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A new species of *Phaeoisaria* from intertidal marine sediment collected in Weihai, China

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Abstract — A culture of a synnematous hyphomycete belonging to *Phaeoisaria* was isolated from surface sediment on the coast of Weihai, China. It is described as a new species, *Phaeoisaria sedimenticola*, based on morphological characters and nuclear ribosomal DNA sequences (large subunit and internal transcribed spacer regions). *Phaeoisaria sedimenticola* is distinguished by its indeterminate synnemata, more or less cylindrical conidiogenous cells, hyaline obovoid to ellipsoidal conidia with pointed bases, aseptate conidia (3.6–7.5 × 2.5–4.4 µm), and 1-septate conidia (4.5–9 × 2–4.5 µm) that are slightly constricted at the septum.

Key words — ITS phylogeny, marine fungi, taxonomy

Introduction

During an ongoing investigation of fungi isolated from marine macroalgae and intertidal sediment in China, several new species and new records for China were encountered. An interesting synnematous hyphomycete, isolated on the coast of Weihai, China, and showing morphological features typical of *Phaeoisaria* Höhn. (Höhnel 1909), could not be assigned to any previously published *Phaeoisaria* species. *Phaeoisaria sedimenticola* is described here based on its morphological characters and phylogenetic analyses of ITS and LSU rDNA sequences.

The hyphomycete genus *Phaeoisaria* is easily recognized by having erect brown (or nearly so) indeterminate synnemata (one species lacks conidiomata) bearing numerous sympodially extending denticulate conidiogenous cells and single dry 0–1-septate conidia (Höhnel 1909; de Hoog & Papendorf 1976). In nature, the genus occurs mainly on leaves, bark, and twigs of plants. Fourteen species currently are accepted in the genus (Mel'nik 2012; Seifert et al. 2011).

Materials & methods

Sampling and Isolation

The uppermost 2–10 cm of marine sediment layers were sampled from the intertidal zone at Weihai, Shandong Province, China. Sediment samples were collected using sterile hollow glass tubes, which were sealed and stored in a freezer at –20 °C until processed. A soil-dilution plate method was adopted for the isolation of fungi. The isolation medium potato dextrose agar (PDA, 200 g of potatoes steamed for 45 min in 200 ml of distilled seawater and then homogenized, 20 g of agar, 20 g of dextrose, and distilled seawater to make a final volume of 1 L) was supplemented with streptomycin and penicillin G at 1g/L to inhibit bacterial growth. Colonies were incubated on PDA at room temperature (22–25 °C) with alternating 12 h/12 h fluorescent light/darkness. Specimens were deposited in the Herbarium of Ocean University of China Marine Biology, Qingdao, China (OUCMB), and cultures in China General Microbiological Culture Collection Center, Beijing, China (CGMCC).

Morphological characterization

Morphological characters were not observed on natural substrates but developed only in pure culture. Microscopic characters and measurements were observed on slides prepared with lactic acid glycerin or stained with Cotton Blue. Detailed incubation and observation methods are presented by Cheng et al. (2011). Means ± standard deviation (s.d.) based on more than 50 measurements are given for conidial dimensions.

DNA extraction, PCR amplification and sequencing

Isolates were grown in 1.5 mL microcentrifuge tubes containing 1.0 mL potato dextrose broth. The broth was decanted, and the mycelium was washed and centrifuged twice in sterile deionized water (Fernández et al. 1999). Genomic DNA was extracted according to Zhang et al. (2010). The primers ITS1 and ITS4 were used to amplify ITS rDNA (White et al. 1990), LR0R and LR7 for LSU rDNA (Fernández et al. 1999).

PCR was performed in a mixture with 12.5 μL of 2 × Taq PCR Master Mix (Tiangen Biotech, Beijing, China), 1 μL each of 10 μM primers, 1 μL of the undiluted DNA extract, with the total volume adjusted to 25 μL with distilled deionized water. PCR was performed using the following thermocycling parameters: initial denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1.5 min, and extension at 72 °C for 2.5 min. A final extension step of 10 min at 72 °C was added. The PCR products were checked on 1% agarose electrophoresis gel stained with ethidium bromide. Sequencing was conducted on an ABI 3730XL automated DNA Analyzer at Sangon Biotech (Shanghai) Co., Ltd.

