



Morphological and molecular characterization of *Distoseptispora bambusae* from Iran

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Abstract: *Distoseptispora* generally is regarded as a saprobic lignicolous fungal genus and presently comprises 64 species. Of these, 42 of them were found in freshwater and 22 in terrestrial habitats. Most *Distoseptispora* species are reported from China and Thailand, which are subtropical and tropical regions. In this study, we report *Distoseptispora bambusae* as a saprobic fungus on decaying leaves of common bamboo (*Bambusa vulgaris*) based on morphological characteristics and sequence data of the ITS–rDNA region. *Distoseptispora bambusae* was described, illustrated, and its morphology and phylogenetic relationships with similar *Distoseptispora* species were discussed. To the best of our knowledge, this is the first report of *D. bambusae* on common bamboo for the mycobiota of Iran and the Middle East.

Keywords: *Distoseptisporaceae*, ITS–rDNA, Morphology, Phylogeny, Taxonomy.

INTRODUCTION

The genus *Distoseptispora* K.D. Hyde, McKenzie & Maharachch., is one of the *Sporidesmium*-like genera introduced by Su et al. (2016) with *D. fluminicola*

McKenzie, Hong Y. Su, Z.L. Luo & K.D. Hyde as the type species. *Distoseptispora* is the only genus in the family *Distoseptisporaceae* (*Distoseptisporales*, *Diaporthomycetidae*, *Sordariomycetes*) based on morphology and multi-locus phylogenetic analyses of the internal transcribed spacer (ITS–rDNA) region, parts of the large subunit ribosomal RNA (*LSU*), translation elongation factor-1 alpha (*tef1-α*) and RNA polymerase II second largest subunit (*RPB2*) genes (Luo et al. 2019). The genus is characterized by having macronematous, mononematous, unbranched, smooth conidiophores, monoblastic or polyblastic, holoblastic, determinate, cylindrical conidiogenous cells, and acrogenous, solitary, olivaceous to brown, euseptate or distoseptate, rarely muriform conidia (Su et al. 2016, Luo et al. 2018, Yang et al. 2018, 2021, Ma et al. 2022, Zhai et al. 2022, Zhang et al. 2022, Li et al. 2023).

Distoseptispora is regarded as a saprobic lignicolous fungal genus, except for *D. caricis* Crous and *D. palmarum* S.N. Zhang, K.D. Hyde & J.K. Liu, which are isolated from the leaves of *Carex* sp. and rachis of *Cocos nucifera* L., respectively (Crous et al. 2019, Hyde et al. 2019, Zhai et al. 2022). At present, the genus comprises 64 species, 42 of which were found in freshwater and 22 in terrestrial habitats (Su et al. 2016, Hyde et al. 2016, 2019, 2020, 2021, Yang et al. 2018, 2021, Luo et al. 2018, 2019, Monkai et al. 2020, Song et al. 2020, Sun et al. 2020, Dong et al. 2021, Li et al. 2021, Shen et al. 2021, Ma et al. 2022, Phukhamsakda et al. 2022, Zhai et al. 2022, Zhang et al. 2022, Afshari et al. 2023, Liu et al. 2023, Jayawardena et al. 2023). Most *Distoseptispora* species have been reported from Asia, primarily in China and Thailand, while two species viz *D. adscendens* (Berk.) R. Zhu & H. Zhang and *D. leonensis* (M.B. Ellis) R. Zhu & H. Zhang were found in Hungary and Malaysia, respectively (Shoemaker & White 1985, McKenzie 1995, Su et al. 2016, Zhai et al. 2022, Liu et al. 2023). Morphological characteristics of the conidia such as septum type (distoseptum vs euseptum) and their numbers, size,

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shape, color, rostrateness and wall thickening, the length of conidiophores, proliferation and conidiogenesis of conidiogenous cells (monoblastic or polyblastic) and habitat have been mainly used to identify species in *Distoseptispora* (Su et al. 2016, Ma et al. 2022, Zhai et al. 2022, Zhang et al. 2022, Liu et al. 2023). However, these characteristics are not significantly correlated with the phylogenetic relationships. Therefore, it is necessary to combine both morphological characteristics and multi-gene phylogenetic analyses to clarify their taxonomic placement (Ma et al. 2022, Zhai et al. 2022, Zhang et al. 2022, Liu et al. 2023). Sequences of the ITS-rDNA, *LSU*, *tef1- α* and *RPB2* have been used for delimitation of *Distoseptispora* species (Su et al. 2016, Hyde et al. 2016, 2019, 2020, 2021, Ma et al. 2022, Zhai et al. 2022, Zhang et al. 2022, Liu et al. 2023).

Up to now, there are no reports of *Distoseptispora* species from Iran and the Middle East. In this study, two isolates of *Sporidesmium*-like taxa were collected from decaying leaves of common bamboo in Bandar-e-Anzali, Guilan Province, Iran. Based on morphological characteristics and sequence data of the ITS-rDNA region, the isolates were identified as *Distoseptispora bambusae* Y.R. Sun, I.D. Goonasekara, Yong Wang bis & K.D. Hyde. Detailed descriptions and illustrations of the species and its comparison with closely allied species are provided.

MATERIALS AND METHODS

Sample collection, fungal isolation, and morphological studies

In this study, dead leaf samples of common bamboo (*Bambusa vulgaris* Schrad. ex J.C.Wendl.) were collected from Shileh-Sar village (Bandar-e-Anzali, Guilan Province, Iran), in 2020. Leaf samples were surface disinfected using 1% sodium hypochlorite solution for 3 min, followed by rinsing in sterile distilled water and incubated in a moist chamber at 25 °C. The incubated leaves were inspected under the stereo microscope (SZ51, Olympus) and single-spore isolation was done following the method described in Ahmadpour et al. (2023). Germinated spores were transferred to potato dextrose agar (PDA: 39 g/l sterile distilled water, Merck, Darmstadt, Germany) plates and incubated at room temperature for 2–4 weeks. The isolates were grown on tap water agar with autoclaved wheat straw (TWA–wheat straw) and PDA at 25 °C in darkness to study the morphological characteristics (Zhai et al. 2022, Zhang et al. 2022, Ahmadpour et al. 2023).

