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Research Article

The Discovery of a New Taxon of *Phaeoisaria* from Deadwood in China

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Abstract

A new fungus named *Phaeoisaria diversa* was found on deadwood in China. It was based on morphological characters and molecular phylogenetic analysis using DNA sequence data of the ITS region and fragments of rDNA LSU. It can be recognized by the diverse conidiogenous cells. The sequences of ITS, LSU, and TUB2 were obtained. It was described and illustrated in detail in this study. The type specimen (dried culture) and living cultures were deposited in the Herbarium of Henan Agricultural University: Fungi (HHAUF).

More Information

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Keywords: *Phaeoisaria*; Phylogenetic analysis; Taxonomy; ITS; LSU

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Introduction

The genus *Phaeoisaria* was first reported in 1909 (Höhn 1909). Its typical morphological features included erect, indeterminate or determinate synnemata, branched conidiophores, numerous sympodially extending denticulate conidiogenous cells, and one-celled, hyaline conidia [1]. So far, a total of 26 records have been recorded (http://www.indexfungorum.org, 2025-2-26), and 20 species of this genus are accepted. Only *P. curvata* [1], *P. glauca* [1], *P. loranthacearum* [2], *P. fasciculata* [3], *P. annesophieae* [4] do not form synnemata. They had enriched the genus by including the strains with or without synnemata. The same condition occurred in the genus of *Cephalotrichum* [5]. In nature, members of the genus occur mainly on leaves, bark, and deadwood, but *P. clematidis* was a cause of keratomycosis [6]. During the investigation of deadwood fungi in China, a fungus with typical morphological characteristics of *Phaeoisaria,* which did not resemble other similar species in this genus, was encountered. Molecular phylogenetic analysis and morphological identification confirmed this isolate as a new taxon.

Materials and methods

Isolation

During the investigation of the wood fungus resource, the spores present on the deadwood were washed with sterile distilled water. The fungus HHAUF170532 was separated by the dilution plate method on PDA culture medium with ampicillin to inhibit bacterial growth, under 25 ℃, alternating day and night alternating incubator, and grown for 15 days. Species identification was based on morphological features of the conidiogenous cells, conidia, and colony characteristics. Morphological characteristics and measurements of the fungi were observed on slides prepared with lactic acid glycerin. Measurements and descriptions of microscopic structures were made using a Nikon N2 light microscope (Nikon Y-IDT, JAPAN). More than 30 conidiogenous cells and 50 conidia were selected randomly for measurement.

DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted from fresh mycelia using the CTAB method [7]. The ITS region (ITS) of nuclear rDNA, spanning the ITS1, 5.8S and ITS2 regions, was amplified using the primer pair ITS1/ITS4 [8]; LR5/LROR for the LSU nrDNA region [9] and Bt2a and Bt2b for the TUB2 [10]. The PCR was performed in a total volume of 20 µL µL. The PCR mixture consisted of 1 µL 50 ng/µL genomic DNA, 1 µL each of 10 µM primers, 10 µL Premix Ex Taq (Version 2.0, TaKaRa, containing 0.625 U DNA polymerase, 200 mM dNTP, and 1.5 mM Mg^{2+}), and 7 µL ddH_2O. Amplification was performed in an Eppendorf Mastercycler gradient thermal cycler (Eppendorf,

Hamburg, Germany) with the following program, initial denaturation step of 5 min at 94 ℃, followed by 35 cycles of 45 s at 94 ℃,30 s at 55 ℃ and 90 s at 72 ℃, and a final elongation step of 10 min at 72 ℃, final incubation at 4 ℃. The PCR products were tested with 1% agarose gel, and the sequence was obtained with an ABI 3730XL automated DNA Analyzer at Sangon Biotech (Shanghai) Co, Ltd.

Sequence alignment and phylogenetic analyses

DNA sequences generated by each primer combination were used to obtain consensus sequences using SeqMan v. 7.1.0 in the DNASTAR Lasergene core suite software (DNASTAR Inc., Madison, WI, USA). Sequences were aligned and edited manually using MEGA v11 Tamura, et al. 2013 and blasted in GenBank. The dataset was analysed with Mybayes v. 3.1.2 [11], parallel Bayesian analyses with four chains each and partitioned by DNA region were run for 10000000 generations, a sample frequency of 100, when the average standard deviation of split frequencies fell below 0.01, it will stop and a burn-in of 25 % [11]. In phylogenetic trees, downloaded sequences are indicated by their GenBank accession numbers. And our sequences were also submitted to GenBank.

