County, alt. 680.5 m, 29.09°N, 115.62°E, on decaying bamboo culms submerged in a freshwater stream, 28 Apr 2020, Z. J. Zhai and W. W. Li, YJS-70 (HFJAU 10007, **holotype**), ex-type living culture, JAUCC 4725 = JAUCC 4726.

**Notes.** In the multi-gene phylogenetic tree (Fig. 1), *D. yongxiuensis* clusters with *D. suoluoensis*. Nonetheless, *D. yongxiuensis* can be distinguished from *D. suoluoensis* by its shorter conidia ( $46\text{--}74(-86) \mu\text{m}$  vs.  $(65\text{--})80\text{--}125(-145) \mu\text{m}$ ) and polyblastic conidiogenous cells (Yang et al. 2018). Additionally, *D. suoluoensis* has the percurrent proliferation of conidia, while it was not observed in *D. yongxiuensis*. *Distoseptispora yongxiuensis* is similar with *D. bambusae* (Sun et al. 2020), *D. palmarum* (Hyde et al. 2019) and *D. meilingensis* for the polyblastic conidiogenous cells, but *D. yongxiuensis* has wider conidia than those of *D. bambusae* ( $10\text{--}13(-16) \mu\text{m}$  vs.  $5.5\text{--}9.5 \mu\text{m}$ ) (Sun et al. 2020), shorter conidia than those of *D. palmarum* ( $46\text{--}74(-86) \mu\text{m}$  vs.  $35\text{--}180 \mu\text{m}$ ) (Hyde et al. 2019) and paler (yellowish-brown or brown vs. bright brown) conidia than those of *D. meilingensis*.