Sequence alignment and phylogenetic analyses

The SeqMan program was used to obtain consensus sequences. BLAST searches were performed to compare the sequences with those deposited in GenBank. Sequences with significant matches were used for phylogenetic analyses. Representative sequences of morphologically similar genera were also included. GenBank accession numbers of involved sequences are included on Fig. 1. The sequences were aligned using MAFFT version 7 online (http://mafft.cbrc.jp/alignment/software/), applying the Lins-i manual strategy with default parameters, followed by manual adjustments as necessary. The final

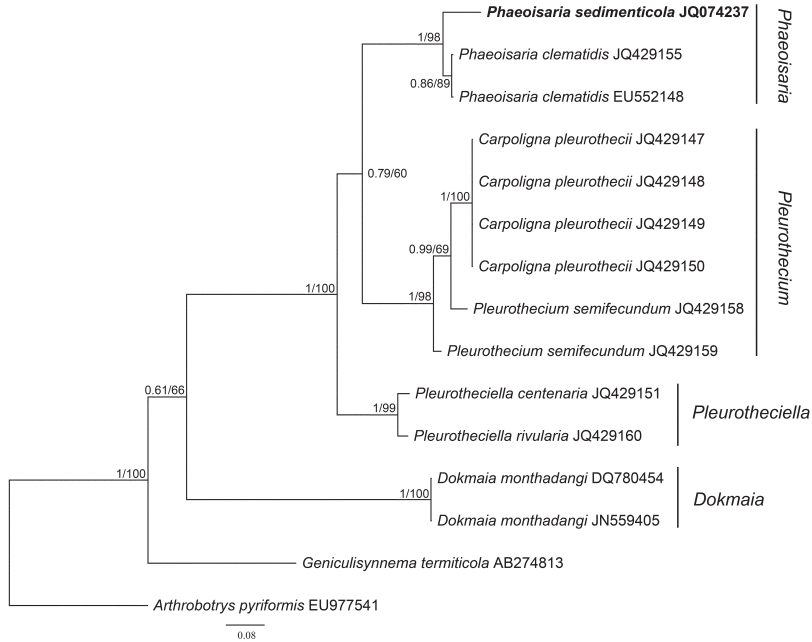

Fig. 1 Phylogenetic tree generated from Bayesian inference based on ITS rDNA sequence data set. Bayesian posterior probability and Maximum likelihood bootstrap are indicated at the nodes.

alignment of the data set was deposited at TreeBASE (submission ID 13811). Phylogenetic relationships were estimated using maximum likelihood (ML) and Bayesian inference (BI) analyses. The best-fit substitution model under the Akaike Information Criterion (AIC) (Posada & Buckley 2004) was determined by using MrModeltest 2.3 (Nylander 2004) and PAUP* 4.0b 10 (Swofford 2003).

ML analysis was performed with PAUP* 4.0b 10 (Swofford 2003). Nodal support was determined by non-parametric bootstrapping with 1000 replicates. The branch-swapping algorithm employed was tree bisection-reconnection (TBR), with MulTrees option in effect and steepest descent option not selected. A bootstrap proportion (BP) above 70% was considered a significant value. BI analysis was conducted in a likelihood framework as implemented by MrBayes 3.2.1 based on Markov chain Monte Carlo approach (Ronquist et al. 2012). One cold and three heated Markov chains were used. The BI analysis was run for 1 000 000 generations, with tree sampled every 100 generations. The first 2500 trees, which represented the burn-in phase of the analysis, were discarded, and the remaining trees were used for calculating the posterior probabilities (PP) in the consensus tree. From those trees that were sampled after the process had reached convergence, a 50% majority-rule consensus tree was computed to get estimates for clade reliability. A probability of 0.95 was considered significant.

Results

Molecular phylogenetics

Based on a megablast search of NCBI's GenBank of nucleotide database, the closest match to our LSU sequence (JQ031561) is *P. clematidis* (strain DAOM 226789, accession no. JQ429231) with 98% identity. The closest hits using the ITS sequence (JQ074237) are with *P. clematidis* (CBS 113340, EU552148) and *P. clematidis* (DAOM 226789, JQ429155) with 90% and 91% identity, respectively, followed by *Pleurotheciella* and *Carpoligna* (anamorph *Pleurothecium*).

The topologies of ITS and LSU rDNA trees were consistent with each other at the species level. Only the phylogenetic tree generated from ITS rDNA (Fig. 1) is shown here. The ITS rDNA data set consisted of 15 aligned sequences (including the outgroup sequence of *Arthrobotrys pyriformis* EU977541) and 558 characters (258 parsimony informative). The best-fitting evolutionary model for the data set was SYM + G model under the AIC. In the ITS phylogeny, our new fungus clusters with *P. clematidis* (JQ429155 and EU552148), the type species of *Phaeoisaria*, with strong support values (BI PP = 1, ML BP =98%). The *Phaeoisaria* lineage appears to be sister to the *Pleurothecium*-group, but did not receive strong branch support in either Bayesian or bootstrap analyses (BI PP = 0.79, ML BP = 60%). *Dokmaia* Promp. and *Geniculisynnema* Okane & Nakagiri occur in two separate clades distinct from the *Phaeoisaria* lineage.