Results

Molecular phylogenetics

The sequence data obtained from NCBI after a blast match had with higher similarity compared to our ITS sequence, with the highest degree of similarity in *P. loranthacearum* (strain CPC 24441, KR611888.1) with 97% identity, and the LSU sequence in *P. fasciculate* (strain CBS 127885, KT278705) with 99% identity. The final dataset comprised 553 characters and 142 parsimony-informative positions from 16 isolates. The resulting ITS phylogeny tree (Figure 1) proved that our two isolates grouped with a bootstrap support value BI PP = 1, two species of *P. loranthacearum* and *P. fasciculata* (0.98 bootstrap support) forming the closest sister clade (1 bootstrap support), and all species of *Phaeoisaria* gathered to one clade, with a strong bootstrap BI PP = 1, the genus of *Pleurothecium* forming a clade, and outgroup in another genus *Pleurotheciella*. The strains of *P. diversia* were claded together with *P. fasciculata* in the LSU phylogeny tree with a strong bootstrap BI PP = 1 (Figure 2). The two strains clustered in a more compact clade with a bootstrap BI PP = 0.99. The species was isolated from dead wood in Zhengzhou. It grows very slowly, but its morphology is unique. Then we observed the conidia and found that they belong to *Phaeoisaria*. Later, molecular systematization studies were conducted, and the phylogenetic results proved that it is a new, unreported isolate of this genus.

Taxonomy

Phaeoisaria diversia Meng Zhang, Huawei Luo, Yuehua Geng & Lele Ma, sp. Nov. (Figure 3).

MycoBank: MB823108

Colonies on the PDA effuse, velvet or powdery, whitish to

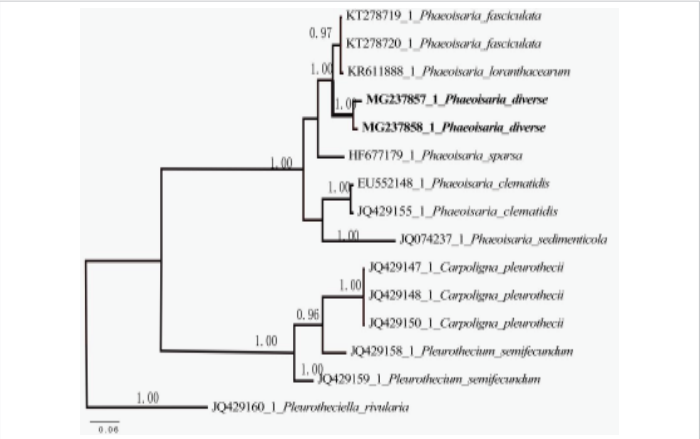

Figure 1: Phylogenetic tree generated from Bayesian inference based on the ITS rDNA sequence data set. Bayesian posterior probabilities ≥ 0.95 are given at the nodes.

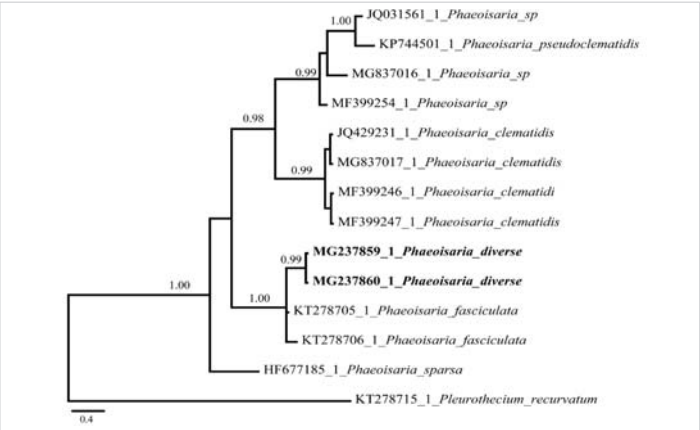

Figure 2: Phylogenetic tree generated from Bayesian inference based on the LSU sequence dataset. Bayesian posterior probabilities ≥ 0.95 are given at the nodes.