***Distoseptispora yunjushanensis* Z. J. Zhai & D. M. Hu, sp. nov.**

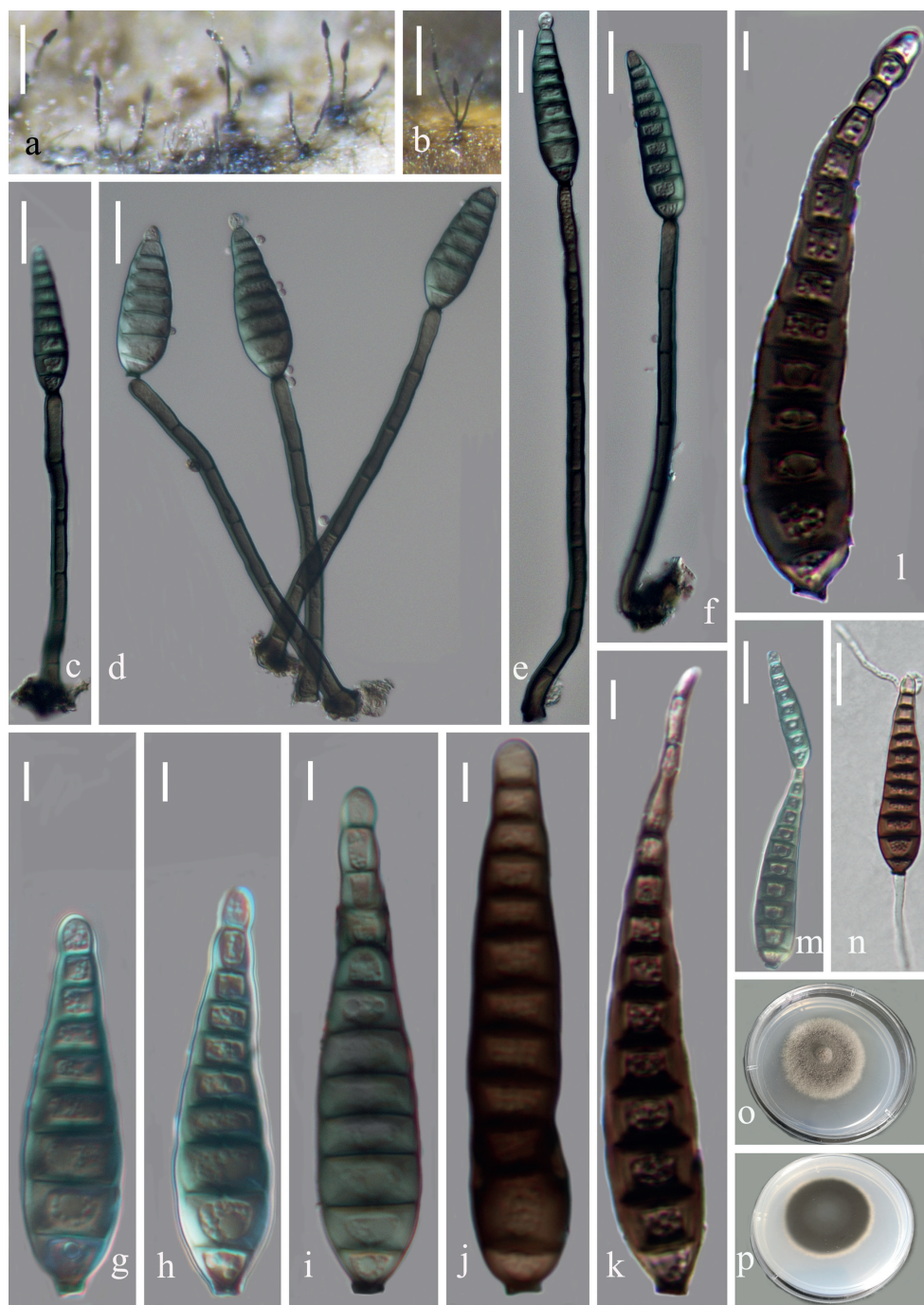
Mycobank No: 842065

Fig. 4

**Etymology.** The epithet refers to the collecting site from the Yunjushan Mountain in China.

**Holotype.** HFJAU10005

**Description.** Saprobiic on decaying bamboo culms submerged in freshwater habitats. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. **Colonies** effuse, olivaceous or dark brown, hairy, velvety. **Mycelium** mostly immersed, consisting of branched, septate, smooth, subhyaline to pale brown hyphae. **Conidiophores**  $100\text{--}175 \mu\text{m} \times 5.5\text{--}10 \mu\text{m}$  ( $\bar{x} = 129 \times 7.1 \mu\text{m}$ ,  $n = 30$ ), single or in groups of 2 or 3, macronematous, mononematous, erect, straight or slightly flexuous, 4–7-septate,



**Figure 4.** *Distoseptispora yunjushanensis* (HFJAU 10005, holotype) **a, b** colonies on bamboo culms **c–f** conidiophores with conidia **g–i** young conidia **j–l** mature conidia **m** conidium with proliferation **n** germinating conidium **o, p** culture on PDA from above and reverse. Scale bars: 100  $\mu\text{m}$  (**a, b**), 20  $\mu\text{m}$  (**c–f, m, n**), 5  $\mu\text{m}$  (**g–l**).

unbranched, olivaceous to dark brown, smooth, cylindrical, rounded at the apex. **Conidiogenous cells** monoblastic, integrated, terminal, determinate, pale to dark brown, cylindrical. **Conidia** 39–67.5(–77)  $\mu\text{m} \times$  (7–)9.5–13.5(–16.5)  $\mu\text{m}$  ( $\bar{x} = 52 \times 12 \mu\text{m}$ ,  $n = 30$ ), acrogenous, solitary, obpyriform or obclavate, thick-walled, tapering towards the rounded apex, slightly curved, truncate at the base, 7–13-distoseptate, guttulate, smooth-walled, olivaceous, dark brown when mature, sometimes with the percurrent proliferation which forms another conidium from the conidial apex.

**Cultural characteristics.** Conidia germinating on PDA within 24 h and germ tubes produced from both ends. Colonies on PDA reaching 12–18 mm diam. at 14 days at 25 °C, in natural light, with fluffy, dense, thin olivaceous mycelium in the centre, becoming sparse and paler at the entire margin; reverse dark brown, pale brown at the smooth margin.

