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New record of *Setomelanomma holmii* on *Picea crassifolia* in China based on morphological and molecular data

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Abstract — *Setomelanomma holmii* (*Phaeosphaeriaceae*) was collected for the first time in China and for the first time on *Picea crassifolia*. This represents a new generic record for Chinese mycobiota. The fungus is identified based on morphological and molecular characters. An illustrated description of the species is provided.

Key words — ITS, SNEED, SSU, taxonomy

Introduction

During an investigation of fungal diversity of *Picea crassifolia* Kom. in different areas of Kangle County, Gansu Province, China, several samples were recovered with the morphological characteristics of *Setomelanomma holmii.* A literature survey indicated that the genus had not been previously reported from China (Tai 1979, Qiao et al. 2011, Zhang et al. 2012, Xu et al. 2013).

Setomelanomma M. Morelet is a monotypic genus typified by *S. holmii*, which was initially reported in France (Morelet 1980). Later, morphological studies and phylogenetic analysis inferred from SSU nrRNA regions indicated that *S. holmii* occurs also in North America (Canada, United States) and possibly South Korea (Rossman et al. 2002, Zhang et al. 2009, Kim et al. 2011, Plewa et al. 2012).

Materials & methods

Morphological studies. — The specimens and pure cultures were deposited at the herbarium of Museum of Beijing Forestry University, Beijing Forestry University (BJFC). Photographs of features were taken using a Nikon P500 digital camera (Nikon, Tokyo).

The microscopic procedures follow Rossman et al. (2002). Microscopic observations and measurements were made from the slide preparations and photographs taken using a Leica 4D07 microscope (Leica, German). Spore sizes are presented as the range ± 5%, and n = number of spores measured. Special color terms follow Petersen (1996).

Molecular procedures and phylogenetic analyses. — Genomic DNA was extracted from pure cultures grown in potato dextrose agar (PDA) at 25°C for 2 weeks. A Wizard® Genomic DNA Purification Kit (Finnzymes) procedure was used to extract total genomic DNA from the fruitbodies for polymerase chain reaction (PCR). DNA sequencing was performed at Beijing Genomics Institute. The nITS region was amplified with primer pairs ITS1 and ITS4 (White et al. 1990) and the nSSU region with primer pairs NS1 and NS4 (http://www.biology.duke.edu/fungi/mycolab/primers.htm). We submitted our newly generated sequences to GenBank as *Setomelanomma holmii* (ITS, KF668244; nSSU, KF668245–KF668247) and aligned them with additional sequences downloaded from GenBank, using BioEdit (Hall 1999) and ClustalX (Thompson et al. 1997). The sequence alignment was deposited at TreeBase (http://purl.org/phylo/treebase/; submission ID: 14727). The SSU nrRNA sequences underwent maximum parsimony analysis using *Sordaria fimicola* (Roberge ex Desm.) Ces. & De Not., *Xylaria hypoxylon* (L.) Grev., and *Hypocrea schweinitzii* (Fr.) Sacc. as outgroups (Rossman et al. 2002). The tree was constructed in PAUP* v.4.0b10 (Swofford 2002) with all characters equally weighted and gaps treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed, and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1000 replicates (Felsenstein 1985). Descriptive tree statistics such as tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC) were calculated for each Maximum Parsimonious Tree (MPT) generated. MrMODELTEST v.2.3 (Posada & Crandall 1998, Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference (BY). Bayesian inference was calculated with MrBayes v.3.1.2 with a general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees for 500,000 generations, and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches that received bootstrap support for maximum parsimony (MP) ≥75% and Bayesian posterior probabilities (BPP) ≥0.95 were considered as significantly supported.

Taxonomy

Setomelanomma holmii M. Morelet, Bull. Soc. Sci. Nat. Arch. Toulon Var 36: 15. 1980. Fig. 1

Ascomata solitary, scattered, initially immersed, subcuticular, becoming erumpent, superficial through periderm of small twigs with needles still attached, perithecioid, 105–279 µm diam, black, globose to subglobose, each with a non-rostrate, periphysate ostiolum, with scattered, sparse to abundant