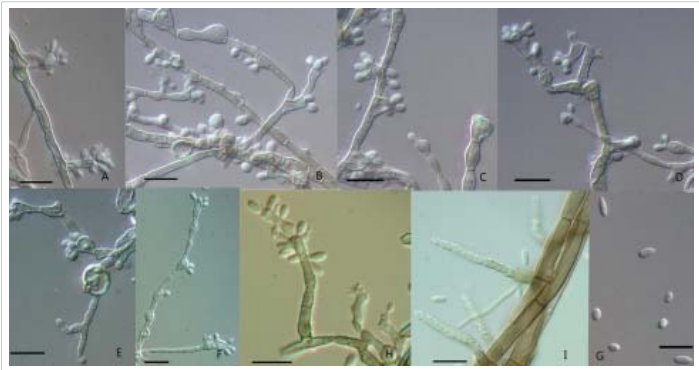

Figure 3: *Phaeoisaria diversia* A-F. Conidiogenous cells are swollen in the middle or at the top of the hyphae. H-I. Conidiophores with denticulate conidiogenous loci. G. conidia. Bar = 10 µm.

beige when sporulating, reverse olive brown, reaching 2 - 3 cm diam. At 25 ℃ in one month. *Mycelium* mostly superficial or immersum, composed of smooth, septate, branch, hyaline to light brown; sometimes mycelium thickening and forming tumor-like knots, 1.2 - 3.5 µm; synnemata absent; *Conidiophores* arising from superficial hyphae, produced in the middle of the hyphae or at the top, erect, 0-3-septate, pale brown, smooth, 5.0 - 14.5(SD = 7.8 ± 1.2 µm) × 2.0 - 4.0 µm. *Conidiogenous cells* polyblastic, with conidiogenous

loci arranged spirally in the middle or at swollen tops of conidiophores, hyaline to light brown, 4.5 - 9.0 × 4.0 - 13.0 µm, producing conidia on the surface of inflated apices, pale brown, 5.8 - 31(SD = 12.3 ± 2.0 µm) × 1.7 - 3.1 µm. Conidiogenous cells with several aggregated denticles along a long rachis covering the length of the conidiophore, with age, 0.4 - 0.6 × 0.5 - 0.7 µm. *Conidia* single, smooth, hyaline, ellipsoidal or long oval, straight, aseptate, 3.5 - 6.8 × 1.5 - 2.8 µm.

The type specimen and ex-type culture are deposited in the Herbarium of the Henan Agricultural University: Fungi (HHAUF).

HOLOTYPE: CHINA, Henan Province, Zhengzhou, deadwood, 26 September 2017, Malele, Holotype ZG0001 = HHAUF 170532 (GenBank access: ITS = MG237857, LSU = MG237859, TUB2 = MG987009), Paratype ZG0023 = HHAUF 170533 (GenBank access: ITS = MG237858, LSU = MG237860, TUB2 = MG987010).

Etymology: Named after the conidiogenous cells, which have diverse morphological characters.

Distribution: China

Comments: A total of six species of *Phaeoisaria* have been described without synnemata so far *they were P. annesophieae, P. curvata, P. loranthacearum, P. fasciculata, P. glauca,* and *P. diversia. Phaeoisaria diversia* differs from the other four species by its diverse conidiogenous cells and conidia. *P. annesophieae* can produce chlamydospores in culture [4], which were different from this new taxon. Conidia of *P. diversia* were smaller (3.5 - 6.8 × 1.9 - 2.8 µm) than those of *P. loranthacearum* (7 - 9 × 1.5 - 3 µm) and *P. fasciculata* (6.0 - 9.0 × 2 µm) (Pedro W. Crous, 2016). The conidiophores in *P. glauca* also do not form obvious synnemata but remain fasciculate, 130 - 319 × 101 - 116 µm [12]. *P. diversia* allied with *P. loranthacearum* in phylogeny, but differed by swollen conidiogenous cells and indistinguishable denticles. While synnemata were present or absent, the strains were all included in this genus, and the result was supported by ITS and LSU phylogeny trees. The case was similar to *Cephalotrichum.* The *C.columnare* without synanamorphs were claded together with *C. microsporum* with evident synanamorphs by the phylogeny tree of combined LSU, ITS, EF-1αand TUB2 [5,13-18].

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