**Material examined.** CHINA, Jiangxi Province, Jiujiang City, Yongxiu County, Yunjushan Mountain, alt. 672.5 m, 29.23°N, 115.59°E, on decaying bamboo culms submerged in a freshwater stream, 28 Apr 2020, Z. J. Zhai and W. W. Li, YJS-42 (HF-JAU 10005, **holotype**), ex-type living culture, JAUCC 4723 = JAUCC 4724.

**Notes.** In the phylogenetic analysis, *D. yunjushanensis* clusters with *D. obclavata* and *D. rayongensis* with moderate support (BS/PP = 81/1.00). However, *D. yunjushanensis* is easily distinguished from *D. obclavata* by its comparatively wider (5.5–10  $\mu\text{m}$  vs. 5–7  $\mu\text{m}$ ) conidiophores and conidia ((7–)9.5–13.5(–16.5)  $\mu\text{m}$  vs. 9–11  $\mu\text{m}$ ) (Luo et al. 2019). Moreover, the percurrent proliferation of conidia was not observed in *D. obclavata* (Luo et al. 2019). *Distoseptispora yunjushanensis* has shorter conidia (39–67.5(–77)  $\mu\text{m}$  vs. (36–)60–106(–120)  $\mu\text{m}$ ) and wider conidiophores (5.5–10  $\mu\text{m}$  vs. 3.5–5.5  $\mu\text{m}$ ) than those of *D. rayongensis* (Hyde et al. 2020). The morphology of *D. yunjushanensis* is similar to *D. guttulata* and *D. songkhlaensis* in having the obclavate conidia, but differs in having wider (5.5–10  $\mu\text{m}$  vs. 3.5–5.5  $\mu\text{m}$  and 4–5.5  $\mu\text{m}$ ) conidiophores, shorter (39–67.5(–77)  $\mu\text{m}$  vs. 75–130(–165)  $\mu\text{m}$  and 44–125  $\mu\text{m}$ ) and proliferating conidia (Yang et al. 2018; Dong et al. 2021). Additionally, *D. yunjushanensis* can be distinguished from *D. guttulata* by its distoseptate conidia (Yang et al. 2018).

## Discussion

Previous reports of *Distoseptispora* were mainly concentrated in tropical areas, such as Thailand (Chiang Rai, Phitsanulok, Phang Nga; Luo et al. 2019) and southwest Yunnan, China (Su et al. 2016; Luo et al. 2018). Nonetheless, several new taxa were found sporadically in subtropical China, for example, *Distoseptispora martinii* (Xia et al. 2017), *D. suoluensis* (Yang et al. 2018) and *D. bambusae* (Sun et al. 2020) in Guizhou Province and *D. euseptata* and *D. yunnansis* in northwest Yunnan (Li et al. 2021). The ongoing discovery of this taxa from other geographic regions in subtropical China will deepen our understanding of the species in this genus. In this study, we introduced another three new species of *Distoseptispora* from Jiangxi Province of subtropical China. It is interesting to note that all these species in subtropical China, except *D. yunjushanensis* and

*D. martinii*, formed a well-supported monophyletic clade in the phylogenetic tree and this clade was at the basal position (Fig. 1). *Distoseptispora yunjushanensis* and *D. martinii* were otherwise phylogenetically placed within other clades (Fig. 1) and, therefore, we suppose that other lineages might also comprise more *Distoseptispora* species distributed in subtropical China. Further discovery of *Distoseptispora* species in more extensive areas in subtropical and other regions of China are needed to be addressed if the phylogenetic position of species reflects their geographical and ecological distribution.

*Distoseptisporaceae* is a holomorphic group of *Sordariomycetes* that are saprobic on decaying wood and plant debris in terrestrial and freshwater habitats (Su et al. 2016). The genus *Distoseptispora* seems not to have specific habitat preferences, as most species were reported from submerged wood in freshwater habitats, while some were introduced from terrestrial habitats (Table 2). So far, only five species of *Distoseptispora* have been found on bamboo, two of them (*Distoseptispora bambusae* and *D. hydei*, Table 2) from terrestrial habitats, the other three (this study) from freshwater. There may be more species in this genus existing on bamboo waiting to be discovered and further studies are needed to clarify if a specific species in *Distoseptispora* is specific to its host.

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