

RESEARCH NOTE

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Morphological and Molecular Characteristics of the Oak Tree Canker Pathogen, *Annulohypoxyton truncatum*

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Cankers are localized dead areas in the bark of stems, branches or twigs of many types of trees and shrubs, and are usually caused by fungi. We observed severe canker symptoms in oak trees located in Gyeongnam province in 2011. A total 31 trees were discovered with cankers of varied size, with an average of 48.5 × 15.2 cm. Black, half-rounded globular mound shaped stromata were associated with the cankers, and the asci of the fungi associated with the cankers were cylindrical shaped with their spore-bearing parts being up to 84 μm in length. The average fungal ascospores size was 7.59 × 4.23 μm. The internal transcribed spacer sequence for the canker causing fungus showed 99% similarity to the sequence of *Annulohypoxyton truncatum*. In this study, the isolated fungus was precisely described and then compared with fungi of similar taxa.

KEYWORDS : Canker, Oak tree, Xylariaceae

The oak tree colony observed in Sancheong-gun, Gyeongnam province, consisted of a total of 44 oak trees. Of these trees, 15 were *Quercus variabilis* and 29 were *Q. acutissima*. Thirty-two of the 44 oak trees exhibited severe canker disease symptoms. The sap from the canker affected trees appeared with dark brown discoloration. Fungal stromata were associated with the cankers (Fig. 1A). The stromata bodies were dark brown to blackish brown in color with a glomerate shape (Table 1, Fig. 1A). Most of the stromata developed individually, but some were aggregated. The position of the fungal ostioles was higher than the surface of the stromata. Inside the stromata, a multitude of perithecia were observed. The perithecia were of the spherical type which included a layer of carbonaceous tissue enclosing the perithecia (Fig. 1B). The fungal asci lengths were 96~154 μm in total, with the spore-bearing parts being 85 μm length on average (Table 1, Fig. 1C) [1, 2]. The ascospores were typically hyaline, unicellular, and ellipsoid-inequilateral in shape with no ornamental surface. The size of the ascospores were 7.6 × 4.2 μm on average (Table 1, Fig. 1D). The Q-value of the spores were 1.79, which indicated that they were ellipsoid in form. Based on the fungal morphological characteristics we suspected it to be *Annulohypoxyton truncatum*.

The fungi *Annulohypoxyton* spp. belongs to the genus, *Hypoxyton* and family, Xylariaceae. Form-genus *Nodulisporium* Preuss is an anamorph of *Hypoxyton*. The *Hypoxyton* genus has two distinguishable sections, *Hypoxyton* and *Annulate* [2]. Those two sections are differentiated by three major characteristics: 1) the presence or absence of a carbonaceous layer in the stromatal tissue, 2) the height of the ostioles (those of *Hypoxyton* are usually lower than the stromatal surface level and cannot be easily observed, and there is no an annulate disc), and 3) most *Hypoxyton* species do not have perispores on their ascospores. *Hypoxyton* spp. have well developed stromata with multiple perithecia and the ascus apical ring stains blue color when an iodine solution is applied. *Hypoxyton* spores are usually hyaline and single celled. Based on the results of β-tubulin and α-actin gene sequences analyses, Hsieh *et al.* [3], relocated *Hypoxyton* into a new genus for which *Annulohypoxyton* received a new name. The fungi of genus *Annulohypoxyton* are ubiquitous with numerous reports of their presence in tropical regions, and they identified as oak tree pathogens.

To support the morphological identification of the fungus using molecular analysis tools, we utilized the primers internal transcribed spacer (ITS)1 and ITS4 [4] to amplify and sequence an ITS rDNA region of the isolate.

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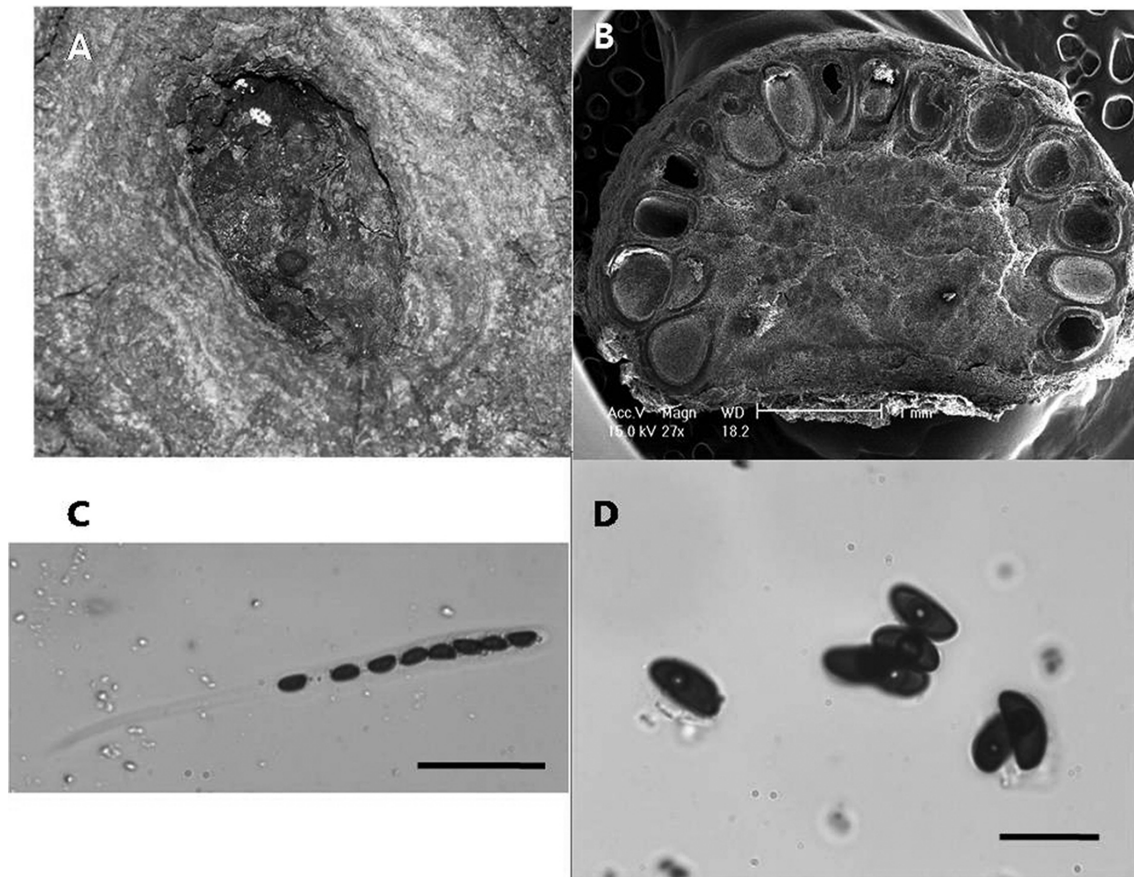