Measurements and microphotographs were prepared from slide mounts in lactophenol using an Olympus AX70 compound microscope with differential interference contrast (DIC) illumination. Adobe Photoshop 2020 v. 2.10.8 software (Adobe Inc., San Jose, California) was used for manual editing. All identified isolates were deposited in the fungal culture collections of the Iranian Research

Institute of Plant Protection (IRAN) and Urmia University (FCCUU).

DNA extraction, PCR amplification, and sequencing

Fresh fungal mycelia were scraped from the surface of colonies growing on PDA with a flame-sterilized scalpel and transferred into a 1.5 mL centrifuge tube. Genomic DNA was extracted as described by Ahmadpour et al. (2021). The ITS-rDNA was amplified using the primer pairs ITS1/ITS4 (White et al. 1990). Each polymerase chain reaction (PCR) mixture contained 0.4 μ M of each primer, 10 μ L of a ready master mix (Taq DNA polymerase 2X Master Mix Red, 2 mM MgCl₂, Ampliqon Company, Denmark), and about 10 ng of template DNA in a final volume of 30 μ L. A touchdown PCR consisted of 35 cycles of 45 s at 95 °C, 45 s at 62–57 °C (annealing temperature decreased 0.5 °C per cycle in the first 10 cycles) and 45 s at 72 °C, and a final extension step at 72 °C for 5 min (Korbie & Mattick 2008). PCR products were evaluated on 1% agarose electrophoresis gel stained with GelRed™ (Biotium, Hayward, CA, USA) and viewed under ultra-violet light. The amplified products were purified and sequenced using the same primer sets used for PCR amplification by Macrogen Corporation (Seoul, South Korea).

Phylogenetic analyses

The newly generated sequences as well as reference sequences of *Distoseptispora* species downloaded from GenBank (Table 1), were aligned using the online multiple alignment program MAFFT version 7 (<https://mafft.cbrc.jp/alignment/server/>) (Katoh et al. 2019) and manually improved, where necessary, in MEGA 6.0 (Tamura et al. 2013). Phylogenetic analyses were done by using Maximum Likelihood (ML) and Bayesian inference (BI) methods. Maximum likelihood (ML) analysis was conducted in the RAxML-HPC BlackBox v. 8.2.8 (Stamatakis 2014) online server of the CIPRES Science gateway portal (<https://www.phylo.org/>) (Miller et al. 2012) for 1000 bootstrapping iterations, using the general time reversible model (GTR) with a discrete gamma distribution. MrModeltest 2.3 (Nylander 2004) was used to carry out the statistical selection of best-fit models of nucleotide substitution using Akaike Information Criterion (AIC). Bayesian analyses (BI) were conducted with MrBayes v. 3.2.7 (Ronquist et al. 2012) to evaluate posterior probabilities (PP) by Markov Chain Monte Carlo sampling (MCMC). Four simultaneous Markov chains were run for one million generations, and trees were sampled every 100 generations. The first 25% of the trees, representing the burn-in phase of the analyses, were discarded, while the remaining trees were used for calculating posterior probabilities in the majority rule consensus tree.

Convergence was determined when the average standard deviation of split frequencies reached less than 0.01. Sequences of *Aquapteridospora aquatica* (MFLUCC 17-2371) and *A. fusiformis* (MFLU 18-

1601) served as the outgroup taxa (Sun et al. 2020, Zhai et al. 2022). The resultant phylogenetic trees were visualized in FigTree v. 1.4.4 (Rambaut 2019), and edited in graphic design software, Adobe Illustrator® CC 2020. All of the sequences used in this study are listed in Table 1 and the newly generated sequences were submitted to GenBank.

RESULTS

Molecular phylogenetic analyses

The ITS sequence dataset consisted of 68 strains from 63 *Distoseptispora* species and one isolate from each *Aquapteridospora aquatica* and *A. fusiformis* as outgroup taxa. The dataset comprised 521 characters, of which 253 were constant, 43 were variable and parsimony uninformative and 225 were parsimony informative. The results of MrModeltest recommended a GTR+I+G model. The RAxML analysis resulted in a best-scoring likelihood tree selected with a final ML optimization likelihood value of -6104.810525. Estimated base frequencies were as follows: A = 0.211374, C = 0.275001, G = 0.279223, T = 0.234402; substitution rates AC = 1.120183, AG = 1.907582, AT = 1.605328, CG = 0.416904, CT = 5.471755, GT = 1.000000; gamma distribution shape parameter $\alpha = 0.730555$. The phylogenetic trees generated from BI analyses were topologically similar to the ones generated via the ML analyses, and the latter is shown in Figure 1. The resulting phylogram (Fig. 1) from the ITS phylogenetic analyses revealed that our isolates clustered well with *Distoseptispora bambusae* with high bootstrap support values (ML/BI= 89/0.99), which is described below.

Taxonomy and morphology

Distoseptispora bambusae Y.R. Sun, I.D. Goonasekara, Yong. Wang bis & K.D. Hyde, Biodiversity Data Journal 8(e53678): 6 (2020) Fig. 2
Description – Mycelium is mostly immersed, composed of branched, septate, brown, smooth hyphae, 2–4 μm wide. Conidiophores macronematous, mononematous, solitary, erect, scattered or in small groups, straight or flexuous, cylindrical, unbranched, slightly constricted at septa, smooth, thick-walled, 18–53 \times 3–5 μm , brown at the base, pale brown towards the apex, 1–4 septate. Conidiogenous cells holoblastic, mono-blastic to polyblastic, integrated, terminal, determinate, brown, cylindrical. Conidia are acrogenous, solitary or rarely catenate (two conidia in a chain), obclavate to rostrate, straight or slightly curved, (3–)4–8(9)-distoseptate, 36–76 \times 4–8 μm (n = 50), pale brown to brown, tapering towards the rounded apex, truncate at the base, smooth, thick-walled. Chlamydospores and sexual morph were not observed.

Culture characteristics – Colonies on PDA effuse, scattered, brown to dark brown, reaching 35 mm diam. after 15 days at 25 °C, entire margin, white to grey aerial mycelium.

Specimen examined – IRAN, Guilan Province, Bandar-e-Anzali, Shileh-Sar village, isolated from

dead leaves of *Bambusa vulgaris* (Bambusoideae, Poaceae, Poales), 20 Sept. 2020, A. Ahmadpour, IRAN 4780C and FCCUU 1700 (Table 1).