Taxonomy

Phaeoisaria sedimenticola X.L. Cheng & Wei Li ter, **sp. nov.** Fig. 2

MycoBank MB 563661

Differs from *Phaeoisaria uniseptata* by its obovoid to ellipsoidal conidia and from *P. clematidis* by its shorter, sometimes 1-septate, conidia.

Type: China. Shandong province, Weihai, intertidal zone, surface marine sediment, 37°27′57.0″N 122°8′21.3″E, 5 Jun 2010, K.M. Sun (**holotype**, OUCMBIII$_{10}$1039; ex-type culture, CGMCC 3.14949).

Etymology: *sedimenticola*, indicating a lifestyle in sediment, from which the fungus was originally isolated.

Colonies on PDA attaining 30 mm diam in 14 d at 25 °C, at first smooth, buff to brown, later often with numerous synnemata; reverse greyish brown at center, olivaceous near margin; exudate and odour absent. Hyphae smooth, hyaline to pale brown, 2–2.5 µm wide. Synnemata erect, cylindrical to subulate, up to 4000 µm high or sometimes longer, 70–90 µm wide at the base, consisting of very regular, parallel, brown hyphae. Conidiogenous cells arising from synnemata with apical fertile portion bent outwards, and also arising from undifferentiated hyphae at approximately right angle. Conidiogenous cells smooth, slightly thick-walled, pale brown near base, subhyaline towards apex, more or less cylindrical, usually about 15–25 µm long, 2–3 µm wide, with conspicuous,

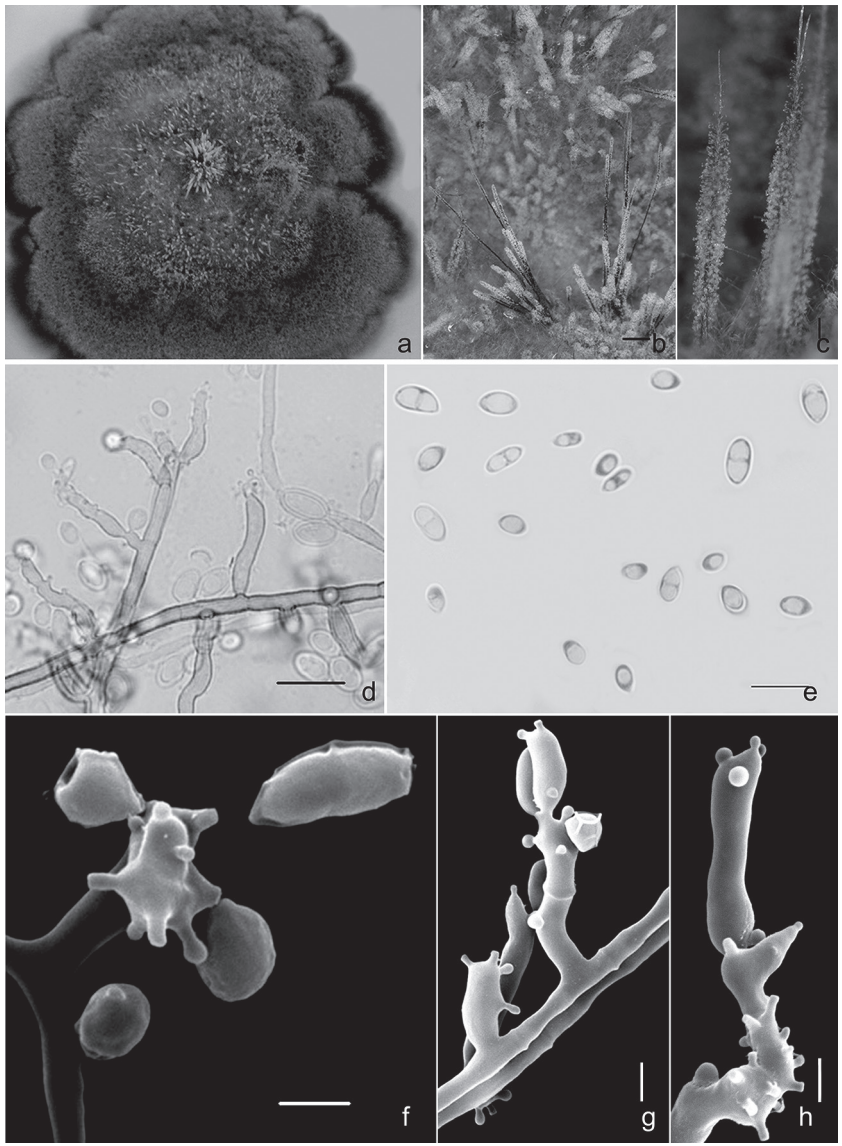

Fig. 2 *Phaeoisaria sedimenticola* (holotype, OUCMBIII$_{10}$1039). a. Colony on PDA; b–c. Synnemata; d, f–h. Conidiogenous cells with denticles; e. 0–1-septate conidia. Scale bars: b, c = 100 μm; d, e = 10 μm; f–h = 2 μm.

