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Article in Phytotaxa · April 2016
DOI: 10.11646/phytotaxa.258.2.9

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A new species of *Zasmidium* associated with sooty blotch and flyspeck

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During a survey to characterize the diversity of sooty blotch and flyspeck fungi in China, we obtained an isolate from a fungal colony exhibiting sooty blotch and flyspeck signs on the petiole of brown bollygum (*Litsea glutinosa*) in Guangxi, China. Based on phylogenetic analysis and morphological characterization, a new species, *Zasmidium litseae* is introduced. The new species is characterized by its swollen, straight or geniculate conidiogenous cells.

**Key words:** ectophyte; Mycosphaerellaceae; multigene; phylogenetic analysis

**Introduction**

Sooty blotch and flyspeck (SBFS) is a fungal disease occurring on a wide range of plant hosts. The disease blemishes the epicuticular wax layer, decreasing the aesthetic appearance and the sale value of fresh-market fruit. Although SBFS fungi have been studied for nearly 200 years, the identification of the causal agents remained uncertain until recent studies revealed that the complex comprised more than 80 species in more than 10 genera (Gleason et al. 2011). It is likely that many additional SBFS species remain to be discovered.

*Zasmidium*, one of the genera that includes SBFS species, was established by Fries in 1849. The type species of *Zasmidium*, *Z. cellare*, clusters in the Mycosphaerellaceae (Fries 1849, Arzanlou et al. 2007). Currently, 196 species of this genus have been documented in MycoBank with most species originating from plant hosts (Shivas et al. 2010, Crous et al. 2014, Quaedvlieg et al. 2014).

Previously, only one species of *Zasmidium*, *Z. angulare*, was reported as a causal agent of SBFS (Li et al. 2012). During a survey of the diversity of SBFS fungi in China, we obtained an isolate from a petiole of brown bollygum, *Litsea glutinosa* (Lour.) C.B. Rob., which was identified as a new species of *Zasmidium* based on phylogeny and morphology analysis. This is the first report of SBFS on *Litsea glutinosa*.

**Material & Methods**

**Isolates**

Petioles of *Litsea glutinosa* (Lour.) C.B. Rob. exhibiting SBFS colonies (Gleason et al. 2011) were collected in Beihai City, Guangxi, China, in October 2013. Thalli were transferred directly from colonies to potato dextrose agar (PDA) slants in a sterile environment and cultured at 25°C for 1 month in darkness (Sun et al. 2003). Mycelium was sub-cultured onto PDA plates in order to measure and observe fungal structures (Li et al. 2011). Microscopic examination was made after 7 days of incubation at 25°C and 30 measurements were obtained per structure. Colony descriptions were made after 2 weeks of growth on PDA plates at 25°C in the dark. Subsequently, pure cultures were stored in glycerol at −80°C.

**Genomic DNA extraction, PCR, and sequencing**

Genomic DNA was obtained according to the protocol of Li et al. (2011). The strain was amplified for two loci (ITS & LSU). The primer pairs for ITS and LSU were ITS1-F/ITS4 (White et al. 1990) and LSU1Fd/LR5 (Crous et al. 2009, Vilgalys & Hester 1990). Amplification reactions consisted of 1 unit of Taq polymerase (Thermo Scientific), 1× Taq buffer, 2 mM MgCl2, 0.2 mM dNTPs, 0.4 μM of the forward and reverse primers, and 2 μL of template DNA, and were made up to a total volume of 25 μL with sterile water. Reactions were performed on a Bio-Rad PCR Sypetiole S1000™ Thermal Cycler (Bio-Rad, USA). The amplification conditions were: initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 52°C for 30 s, extension at 72°C for 1 min, and a final extension step at 72°C for 10 min. The PCR products were sequenced by BGI (Beijing, China).
Sequence alignment

The sequences generated in this study were added to other sequences that were downloaded from GenBank (Table 1) and imported into BioEdit5.0.9.1 (Hall 1999). Preliminary alignments were performed using CLUSTAL-X (Thompson et al. 1997), then adjusted manually. Phylogenetic analysis of aligned DNA sequences was carried out with PAUP v.4.0b 10 for 32-bit Microsoft Windows (Swofford 2003). Heuristic searches were performed with 1,000 random sequence additions. Clade stability was assessed by 1,000 bootstrap replications. The sequences described in this study were deposited in GenBank; alignment and a representative tree were saved in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S17870).

<table>
<thead>
<tr>
<th>Species</th>
<th>Host or substrate</th>
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<th>LSU</th>
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Results

DNA phylogeny

We constructed trees using ITS and LSU sequences by maximum parsimony methods (Fig. 1). The tree was rooted to Teratosphaeria cryptica. A total of 18 sequences were included in the alignment of ITS and LSU regions, which contained 1,148 characters. In the analysis, 865 characters were constant, 157 characters were parsimony informative, and 126 variable characters were parsimony uninformative. The first (TL=529, CI=0.7259, RI=0.7452, RC=0.5409, HI=0.2741) of the most parsimonious trees is presented (Fig. 1).

The ZWY137 strain clustered together with Z. syzygii forming a clade with a bootstrap value of 100%. Meanwhile the clade stayed close with the type species of Zasmidium, Z. cellare. This phylogram provided a preliminary guide for identification of the isolate used in the research.

Taxonomy

Zasmidium litseae G.Y. Sun & Wanyu Zhao, sp. nov. (Fig. 2) MycoBank MB 812971

Etymology—Named after its host genus.

Mycelium consisting of septate, branched, 1.06–3.16 μm diam. hyphae. Conidiophores lateral on mycelium, solitary, erect, unbranched, straight to somewhat flexuous, subcylindrical, 27.36–59.45 × 2.03–3.09 μm, 0–5-septate. Conidiogenous cells integrated, terminal, subcylindrical, unbranched, but apex frequently swollen, straight or once geniculate, with one to several conidiogenous loci, proliferating sympodially. Conidia ellipsoidal, subcylindrical or fusiform, straight to somewhat flexuous, apex obtusely rounded, base truncate, 0–2-septate, 4.20–16.72 × 1.61–2.76 μm.
**Cultural characteristics**—On PDA convex, margin entire, with moderate aerial mycelium, reaching 19 mm diam. after 2 weeks.

**Mycelial type**—Discrete speck.

**Holotype**—CHINA. Guangxi Province: Beihai City, 21°27’26”N, 109°3’37”E, on the petiole of *Litsea glutinosa* (Lour.) C.B. Rob, Oct. 2013, Wanyu Zhao and Liu Gao, HMAS 246718 (=ZWY137) (dried culture), ex-type CGMCC3.17701 (=ZWY137).

**Notes**—*Zasmidium litseae* clusters close to *Z. syzygii* phylogenetically, but they are distinct in morphology. *Z. litseae* has shorter conidiophores and conidia, and its conidia have fewer septa. The conidiogenous cells of *Z. syzygii* taper apically towards a rounded or flattened apex, whereas the conidiogenous cells of *Z. litseae* are often swollen and geniculate towards the apex.
Acknowledgement
This work was supported by the National Natural Science Foundation of China (31371887, 31171797), the 111 Project from Education Ministry of China (B07049), CARS 28.

References