Notes – *Distoseptispora bambusae* was originally described on culms of bamboo as a saprobic fungus from China (Sun et al. 2020). According to the phylogenetic tree, Two Iranian isolates obtained in this study (IRAN 4780C and FCCUU 1700) were clustered well with the type and representative isolates of *D. bambusae* (MFLUCC 20-0091^T and MFLUCC 14-0583) in a distinct subclade with 89% ML bootstrap and 0.99 BI posterior probabilities values (Fig. 1).

This species is phylogenetically closely related to *D. meilingensis* Z.J. Zhai & D.M. Hu, *D. suoluensis* J. Yang, Maharachch. & K.D. Hyde, *D. verrucosa* J. Yang & K.D. Hyde, *D. yongxiuensis* Z.J. Zhai & D.M. Hu and *D. lancangjiangensis* H.W. Shen, H.Y. Su, K.D. Hyde & Z.L. Luo (Fig. 1), but can be distinguished concerning the shape and size of conidia and the number of transverse septa (Yang et al. 2018, 2021, Shen et al. 2021, Zhai et al. 2022). *Distoseptispora bambusae* is morphologically similar to *D. lancangjiangensis*, *D. meilingensis* and *D. suoluensis*, but it has shorter conidiophores (40–96 μm vs. 144–204 μm in *D. lancangjiangensis*, 69–192 μm in *D. meilingensis* and 80–250 μm in *D. suoluensis*) and shorter conidia (45–74 \times 5.5–10 μm vs. 64–84 \times 9–10 μm in *D. lancangjiangensis*, 32–64.5 \times (7–) 9–12.5 μm in *D. meilingensis* and (65–) 80–125(–145) \times 8–13 μm in *D. suoluensis*) (Yang et al. 2018, 2021, Shen et al. 2021, Zhai et al. 2022). *Distoseptispora bambusae* is a new record on *Bambusa vulgaris* for the fungal of Iran and the Middle East (Ershad 2022, Farr & Rossman 2023). *Distoseptisporaceae* typified by *Distoseptispora* (Su et al. 2016). Later on, Hyde et al. (2021) introduced the family *Aquapteridosporaceae* to accommodate the genus *Aquapteridospora*, a genus with morphological similarities to *Distoseptispora*, and placed this family sister to *Distoseptisporaceae* in *Distoseptisporales*.

Distoseptisporaceae is a monotypic family and currently includes 64 valid species, most of them are known from dead parts of plants in aquatic or terrestrial habitats, however, the species do not seem to have habitat preferences (Hyde et al. 2019, Sun et al. 2020, Zhang et al. 2022, Liu et al. 2023). They play important roles in the decomposition of lignocelluloses in wood (Wong et al. 1998, Krauss et al. 2011, Hyde et al. 2016). There are only two species, *D. hyaline* J. Yang and K.D. Hyde and *D. licualae* Konta & K.D. Hyde, which have been described based on sexual morphs and phylogeny, but the connection between sexual and asexual morphs need to be confirmed (Yang et al. 2021, Konta et al. 2023).

Table 1. Characteristics of fungal taxa used in the phylogenetic analyses (Iranian isolates are shown in bold type).

