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Full paper

Diaporthosporellaceae, a novel family of Diaporthales (Sordariomycetes, Ascomycota)

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ABSTRACT

Diaporthosporella cercidicola, collected from diseased branches of *Cercis chinensis* in China, is herein described and illustrated. Evidence for this new genus and species is provided by both holomorphic morphology and phylogenetic analysis. *Diaporthosporella* cannot be classified into any existing family. Thus, Diaporthosporellaceae is introduced as a new family to accommodate *Diaporthosporella*, typified by *D. cercidicola*. Morphologically, Diaporthosporellaceae can be distinguished from other diaporthalean families by irregularly uniseriate, allantoid or subreniform ascospores, conidiophores acropleurogenous, branched or sympodially branched, cylindrical, aseptate, and ellipsoidal, biguttulate conidia. Phylogenetic analysis using the internal transcribed spacers (ITS) and large subunit (LSU) ribosomal (r) DNA sequence data shows that Diaporthosporellaceae forms a distinct family within Diaporthales.

1. Introduction

The order Diaporthales includes plant pathogens, plant endophytes, saprobes, human-animal pathogens, and soil inhabitants, some of which have extensive host ranges and geographical distributions (Castlebury, Rossman, Jaklitsch, & Vasilyeva, 2002; Rossman, Farr, & Castlebury, 2007; Maharachchikumbura et al., 2015, 2016). One of the most notorious pathogens in the Diaporthales is *Cryphonectria parasitica* (Murrill) M.E. Barr, which has destroyed the American chestnut population in North America (Anagnostakis, 1987; Gryzenhout, Myburg, Wingfield, & Wingfield, 2006). Another species in the Diaporthales, *Diaporthe phaseolorum* (Cooke & Ellis) Sacc., causes stem canker in soybeans, resulting in decreased yields (Udayanga, Castlebury, Rossman, Chukeatirote, & Hyde, 2015), while species of *Cytospora* in the Valsaceae cause diseases of *Eucalyptus* (Adams, Wingfield, Common, & Roux, 2004).

The sexual morphs of Diaporthales are characterized by immersed ascomata in a stroma or in the substrate with brown to black perithecia; deliquescent true paraphyses at maturity; and unitunicate asci, with a refractive ring in the apex, that float freely within the centrum at maturity (Barr, 1978; Castlebury et al., 2002; Voglmayr, Rossman, Castlebury, & Jaklitsch, 2012; Maharachchikumbura et al., 2015). The asexual morphs of Diaporthales are generally coelomycetous, producing phialidic, often annellidic conidiogenous cells, with usually non- or one-septate conidia in the acervuli or pycnidia with or without a well-developed stroma (Rossman et al., 2007).

Familial classifications within the Diaporthales have changed substantially over the years. The LSU sequence analysis of Castlebury et al. (2002) supported six major lineages: the *Cryphonectria-Endothia* complex, Diaporthaceae *sensu stricto*, Gnomoniaceae *sensu stricto*, Melanconidaceae *sensu stricto*, the Schizoparme complex, and Valsaceae *sensu stricto*. Since then, three additional lineages were added in Diaporthales by examining the types of most genera (Rossman et al., 2007). A total of ten families were listed in the Diaporthales by Kirk, Cannon, Minter, and Stalpers (2008), i.e., Cryphonectriaceae, Diaporthaceae, Gnomoniaceae, Melanconidaceae, Melogrammataceae, Pseudovalsaceae, Schizoparmeaceae, Sydowiellaceae, Togniniaceae and Valsaceae. Subsequently, the families Harknessiaceae, Lamproconiaceae, Pseudoplagiostomataceae, and Tirisporellaceae were added (Cheewangkoon et al., 2010; Crous, Summerell, Shivas, Carnegie, & Groenewald, 2012; Norphanphoun et al., 2016; Suetrong et al., 2015). Voglmayr, Castlebury, and Jaklitsch (2017) demonstrated that *Melanconis* species occurring on Juglandaceae are phylogenetically distinct from *Melanconis* s. str., and described a new family, Juglanconidaceae. Senanayake et al. (2017) updated the families of Diaporthales based on morphological and phylogenetic

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evidence and added seven additional families. Currently, there are 21 families accepted in Diaporthales.

In the present study, we provide morphological and phylogenetic evidence for *Diaporthosporella cercidicola* sp. nov. based on three specimens collected from *Cercis chinensis* in Jiangsu Province, China. These three specimens form a well-supported, monophyletic clade in molecular phylogenetic analyses of the internal transcribed spacers (ITS) and large subunit (LSU) ribosomal (r) DNA. We therefore introduce *Diaporthosporella* gen. nov. and Diaporthosporellaceae fam. nov. to accommodate this new species.

2. Materials and methods

2.1. Isolates

Fresh specimens of diaporthalean fungi were collected from infected branches or twigs during collecting trips in Jiangsu Province, China (Table 1). Three isolates were made by removing a mucoid spore mass from the conidiomata or ascomata, and spreading the suspension onto the surface of 1.8% potato dextrose agar (PDA) in a Petri dish. Petri dishes were incubated at 25 °C for up to 24 h. Single germinating conidium or ascospore was transferred onto fresh PDA plates. All specimens were deposited in the Museum of the Beijing Forestry University (BJFC). Axenic cultures (CFCC 51994, CFCC 51995, CFCC 51996) are held in the China Forestry Culture Collection Center (CFCC).