cylindrical denticles 0.5–1 μm long, occurring scattered or clustered in the apical region. Conidia smooth-walled, hyaline, with a pointed base, usually aseptate when attached to the conidiogenous cells, 0–1-septate after release; aseptate conidia, obovoid to ellipsoidal, (3.5–)4.5–5.5(–7.5) × (2.5–)3–4(–4.5) μm (mean ± s.d. = 5.0 ± 0.8 × 3.3 ± 0.4 μm); 1-septate conidia, obovoid, slightly constricted at septum, (4.5–)5.5–6.5(–9) × (2–)2.5–3.5(–4.5) μm (mean ± s.d. = 6.2 ± 1.0 × 3.1 ± 0.6 μm).

Discussion

Phaeoisaria sedimenticola is assigned to *Phaeoisaria* based on its typical indeterminate synnemata (Fig. 2b,c) with monomitic parallel stipe (Seifert & Okada 1990). Some synnematous hyphomycete genera, such as *Chryseidea* Onofri, *Dokmaia*, *Drumopama* Subram., *Geniculisynnema*, *Harpographium* Sacc., *Nodulisporium* Preuss, and *Phialophaeoisaria* Matsush. morphologically resemble *Phaeoisaria,* producing indeterminate synnemata, sympodial conidiogenous cells, and dry, usually aseptate, conidia (Seifert et al. 2011). They can be clearly separated from *Phaeoisaria* by their morphology. *Chryseidea*, *Dokmaia*, and *Phialophaeoisaria* differ from *Phaeoisaria* by their conidiogenous cells. *Chryseidea* and *Dokmaia* produce ampulliform (rather than cylindrical or clavate) conidiogenous cells (Promputtha et al. 2003; Onofri et al. 1981), whereas *Phialophaeoisaria* has phialidic or polyphialidic conidiogenesis (Matsushima 1995). *Drumopama* and *Harpographium* differ from *Phaeoisaria* by producing citriform (*Drumopama*) and fusiform to falcate (*Harpographium*) conidia (Seifert et al. 2011). *Geniculisynnema* is distinguished by antler-like synnemata and dichotomously branching conidiophores (Okane & Nakagiri 2007). The distinction between synnematous asexual stages of *Xylaria* (formerly classified in *Nodulisporium*) and *Phaeoisaria* is based on apically aggregated scars and rhexolytic conidial secession.

The taxonomic significance of the presence of synnemata must be evaluated on a case-by-case basis (Seifert & Okada 1990). Genera such as *Pleurothecium* Höhn. (anamorphic *Carpoligna*), *Ramichloridium* Stahel ex de Hoog, *Rhinocladiella* Nannf., and *Sporothrix* Hektoen & C.F. Perkins have denticulate sympodial conidiogenous cells (similar to *Phaeoisaria* species) but do not produce synnemata. *Phaeoisaria sedimenticola* was confused with *Pleurothecium* in early stages of cultivation, before synnemata appeared. In fact, *Pleurothecium* species typically have distinctive recurved sporogenous cells, with the production of successive conidia, i.e., "helicoid cyme" (Goos 1969). *Ramichloridium* and *Rhinocladiella* are distinguished by minute denticles on conidiogenous cells and also lack conidioma (Arzanlou et al. 2007). Some species of *Sporothrix* also resemble *Phaeoisaria*, but differ in cottony colonies and the absence of pigmentation from the fertile hyphae (de Hoog & Papendorf

1976). The morphological distinctions among all of these genera are also well supported by LSU rDNA sequence analyses. *Phaeoisaria sedimenticola* can be easily distinguished from previously described species of *Phaeoisaria* by its morphology. In comparison with *P. sedimenticola*, *P. clematidis* differs by its aseptate conidia, (4.5–)6–8(–10) × 1.5–4 μm (de Hoog & Papendorf 1976); *P. magnifica* differs by its very pale olivaceous, aseptate conidia, 5–6.5 × 4–4.5 μm (Deighton 1974); and *P. uniseptata* differs by its fusiform, elliptical to cylindrical conidia, usually 1-septate, 3.6–10.2 × 1.6–3.4 μm (Mercado 1984).

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