Species	Strain number	Habitat	Host	ITS accession numbers	References
<i>Aquapteridospora aquatica</i>	MFLUCC 17-2371 ^T	Freshwater	submerged wood	MW286493	Dong et al. (2021)
<i>A. fusiformis</i>	MFLU 18-1601 ^T	Freshwater	decaying wood submerged	MK828652	Luo et al. (2019)
<i>Distoseptispora amniculi</i>	MFLUCC 17-2129 ^T	Freshwater	submerged decaying wood	MZ868770	Yang et al. (2021)
<i>D. appendiculata</i>	MFLUCC 18-0259 ^T	Freshwater	Unidentified submerged wood	MN163009	Luo et al. (2019)
<i>D. aqualignicola</i>	KUNCC 21-10729 ^T	Freshwater	decaying wood	OK341186	Zhang et al. 2022
<i>D. aquamyces</i>	KUNCC 21-10732 ^T	Freshwater	decaying wood	OK341187	Zhang et al. 2022
<i>D. aquatica</i>	MFLUCC 18-0646 ^T	Freshwater	Unidentified submerged wood	MK828648	Su et al. (2016)
<i>D. aquisubtropica</i>	GZCC 22-0075 ^T	Freshwater	decaying wood submerged	ON527933	Ma et al. (2022)
<i>D. atroviridis</i>	GZCC 20-0511 ^T	Freshwater	submerged decaying wood	MZ868772	Yang et al. (2021)
<i>D. bambusae</i>	MFLUCC 20-0091 ^T	Terrestrial	Dead bamboo culms	MT232713	Sun et al. (2020)
<i>D. bambusae</i>	MFLUCC 14-0583	Terrestrial	Dead bamboo culms	MT232712	Sun et al. (2020)
<i>D. bambusae</i>	IRAN 4780C	Terrestrial	Dead bamboo leaf	OQ119795	This study
<i>D. bambusae</i>	FCCUU 1700	Terrestrial	Dead bamboo leaf	OQ119796	This study
<i>D. bambusicola</i>	GZCC21-0667 ^T	Freshwater	decaying stems of bamboo	MZ474873	Jayawardena et al. (2022)
<i>D. bangkokensis</i>	MFLUCC 18-0262 ^T	Freshwater	Unidentified submerged wood	MZ518205	Shen et al. (2021)
<i>D. cangshanensis</i>	MFLUCC 16-0970 ^T	Freshwater	Unidentified submerged wood	MG979754	Luo et al. (2018)
<i>D. caricis</i>	CPC 36498 ^T	Terrestrial	Leaves of <i>Carex</i> sp.	MN562124	Crous et al. (2019)
<i>D. chinensis</i>	GZCC 21-0665 ^T	Terrestrial	decaying wood	MZ474871	Hyde et al. (2021)
<i>D. clematidis</i>	MFLUCC 17-2145 ^T	Terrestrial	Dried branches of <i>Clematis sikkimensis</i>	MT310661	Phukhamsakda et al. (2020)
<i>D. crassispora</i>	KUMCC 21-10726 ^T	Freshwater	decaying wood	OK310698	Zhang et al. (2022)
<i>D. curvularia</i>	KUMCC 21-10725 ^T	Freshwater	decaying wood	OK310697	Zhang et al. (2022)
<i>D. cylindricospora</i>	DLUCC 1906 ^T	Freshwater	submerged decaying wood	OK491122	Phukhamsakda et al. (2022)
<i>D. dehongensis</i>	KUMCC 18-0090 ^T	Freshwater	Unidentified submerged wood	MK085061	Hyde et al. (2019)
<i>D. dipteroearpi</i>	MFLUCC 22-0104 ^T	Terrestrial	woody litter of <i>Dipteroearpus</i> sp.	OP600053	Afshari et al. (2023)
<i>D. effusa</i>	GZCC 19-0532 ^T	Freshwater	decaying wood	MW133916	Yang et al. (2021)
<i>D. euseptata</i>	DLUCC S2024	Freshwater	Unidentified submerged wood	MW081540	Li et al. (2021)
<i>D. fasciculata</i>	KUMCC 19-0081 ^T	Freshwater	Unidentified submerged wood	MW286501	Dong et al. (2021)
<i>D. fluminicola</i>	MFLUCC 15-0417 ^T	Freshwater	Unidentified submerged wood	MF077553	Su et al. (2016)
<i>D. fusiformis</i>	GZCC 20-0512 ^T	Freshwater	submerged decaying wood	MZ868773	Yang et al. (2021)
<i>D. guizhouensis</i>	GZCC 21-0666 ^T	Terrestrial	decaying wood	MZ474868	Hyde et al. (2021)
<i>D. guttulata</i>	MFLUCC 16-0183 ^T	Freshwater	Unidentified submerged wood	MF077543	Yang et al. (2018)
<i>D. hyalina</i>	MFLUCC 17-2128 ^T	Freshwater	decaying submerged wood	MZ868769	Yang et al. (2021)
<i>D. hydei</i>	MFLUCC 20-0115 ^T	Terrestrial	Dead bamboo culms	MT1734661	Monkai et al. (2020)
<i>D. lancangjiangensis</i>	DLUCC 1864 ^T	Freshwater	Unidentified submerged wood	MW723055	Shen et al. (2021)
<i>D. liculalae</i>	MFLUCC 141163A ^T	Terrestrial	dead leaves of <i>Licuala glabra</i>	ON650686	Konta et al. (2023)
<i>D. lignicola</i>	MFLUCC 18-0198 ^T	Freshwater	Unidentified submerged wood	MK828651	Luo et al. (2019)
<i>D. longispora</i>	HFAU 0705 ^T	Freshwater	Unidentified submerged wood	MH555359	Song et al. (2020)
<i>D. martinii</i>	CGMCC 3.18651 ^T	Terrestrial	Unidentified dead branches	KU999975	Xia et al. (2017)
<i>D. meilingensis</i>	JAUC 4727 ^T	Freshwater	Dead bamboo culms	OK562390	Zhai et al. (2022)
<i>D. mengsongensis</i>	HJAUP C2126 ^T	Terrestrial	Dead branches	OP787876	Liu et al. (2023)
<i>D. multiseptata</i>	MFLUCC 15-0609 ^T	Freshwater	Unidentified submerged wood	KX710145	Hyde et al. (2016)
<i>D. nabanheensis</i>	HJAUP C2003 ^T	Terrestrial	Dead branches	OP787873	Liu et al. (2023)
<i>D. neorostrata</i>	MFLUCC 18-0376 ^T	Freshwater	Unidentified submerged wood	MN163008	Luo et al. (2019)
<i>D. nonrostrata</i>	KUNCC 21-10730 ^T	Freshwater	decaying wood	OK310699	Zhang et al. 2022
<i>D. obclavata</i>	MFLUCC 18-0329 ^T	Freshwater	Unidentified submerged wood	MN163012	Luo et al. (2019)
<i>D. obpyriformis</i>	DLUCC 0867	Freshwater	Unidentified submerged wood	MG979757	Luo et al. (2018)
<i>D. pachyconidia</i>	KUMCC 21-10724 ^T	Terrestrial	decaying wood	OK310696	Zhang et al. (2022)
<i>D. palmarum</i>	MFLUCC 18-1446 ^T	Terrestrial	Rachis of <i>Cocos nucifera</i>	MK085062	Hyde et al. (2019)
<i>D. phangngaensis</i>	MFLUCC 16-0857 ^T	Freshwater	Unidentified submerged wood	MF077545	Yang et al. (2018)
<i>D. rayongensis</i>	MFLUCC 18-0415 ^T	Freshwater	Unidentified submerged wood	MH457172	Hyde et al. (2020)
<i>D. rostrata</i>	MFLUCC 16-0969 ^T	Freshwater	Unidentified submerged wood	MG979758	Luo et al. (2018)
<i>D. saprophytica</i>	MFLUCC 18-1238 ^T	Freshwater	Unidentified submerged wood	MW286506	Dong et al. (2021)
<i>D. septata</i>	GZCC 22-0078 ^T	Freshwater	decaying wood	ON527939	Ma et al. (2022)
<i>D. sinensis</i>	HJAUP C2044 ^T	Terrestrial	Dead branches	OP787878	Liu et al. (2023)
<i>D. songkhlaensis</i>	MFLUCC 18-1234 ^T	Freshwater	Unidentified submerged wood	MW286482	Dong et al. (2021)
<i>D. submersa</i>	MFLUCC 16-0946 ^T	Freshwater	Unidentified submerged wood	MG979760	Luo et al. (2018)
<i>D. suoluensis</i>	MFLUCC 17-0224 ^T	Freshwater	Unidentified submerged wood	MF077546	Yang et al. (2018)
<i>D. tectonae</i>	MFLUCC 12-0291 ^T	Terrestrial	Dead twig of <i>Tectona grandis</i>	KX751711	Hyde et al. (2016)
<i>D. tectonigena</i>	MFLUCC 12-0292 ^T	Terrestrial	Dead twig of <i>Tectona grandis</i>	KX751712	Hyde et al. (2016)
<i>D. thailandica</i>	MFLUCC 16-0270 ^T	Terrestrial	Dead leaves of <i>Pandanus</i> sp.	MH275060	Tibpromma et al. (2018)
<i>D. thysanolaenae</i>	KUMCC 18-0182 ^T	Terrestrial	Dead culms of <i>Thysanolaena maxima</i>	MK045851	Phookamsak et al. (2019)
<i>D. tropica</i>	GZCC 22-0076 ^T	Terrestrial	dead wood	ON527935	Ma et al. (2022)
<i>D. verrucosa</i>	GZCC 20-0434 ^T	Freshwater	submerged decaying wood	MZ868771	Yang et al. (2021)
<i>D. wuzhishanensis</i>	GZCC 22-0077 ^T	Freshwater	decaying wood	ON527938	Ma et al. (2022)
<i>D. xishuangbannaensis</i>	KUMCC 17-0290 ^T	Terrestrial	Dead leave of <i>Pandanus utilis</i>	MH275061	Tibpromma et al. (2018)
<i>D. yongxiensis</i>	JAUC 4725 ^T	Freshwater	Dead bamboo culms	OK562388	Zhai et al. (2022)
<i>D. yunjuhanensis</i>	JAUC 4724 ^T	Freshwater	Dead bamboo culms	OK562393	Zhai et al. (2022)
<i>D. yunnanensis</i>	MFLUCC 20-0153 ^T	Freshwater	Unidentified submerged wood	MW081541	Li et al. (2021)