2.2. DNA amplification and sequencing

Genomic DNA was extracted from 7 d old colonies grown on PDA with cellophane using a modified CTAB method (Doyle & Doyle, 1990). DNA was checked by electrophoresis in 1% agarose gels, and the quality was measured with a NanoDrop 2000 (Thermo Fisher Scientific, Waltham), following the manufacturer's instructions (Desjardins, Hansen, & Allen, 2009). PCR amplifications were performed in a DNA Engine (PTC-200) Peltier Thermal Cycler (Bio-Rad Laboratories, Waltham), using primer pairs ITS1/ITS4 (White, Bruns, Lee, & Taylor, 1990) and NL1/NL4 (O'Donnell, 1993) for the ITS and LSU rDNA, respectively. PCR products were checked visually via electrophoresis in 2% agarose gels. DNA sequencing was performed using an ABI PRISM 3730XL DNA Analyzer with a BigDye Terminator Kit v.3.1 (Invitrogen, Waltham) at the Shanghai Invitrogen Biological Technology Company (Beijing, China).

2.3. Phylogenetic analysis

To identify the phylogenetic position of the collected specimens within the Diaporthales, we used ITS and LSU sequence data to construct a phylogeny. Sequences of representative species of Diaporthales species were selected from Norphanphoun et al. (2016) and supplemented with sequences from GenBank (Table 1). *Pyricularia grisea* (Ina168) and *Nakataea oryzae* (CBS 243.76) were selected as outgroups. Our novel sequences were aligned using MAFFT v.6 (Katoh & Toh, 2010) and edited manually with MEGA6 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). Three methods of phylogenetic reconstruction were performed: maximum parsimony (MP) in PAUP v.4.0b10 (Swofford, 2003); maximum likelihood (ML) in PhyML v.7.2.8 (Guindon et al., 2010); and Bayesian Inference (BI) in MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003). In the MP analysis, a heuristic search was performed with 1000 random-addition of sequences with the tree bisection and reconnection (TBR) algorithm. Maxtrees was set to 5000, branches of zero length were collapsed, and all of the most parsimonious trees were saved. Tree length (TL), consistency index (CI), retention index (RI), and rescaled consistency (RC) were recorded. For the ML analysis, a GTR site substitution model was used (Guindon et al., 2010). Branch support was evaluated with a bootstrapping (BS) method of 1000 replicates (Hillis & Bull, 1993).

MrModeltest v. 2.3 (Posada & Crandall, 1998) was used to estimate the best-fit model of nucleotide substitution model for each gene. Using this model, we performed BI on each individual DNA dataset with a Markov Chain Monte Carlo (MCMC) algorithm in MrBayes v. 3.1.2 (Ronquist & Huelsenbeck, 2003). Two MCMC chains were run from random trees for 10 million generations and stopped when average standard deviation of split frequencies fell below 0.01. Trees were saved each 1000 generations. The first 25% of trees were discarded as the burn-in phase of each analysis, and the Bayesian posterior probabilities (BPP) were calculated from the remaining trees (Rannala & Yang, 1996). Phylograms were drawn with Figtree v. 1.3.1 (Rambaut & Drummond, 2010). Sequences were submitted to GenBank (Table 1). The sequence alignment file was submitted to TreeBASE (www.treebase.org; accession number S21126). Our novel taxonomic descriptions were submitted to MycoBank (Crous, Gams, Stalpers, Robert, & Stegehuis, 2004).

2.4. Morphology

Species identification was based on the morphological features of the fruiting bodies produced on infected plant tissues, supplemented with cultural characteristics. Morphological characteristics of the fruiting bodies were examined using a stereomicroscope (M205 FA, Leica, Wetzlar). The features recorded included the size and shape of the stromata and locules. Micromorphological observations were made with a compound microscope (DM 2500, Leica). More than 20 fruiting bodies were sectioned, and 50 spores were selected randomly for measurement. Cultural characteristics of isolates incubated on PDA in the dark at 25 °C were observed and recorded, including colony colour, texture, and the arrangement of the conidiomata.

3. Results

3.1. Molecular phylogeny

The phylogenetic tree based on a combined analysis of ITS and LSU sequence data was used to resolve the position of *Diaporthosporella* within Diaporthales. The sequence alignment of Diaporthales included three strains from this study and 73 reference strains from recent studies (Castlebury et al., 2002; Gomes et al., 2013; Gryzenhout et al., 2006; Hongsanan et al., 2017; Mejía, Castlebury, Rossman, Sogonov, & White, 2008; Norphanphoun et al., 2016; Senanayake et al., 2017; Udayanga et al., 2011; Voglmayr et al., 2017, 2012). The alignment consisted of 1311 characters including gaps, of which 599 characters were constant, 71 variable characters were parsimony-uninformative, and 641 variable characters were parsimony informative. The MP analysis yielded 97 most parsimonious trees (TL = 2641, CI = 0.443, RI = 0.752, RC = 0.333; first tree shown in Fig. 1). Tree topologies of all MP trees were identical with few exceptions (see nodes marked with asterisks in Fig. 1). The 76 isolates clustered in 23 clades corresponding to 22 families in Diaporthales, and one additional clade referring to outgroup. The three isolates sequenced herein formed a well-supported, monophyletic clade in all analyses (MP/ML/BI = 100/100/1). Tree topologies of all genera recovered by MP, ML, and BI analyses were congruent in the combined dataset.

3.2. Taxonomy

Diaporthosporellaceae C.M. Tian & Q. Yang, fam. nov.
MycoBank no.: MB822663.

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