Fig. 1. A, The stromata as appearing inside a canker; B, Cross section of the stromata; C, Ascus of *Annulohyphoxylon truncatum*; D, The ascospores were hyaline and one-celled (scale bars: C = 50 µm, D = 10 µm).

Table 1. Comparison of the morphological characteristics of *A. truncatum* observed in this study

	<i>A. thouarsianum</i>	<i>A. truncatum</i>	<i>A. truncatum</i>
Reference	Whalley [1]	Ju and Rogers [2]	In this study
Host	<i>Quercus</i> wood	<i>Quercus</i> wood	<i>Quercus</i> wood
Stromata	Hemispherical to spherical, blackish brown	Glomerate to effused-pulvinate, dark reddish brown	Glomerate, dark brown to blackish brown
Perithecia	Obovoid	Spherical	Spherical
Ostioles	Papillate	Papillate	Papillate
Asci	Not observed	120~180 µm, spore-bearing parts 70~90 µm	96~154 µm, spore-bearing parts avg. 85 µm
Ascospores	14.0~24.0 × 4.5~6.0 µm, smooth, ellipsoie-inequilateral, unicellular, hyaline	8~10.5 × 4~5 µm, smooth, ellipsoie-inequilateral, unicellular, hyaline	avg. 7.5 × 4.2 µm, smooth, ellipsoie-inequilateral, unicellular, hyaline

The sequence was deposited in GenBank (accession No. JQ303335). The ITS sequences shared 99% similarity with sequences of the *Annulohyphoxylon truncatum* strain xsd08029 (accession No. FJ478107). Phylogenetic analysis was conducted using MEGA5 with a neighbor-joining algorithm. The ITS sequences of closely related fungi sections, *Annulate* and *Xylariaceae*, served as references. *Phytophthora ramorum*, the pathogen responsible for

Sudden Oak Death, was identified as an outgroup taxon (Fig. 2).

Lee and Whalley [5,6] fully described the genus *Hypoxylon* as it occurred in Asia. They reported observing *H. acrhri*, *H. bovei*, *H. stygium*, *H. fragiforme*, *H. fuscum*, *H. howeianum*, *H. rubiginosum*, *H. subgilvum*, *H. crocopleum*, and *H. jecorinum*, in Asia. However, *A. truncatum* had not been clearly described in Korea. In this

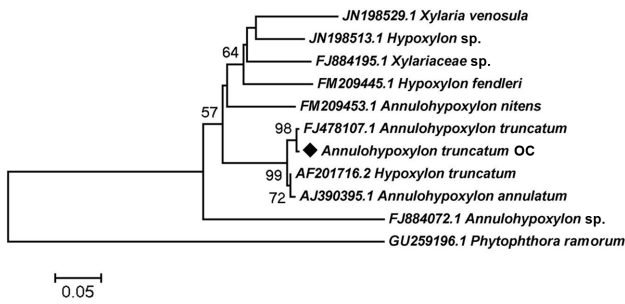


Fig. 2. A phylogenetic tree generated from the internal transcribed spacer sequences using the MEGA5 program with the neighbor-joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test is shown next to the branches (1,000 replicates). All taxa in the tree belonged to the Family Xylariaceae, except *Phytophthora ramorum*, which is a Sudden Oak Death pathogen. *P. ramorum* was used as an outgroup taxon. The present isolate was marked with a black diamond, ◆.

study, we described the characteristics of the *A. truncatum* OC isolate in terms of its morphological and molecular aspects.

References

1. Whalley AJ. Numerical taxonomy of some species of *Hypoxylon*. Mycopathologia 1976;59:155-61.
2. Ju YM, Rogers JD. A revision of the genus *Hypoxylon*. Mycologia Memori No. 20. St. Paul: APS Press; 1996.
3. Hsieh HM, Ju YM, Rogers JD. Molecular phylogeny of *Hypoxylon* and closely related genera. Mycologia 2005;97: 844-65.
4. White TJ, Bruns T, Lee S, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. New York: Academic Press, Inc.; 1990. p. 315-33.
5. Lee YS, Whalley AJ. The genus *Hypoxylon*, wood decay fungi. II. Teleomorph of *Annulata* section. Mycobiology 2000;28:1-4.
6. Lee YS, Whalley AJ. The genus *Hypoxylon*, wood decay fungi. I. Teleomorph of *Hypoxylon* section. Mycobiology 2000;28:5-10.