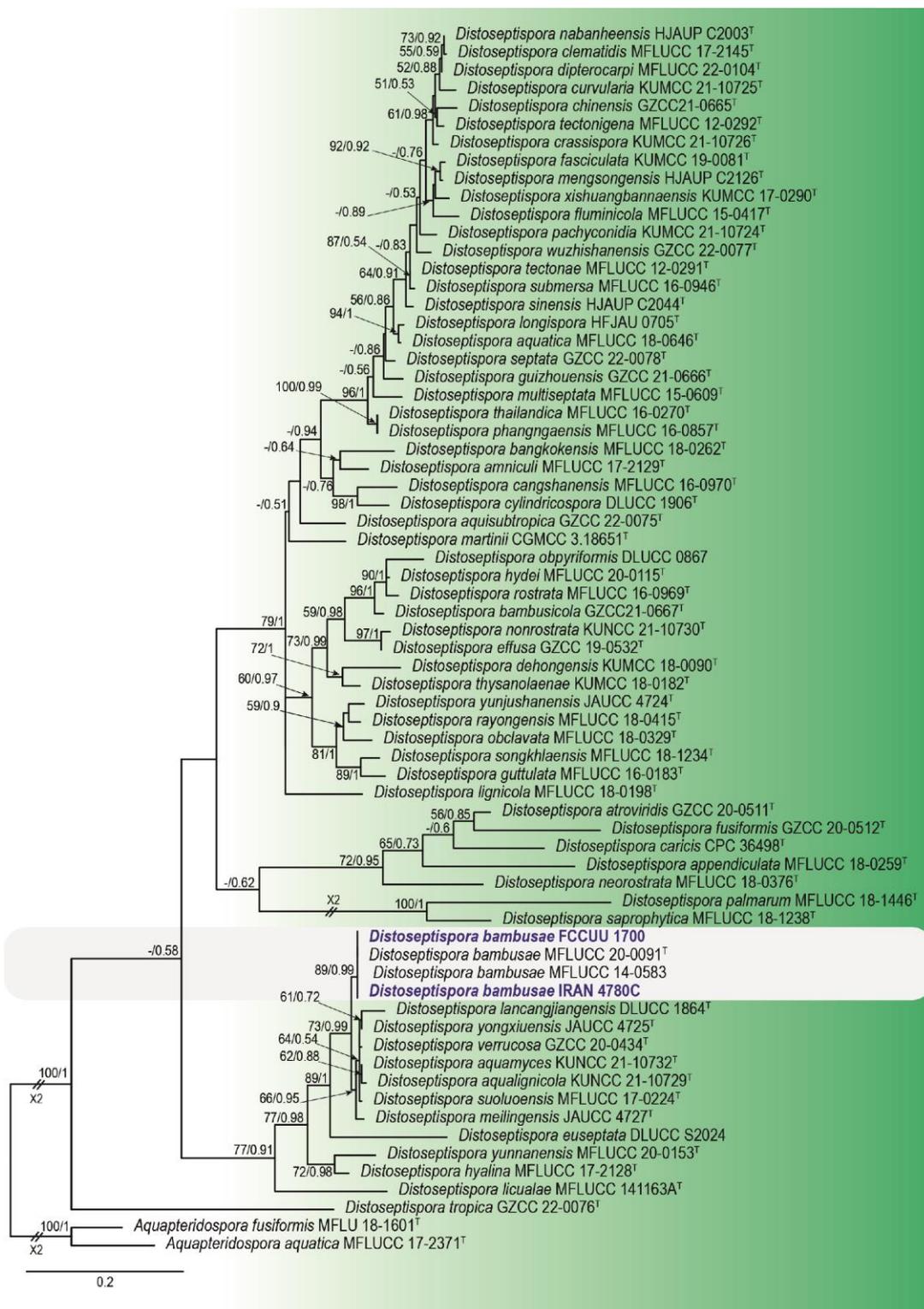


Fig. 1 Phylogenetic tree inferred from Maximum Likelihood (ML) analysis based on ITS sequence data of *Distoseptispora* species. The Maximum Likelihood bootstrap supports (BS) values and Bayesian posterior probabilities (PP) >50% are given at the nodes (BS/PP). The tree was rooted to *Aquapteridospora aquatica* (MFLUC 17-2371) and *A. fusiformis* (MFLU 18-1601) and newly identified strains are in blue bold face. The scale bar indicates the number of nucleotide substitutions. ^T indicates ex-type strains.