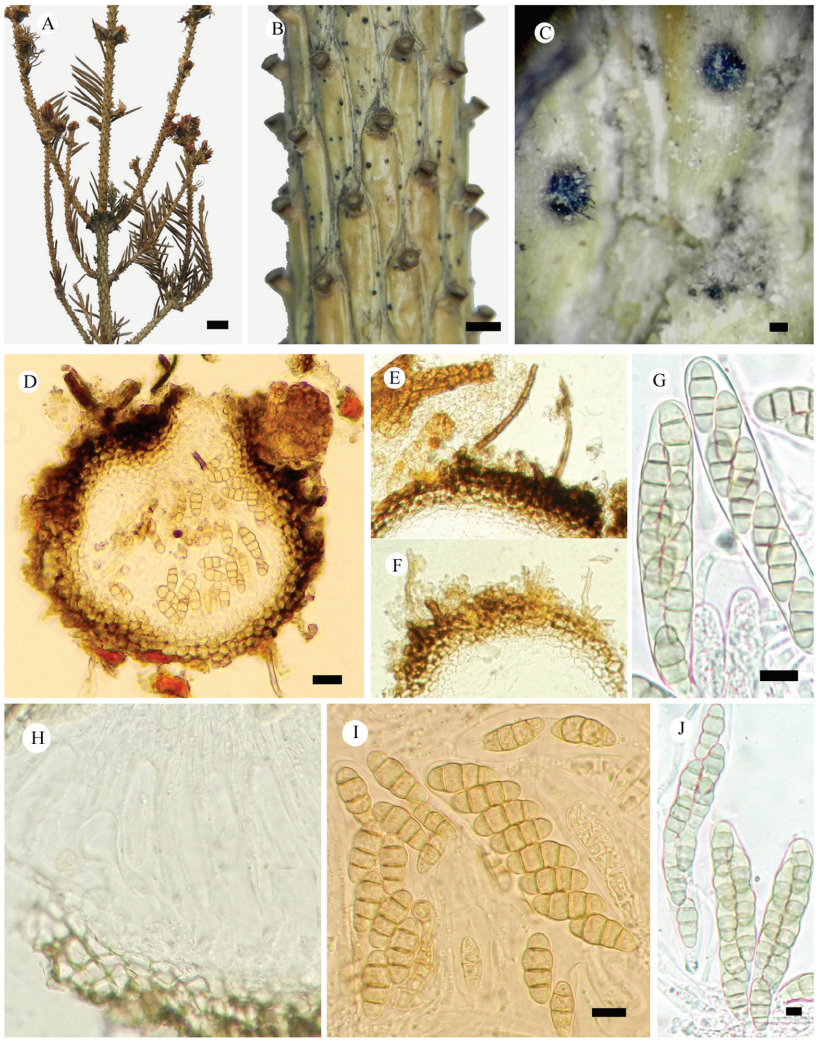

Figure 1. *Setomelanomma holmii*. A: Infected twig of *Picea crassifolia* (BJFC-S425). B–C: Ascomata on twig (BJFC-S423). D: Longitudinal section of ascomata (BJFC-S425). E–F: Setae on ascomata (BJFC-S424). G, J: Asci (BJFC-S425). H: Interthecial elements among immature asci (BJFC-S428). I: Ascospores (BJFC-S425). Scale bars: A = 1 cm; B–C = 100 μm; D = 20 μm; G–I = 10 μm; J = 5 μm.

setae; setae short, pale brown to reddish brown, straight to flexuous, slightly tapered, blunt, 17–54 × 3–6 μm. Ascomatal wall 19–36 μm thick, of 3–6 layers of cells that are dark brown and thick-walled toward outside, becoming thin-

walled, hyaline toward centrum, wall slightly thicker around non-papillate ostiolum, of 5–9 layers of cells.

Asci bitunicate, 53–77 × 10–18 μm, cylindrical to broadly cylindrical with rounded apex, with short stalk, attached at base of ascomata, eight-spored. Interthecial elements arising from basal portion of ascomatal centrum, multicellular, 2.5–4 μm wide, septate, hyaline, anastomosing above asci.

Ascospores broadly ellipsoidal, 16–20.8 × 5.9–8.4 μm (ave. 19.7 × 7.4 μm, n = 63), pale to medium brown, three-septate, slightly constricted at median septum, first and third secondary septa, cells about equal in length, widest at penultimate cell, apex broadly rounded, base rounded, surface smooth, gelatinous sheath not observed.

Specimens examined: **CHINA. Gansu Province**, Kangle County, Fucheng, Zhangjian Village, on living twigs of *Picea crassifolia*, 17 April 2013, Y.M. Liang (BJFC-S462); Huguan, Ershilipu Village, on living twigs of *Picea crassifolia*, 17 April 2013, Y.M. Liang (BJFC-S463); Minglu, Mingguan Village, on dying twigs of *Picea crassifolia* 17 April 2013, Y.M. Liang (BJFC-S464, BJFC-S465); Xiajiazhai Nursery, on dying twigs of *Picea crassifolia*, 31 July 2013, Y.M. Liang (BJFC-S466; culture BJFC-LSH01; GenBank KF668244, KF668245); Suji Town, on living twigs of *Picea crassifolia*, 31 July 2013, Y.M. Liang (BJFC-S467; culture BJFC-LSH02; GenBank KF668246); Sanchaping Forestry Farm, on dying twigs of Picea crassifolia, 1 August 2013, Y.M. Liang (BJFC-S468; culture BJFC-LSH03; GenBank KF668247).

Phylogeny

The SSU nrRNA region dataset included sequences from 44 fungal specimens representing 40 taxa. The dataset had an aligned length of 1,056 characters of which 836 characters are constant, 86 are variable and parsimony-uninformative, and 134 are parsimony-informative. Maximum parsimony analysis yielded 52 equally parsimonious trees (TL = 391, CI = 0.659, RI = 0.833, RC = 0.549). Best model for SSU nrRNA region was estimated and applied in the Bayesian analysis: GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). The Bayesian analysis resulted in the same topology with an average standard deviation of split frequencies = 0.008946.

