Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Black Spot Needle Blight of *Pinus sylvestris* var. *mongolica* Caused by *Heterotruncatella spartii* in China

Shu-Ren Wang,¹ Hai-Xiao Zhang,¹ Yun-Ze Chen,^{1,2} Yun-Di Zhang,¹ Da-Bo Li,³ Ying Huang,³ Guo-Cai Zhang,^{1,†} and Jing Yang^{1,4,†}

¹ Heilongjiang Province Key Laboratory of Forest Protection, School of Forest, Northeast Forestry University, Xiangfang District, Harbin 150040, P.R. China

² School of Biological Sciences, Guizhou Education University, Wudang District, Guiyang 550018, P.R. China

³ Forest Pest Control and Quarantine Station of Honghua'erji Forestry Bureau, Hulunbuir, 021112, P.R. China

⁴ College of Forestry, Guizhou University, Huaxi District, Guiyang 550025, P.R. China

S.-R. Wang and H.-X. Zhang contributed equally to this work.

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Pinus sylvestris var. *mongolica* Litv. (Pinales: Pinaceae) is an excellent tree for soil and water conservation in Northeast China. The Honghua'erji area in Inner Mongolia is the "hometown of *P. sylvestris* var. *mongolica*." However, in recent years, coniferous diseases of *P. sylvestris* var. *mongolica* have frequently occurred here. During an investigation, some black spot needle blight was observed in addition to the common blight caused by *Sphaeropsis sapinea*. From May to September 2020, black spot needle blight was found on hundreds of *P. sylvestris* var. *mongolica* trees in four forest farms, and the infection rate among the forests was 24.58% ($n = 240$). This disease first appeared on the upper part of the needles, and the needles then became withered and gradually showed light black spots, although they remained green. As the disease progressed, the needles eventually died and turned gray with many dark black spots. A fungal isolate named YJ-1 was obtained from infected needles of symptomatic pine trees, and a voucher specimen was deposited in Heilongjiang Province Key Laboratory of Forest Protection. Microscopic observation showed the conidia were three-septate (four cells) clavate spindles that measured $23.9 (20.8 \text{ to } 25.9) \times 5.9 (4.5 \text{ to } 8.2) \mu\text{m}$ ($n = 50$). The middle two cells were dark brown, and the septa

were darker than the cells. Both apical and basal cells were hyaline. The apical cell had 2 to 4 appendages (mostly 3), and the basal cell had a truncate base ($n = 50$). The cultural characteristics on potato dextrose agar medium were flat off-white and dense after 3 to 5 days. At approximately 5 to 7 days, the reverse side of the colony turned pale to slightly luteous. Superficial black acervuli were distributed in the center of the mature colonies after 10 d. Morphological, cultural, and microscopic characteristics observed were similar to *Heterotruncatella spartii* (basionym: *Truncatella spartii*) reported by Hlaiem et al. (2019). For further identification, total DNA was extracted and the internal transcribed spacer region (ITS-rDNA) was amplified by PCR using the primers ITS1/ITS4 and sequenced for BLASTn analysis and phylogenetic tree construction. The resulting 564 bp sequence (GenBank accession no. OL662864) had 99.24% identity (521/525) to *H. spartii* MFLUCC 15-0537, with bootstrap support of at least 94% using the neighbor-joining algorithm by MEGA-X (Felsenstein 1985). The fungus was identified as *H. spartii* based on morphology and molecular methods. A pathogenicity test was conducted by preparing a conidial suspension of 2.0×10^7 conidia/ml. The suspension was sprayed onto the needles of 20 potted one-year-old *P. sylvestris* var. *mongolica* seedlings, and the control was sprayed with sterile water. Then the seedlings were placed in a constant temperature room at 25°C. After 30 days, typical symptoms appeared on 11 inoculated needles, while the control needles remained symptomless. After 50 days, the reisolation infection rate reached 66.7%. The fungus present on the inoculated seedlings was morphologically identical to that originally observed on diseased pines, fulfilling Koch's postulates. The fungus was isolated from *Spartium junceum* for the first time and designated *Truncatella spartii* (Senanayake et al. 2015). It was then renamed *H. spartii* (Liu et al. 2019) and has been reported to infect *P. pinea* in Tunisia (Hlaiem et al. 2019). To our knowledge, this is the first report of *H. spartii* causing black spot needle blight on *P. sylvestris* var. *mongolica* in China and worldwide.

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[†]Indicates the corresponding authors.

G.-C. Zhang; zhang640308@126.com, and J. Yang; jingyang@gzu.edu.cn