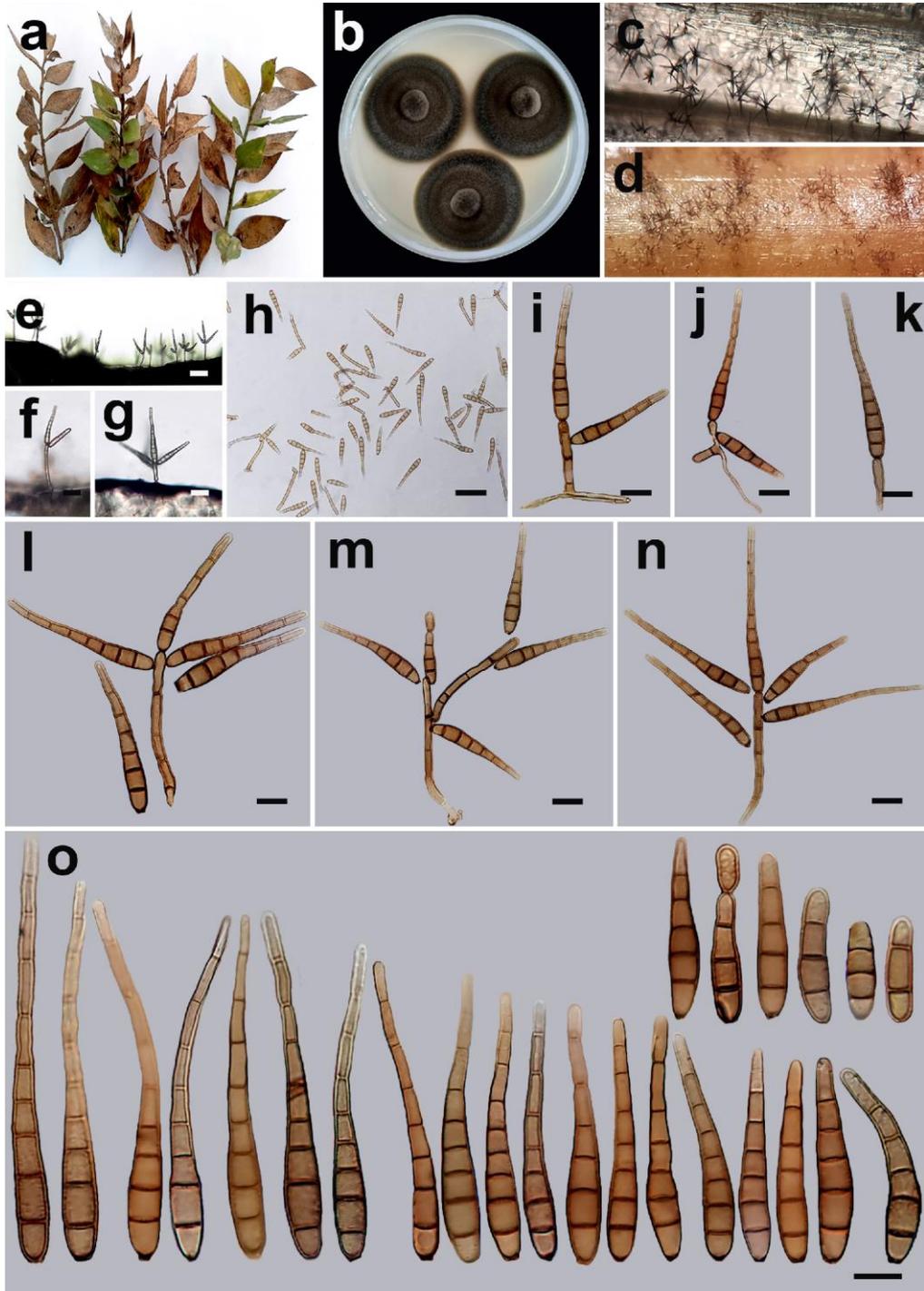


Fig. 2 *Distoseptispora bambusae* (IRAN 4780C). **a.** Host. **b.** Colony on PDA after two weeks. **c–g.** Sporulation pattern on TWA. **i–n.** Conidiophores. **o.** Conidia. Scale Bars: e–g = 40 μ m, h–o = 20 μ m.

DISCUSSION

Distoseptisporales was introduced by Luo et al. (2019) to accommodate the family. In this study, the morphological characteristics of the studied Iranian isolates from common bamboo leaves matched well with the generic concept of *Distoseptispora* (Su et al. 2016). In the phylogenetic tree based on ITS-rDNA sequences, two Iranian isolates were clustered along with the type and representative isolates of *D. bambusae* in a well-separated subclade with close affinity to *D. lancangjiangensis*, *D. meilingensis*, *D. suoluoenensis*, *D. verrucosa* and *D. yongxiuensis* (Fig. 1), although these species could be differentiated based on the shape and size of conidia and the number of transverse septa (Yang et al. 2018, 2021, Shen et al. 2021, Zhai et al. 2022). So far, six species of *Distoseptispora* have been reported on decaying/dead bamboo culms (*D. bambusae*, *D. bambusicola* X. Tang, Jayaward., J.C. Kang & K.D. Hyde, *D. hydei* Monkai & Phookamsak, *D. meilingensis*, *D. yongxiuensis*, and *D. yunjushanensis* Z.J. Zhai & D.M. Hu) (Monkai et al. 2020, Sun et al. 2020, Zhai et al. 2022, Jayawardena et al. 2023). Of these, *D. bambusae* and *D. hydei* species were associated with terrestrial habitats while the rest of the species have been reported from freshwater (Table 1). The fact that most of the reports about *Distoseptispora* species are mainly from China and Thailand does not seem to be closely related to the geographic regions. Extensive studies within different geographic regions, ecological environments, and climatic conditions are needed to explore the species diversity and geographic distribution of *Distoseptispora* (Liu et al. 2023). Here, we report *D. bambusae* on common bamboo for the first time for the fungal of Iran and the Middle East, broadening our understanding of the geographic distribution of the species.

Distoseptispora species are not restricted to any particular hosts, and have been recorded from a diverse range of monocotyledonous and dicotyledonous plants such as *Cocos*, *Pandanus* sp., *Tectona grandis*, *Bambusa*, *Clematidis* sp. and *Carex* sp. (Hyde et al. 2016, Tibpromma et al. 2018, Crous et al. 2019, Zhai et al. 2022, Zhang et al. 2022). Some species, viz. *D. bambusae*, *D. clematidis* Phukhams., M.V. de Bult & K.D. Hyde, *D. tectonae* Doilom & K.D. Hyde and *D. thysanolaenae* Goonas., Dayar., Phookamsak & K.D. Hyde have been recorded in both freshwater and terrestrial habitats (Zhang et al. 2022). In this study, we obtained isolates from dead bamboo leaves, a terrestrial habitat. This study highlights the need for further studies to explore *Distoseptispora* species diversity in different regions and diverse climatic conditions in Iran.