The fungus was readily recognized as a member of *Phaeosphaeriaceae* (*Pleosporales*) based on a primary BLAST search of GenBank using the rRNA ITS1-5.8S-ITS2 region. The phylogeny inferred from SSU nrDNA region sequences resulted in seven major clades for the 44 species (Fig. 2). Three isolates of *Setomelanomma holmii* formed a strongly supported clade (MP = 98%; 1.00 BBP = 1.00) with the AF525675 sequence from the *S. holmii* holotype strain PC99.4334.

Discussion

Our collections are identified as *Setomelanomma holmii* based on morphological observations and molecular phylogenetic analyses.

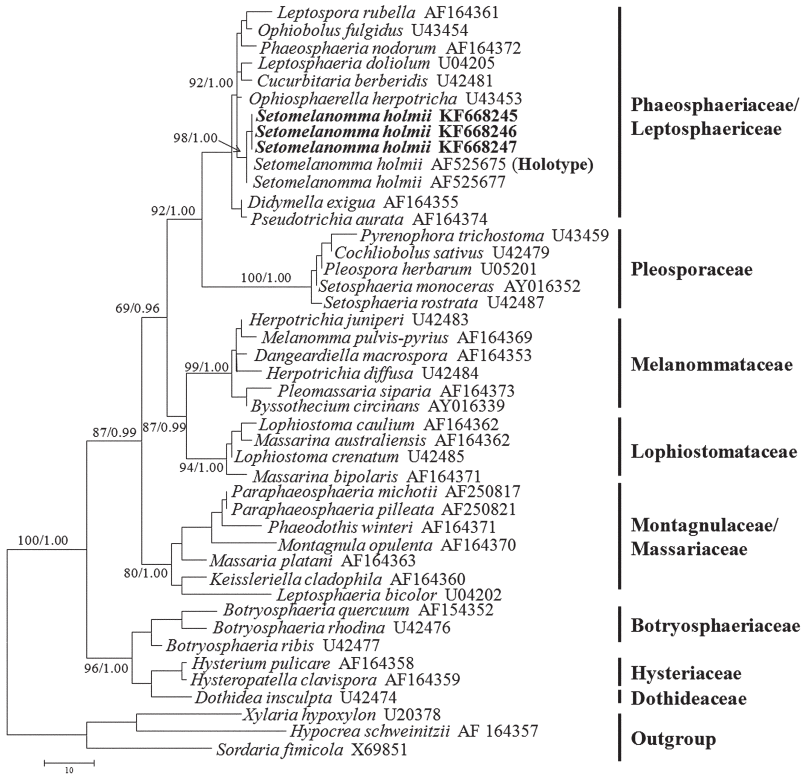

Figure 2. One of the 52 parsimonious trees illustrating the phylogeny of *Setomelanomma holmii* and related species based on nSSU sequences. Parsimony bootstrap proportions greater than 50% (before the slash marks) and Bayesian posterior probabilities greater than 0.95 (after the slash marks) are indicated along branches.

Morphologically, they are characterized by setaceous ascomata, bitunicate asci, and broadly ellipsoidal 3-septate ascospores measured 16–20.8 × 5.9–8.4 µm.

Setomelanomma was established as a monotypic genus based on *S. holmii* (Morelet 1980) and belongs to the *Phaeosphaeriaceae*. It can be distinguished from other genera of *Phaeosphaeriaceae* by the brown, three-septate ascospores, setose ascomata, and occurrence on conifers (Barr 1992). The genus is morphologically similar to *Kalmusia* Niessl and *Phaeosphaeria* I. Miyake to which it appears related (Hedjaroude 1968, Rossman et al. 2002, Zhang et al. 2012). *Kalmusia* differs in having immersed ascomata at maturity and well-developed apical papillae (Rossman et al. 2002). *Phaeosphaeria* is distinguished from *Setomelanomma* by absence of setae, causing diseases of angiosperm

hosts, and presence of coelomycetous anamorphs (Holm 1957, Hedjaroude 1968, Leuchtmann 1984).

In the phylogenetic tree (Fig. 2), the three isolates of *S. holmii* from China nested within the *Phaeosphaeriaceae*, and grouped closely with the type strain (PC99.4344) of *S. holmii*, forming a well supported lineage distinct from other species with a 92% bootstrap value and 1.00 Bayesian posterior probability.

Setomelanomma holmii causes a disease called spruce needle drop (SNEED). It has been reported in association with *Picea pungens* Engelm. and *P. glauca* (Moench) Voss (Morelet 1980; Rossman et al. 2002) in France and North America. This is the first report of this species in China, and the first report on the new host *P. crassifolia*. Although Kim et al. (2011) reported *S. holmii* from soybean paste in Korea, this report was based solely on a sequence similarity.

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