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REFERENCES

- Afshari, N., Gomes de Farias, A.R., Bhunjun, C.S., Phukhamsakda, C., Lumyong, S. and Hyde, K.D. 2023. *Distoseptispora dipterocarpi* sp. nov. (Distoseptisporaceae), a lignicolous fungus on decaying wood of *Dipterocarpus* in Thailand. *Current Research in Environmental & Applied Mycology* 13: 68–78.
- Ahmadpour, A., Ghosta, Y. and Poursafar, A. 2021. Novel species of *Alternaria* section *Nimbya* from Iran as revealed by morphological and molecular data. *Mycologia* 113: 1073–1088.
- Ahmadpour, A., Heidarian, Z., Alavi, Z., Alavi, F. and Ghosta, Y. 2023. *Exserohilum persianum* sp. nov., a new species from Iran based on morphological and molecular characters. *Mycologia Iranica* 10: 35–44.
- Crous, P.W., Wingfield, M.J., Lombard, L., Roets, F., Swart, W.J., et al. 2019. *Fungal Planet* description sheets: 951–1041. *Persoonia* 43: 223–425.
- Dong, W., Hyde, K.D., Jeewon, R., Doilom, M., Yu, X.D., et al. 2021. Towards a natural classification of annulatasceae-like taxa II: Introducing five new genera and eighteen new species from freshwater. *Mycosphere* 12: 1–88.
- Ershad, D. 2022. *Fungi and fungal analogues of Iran*. Ministry of Agriculture, Agricultural Research, Education and Extension Organization, Iranian Research Institute of Plant Protection, Iran. 695 pp.
- Farr, D.F. and Rossman, A.Y. 2023. *Fungal databases*, U.S. National Fungus Collections, ARS, USDA. <https://nt.ars-grin.gov/fungaldatabases/>. Retrieved January 25, 2023.
- Hyde, K.D., Bao, D.F., Hongsanan, S., Chethana, K.W.T., Yang, J. and Suwannarach, N. 2021. Evolution of freshwater Diaporthomycetidae (Sordariomycetes) provides evidence for five new orders and six new families. *Fungal Diversity* 107: 71–105.
- Hyde, K.D., Hongsanan, S., Jeewon, R., Bhat, D.J., McKenzie, E.H.C., et al. 2016. Fungal diversity notes 367–490: Taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity* 80: 1–270.
- Hyde, K.D., Norphanphoun, C., Maharachchikumbura, S.S.N., Bhat, D.J., Jones, E.B.G., et al. 2020. Refined families of *Sordariomycetes*. *Mycosphere* 11: 305–1059.
- Hyde, K.D., Tennakoon, D.S., Jeewon, R., Bhat, D.J., Maharachchikumbura, S.S., et al. 2019. Fungal diversity notes 1036–1150: Taxonomic and phylogenetic contributions on genera and species of fungal taxa. *Fungal Diversity* 96: 1–242.
- Jayawardena, R.S., Hyde, K.D., Wang, S., Sun, Y.R., Suwannarach, N., et al. 2023. Fungal diversity notes 1512–1610: Taxonomic and phylogenetic

- contributions on genera and species of fungal taxa. *Fungal Diversity* 23: 1–272.
- Katoh, K., Rozewicki, J. and Yamada, K.D. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 108: 1–7.
- Konta, S., Tibpromma, S., Karunarathna, S.C., Samarakoon, M.C., Steven, L.S., et al. 2023. Morphology and multigene phylogeny reveal ten novel taxa in Ascomycota from terrestrial palm substrates (Arecaceae) in Thailand. *Mycosphere* 14: 107–152.
- Korbie, D.J. and Mattick, J.S. 2008. Touchdown PCR for increased specificity and sensitivity in PCR amplification. *Nature Protocols* 9: 1452–1456.
- Krauss, G.J., Sole, M., Krauss, G., Schlosser, D., Wesenberg, D. and Baerlocher, F. 2011. Fungi in freshwaters: Ecology, physiology and biochemical potential. *FEMS Microbiology Reviews* 35: 620–651.
- Li, W.L., Liu, Z.P., Zhang, T., Dissanayake, A.J., Luo, Z.L. et al. 2021. Additions to *Distoseptispora* (Distoseptisporaceae) associated with submerged decaying wood in China. *Phytotaxa* 520: 75–86.
- Liu, J., Hu, Y., Luo, X., Xu, Z., Castañeda-Ruiz, R.F. et al. 2023. Morphological and phylogenetic analyses reveal three new species of *Distoseptispora* (Distoseptisporaceae, Distoseptisporales) from Yunnan, China. *Journal of Fungi* 9: 470.
- Luo, Z.L., Hyde, K.D., Liu, J.K., Bhat, D.J., Bao, D.F. et al. 2018. 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* 9: 444–461.
- Luo, Z.L., Hyde, K.D., Liu, J.K., Maharachchikumbura, S.S.N., Jeewon, R. et al. 2019. Freshwater *Sordariomycetes*. *Fungal Diversity* 99: 451–660.
- Ma, J., Zhang, J.Y., Xiao, X.J., Xiao, Y.P., Tang, X. et al. 2022. Multi-gene phylogenetic analyses revealed five new species and two new records of *Distoseptisporales* from China. *Journal of Fungi* 8: 1202.
- Maddison, W.P. and Maddison, D.R. 2019. Mesquite: a modular system for evolutionary analysis. Version 3.61. Available from: <http://www.mesquiteproject.org>.
- McKenzie, E.H.C. 1995. Dematiaceous hyphomycetes on Pandanaceae. 5. *Sporidesmium* sensu lato. *Mycotaxon* 56: 9–29.
- Miller, M.A., Pfeiffer, W. and Schwartz T. 2012. The CIPRES science gateway: enabling high-impact science for phylogenetics researchers with limited resources. Paper presented at: Proceedings of the 1st Conference of the Extreme Science and Engineering Discovery Environment: Bridging from the extreme to the campus and beyond (ACM).
- Monkai, J., Boonmee, S., Ren, G.C., Wei, D.P., Phookamsak, R. and Mortimer, P.E. 2020. *Distoseptispora hydei* sp. nov. (Distoseptisporaceae), a novel lignicolous fungus on decaying bamboo in Thailand. *Phytotaxa* 459: 93–107.
- Nylander, J.A.A. 2004. MrModeltest v2.0. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden.
- Phukhamsakda, C., McKenzie, E.H.C., Phillips, A.J.L., Jones, E.B.G., Bhat, D.J. et al. 2020. Microfungi associated with *Clematis* (Ranunculaceae) with an integrated approach to delimiting species boundaries. *Fungal Diversity* 102: 1–203.
- Phukhamsakda, C., Nilsson, R.H., Bhunjun, C.S., de Farias, A.R., Sun, Y.R. et al. 2022. The numbers of fungi: contributions from traditional taxonomic studies and challenges of metabarcoding. *Fungal Diversity* 114: 327–386.
- Rambaut, A. 2019. FigTree, a graphical viewer of phylogenetic trees. Available from: <http://tree.bio.ed.ac.uk/software/figtree>.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A. et al. 2012. MrBayes 3: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.
- Shen, H.W., Bao, D.F., Hyde, K.D., Su, H.Y., Bhat, D.J. and Luo, Z.L. 2021. Two novel species and two new records of *Distoseptispora* from freshwater habitats in China and Thailand. *Mycology* 84: 79.
- Shoemaker, R.A. and White, G.P. 1985. *Lasiosphaeria caesariata* with *Sporidesmium hormiscioides* and *L. triseptata* with *S. adscendens*. *Sydowia* 38: 278–283.
- Song, H.Y., Sheikha, A.F., Zhai, Z.J., Zhou, J.P., Chen, M.H. et al. 2020. *Distoseptispora longispora* sp. nov. from freshwater habitats in China. *Mycotaxon* 1353: 513–523.
- Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.
- Su, H.Y., Hyde, K.D., Maharachchikumbura, S.S.N., Ariyawansa, H.A., Luo, Z.L. et al. 2016. The families Distoseptisporaceae fam. nov., Kirschsteinioteliaceae, Sporormiaceae and Torulaceae, with new species from freshwater in Yunnan Province, China. *Fungal Diversity* 80: 375–409.
- Sun, Y.R., Goonasekara, I.D., Thambugala, K.M., Jayawardena, R.S., Wang, Y. et al. 2020. *Distoseptispora bambusae* sp. nov. (Distoseptisporaceae) on bamboo from China and Thailand. *Biodiversity Data Journal* 8: e53678.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. 2013. MEGA6: molecular evolutionary

- genetics analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–2729.
- Tibpromma, S., Hyde, K.D., McKenzie, E.H.C., Bhat, D.J., Phillips, A.J.L. et al. 2018. Fungal diversity notes 840–928: Micro-fungi associated with Pandanaceae. *Fungal Diversity* 93: 1–160.
- White, T.J., Bruns, T., Lee, S.J.W.T. and Taylor, J.W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: a guide to methods and applications* (M.A. Innis, D.H. Gelfand, J.J. Sninsky & T.J. White, eds): 315–322. Academic Press, New York.
- Wong, K.M.K., Goh, T.K., Hodgkiss, I.J., Hyde, K.D., Ranghoo, V.M. et al. 1998. Role of fungi in freshwater ecosystems. *Biodiversity and Conservation* 7: 1187–1206.
- Xia, J.W., Ma, Y.R., Li, Z. and Zhang, X.G. 2017. Acrodictys-like wood decay fungi from southern China, with two new families Acrodictyaceae and Junewangiaceae. *Scientific Reports* 7: e7888.
- Yang, J., Liu, L.L., Jones, E.B.G., Li, W.L., Hyde, K.D. and Liu, Z.Y. 2021. Morphological variety in *Distoseptispora* and introduction of six novel species. *Journal of Fungi* 7: 945.
- Yang, J., Maharachchikumbura, S.S., Liu, J.K., Hyde, K.D., Gareth Jones, E.B. et al. 2018. *Pseudostanjehughesia aquitropica* gen. et sp. nov. and *Sporidesmium* sensu lato species from freshwater habitats. *Mycological Progress* 17: 591–616.
- Zhai, Z.J., Yan, J.Q., Li, W.W., Gao, Y., Hu, H.J. et al. 2022. Three novel species of *Distoseptispora* (Distoseptisporaceae) isolated from bamboo in Jiangxi Province, China. *Mycology* 88: 35–54.
- Zhang, H., Zhu, R., Qing, Y., Yang, H., Li, C. et al. 2022. Polyphasic identification of *Distoseptispora* with six new species from fresh water. *Journal of Fungi* 8: 1063.

ویژگی‌های ریخت‌شناختی و مولکولی *Distoseptispora bambusae* از ایران

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چکیده: اعضای جنس *Distoseptispora* قارچ‌هایی پوده‌رست لیگنین‌دوست با ۶۴ گونه معتبر است که ۴۲ گونه از آنها از زیستگاه‌های آبی و ۲۲ گونه از زیستگاه خاک جداسازی شده‌اند. اغلب گونه‌های این جنس از کشورهای تایلند و چین از مناطق استوایی و نیمه‌استوایی گزارش شده‌اند. در مطالعه حاضر، گونه *Distoseptispora bambusae* از برگ‌های در حال تجزیه گیاه بامبو (*Bambusa vulgaris*) بر اساس تلفیق صفات ریخت‌شناختی و داده‌های توالی حاصل از ناحیه ITS-rDNA گزارش می‌شود. توصیف دقیق ریخت‌شناختی، عکس‌ها و مقایسه آن با گونه‌های نزدیک از نظر ویژگی‌های ریخت‌شناختی و تبارشناختی ارائه شده‌اند. براساس دانش ما، این اولین گزارش از گونه *D. bambusae* از گیاه بامبو برای فونگای ایران و خاورمیانه می‌باشد.

کلمات کلیدی: *Distoseptisporaceae*، ITS-rDNA، ریخت‌شناسی، تبارشناسی، آرایه‌بندی.