

New Record of *Xylaria persicaria* on *Liquidambar* Fruits in Korea

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(Received May 25, 2007)

Some *Xylaria* materials growing on the fruits of *Liquidambar* spp. were collected. They were identified as *X. persicaria* on the basis of morphological characteristics and sequence analysis of the complete ITS region (ITS1-5.8S-ITS2) of rDNA. This is the first record of this species from Korea.

KEYWORDS: Identification, ITS, Korea, *Liquidambar*, *Xylaria*

Xylaria Hill ex Schrank is one of the largest genera in the Xylariaceae accommodating about 100 species (Kirk *et al.*, 2001). Up to now, only six species, viz. *X. carpophila*, *X. filiformis*, *X. hypoxylon*, *X. longipes*, *X. oxyacanthae* and *X. polymorpha*, have been recorded in Korea (Lee and Lee, 2000).

In the course of mycofloristic investigation, *Xylaria* materials growing on the fruits of *Liquidambar* spp. were found. All collections were air-dried at room temperature and housed in the herbarium of Korea University (KUS). Morphological observation and sequence analysis of ITS rDNA (ITS1-5.8S-ITS2) were performed to identify the *Xylaria* materials. Sequence data used in the present study and their sources were listed in Table 1.

Each specimen was examined in the morphological characteristics of asci, ascospores, paraphyses and other structures of taxonomic value. Melzer's reagent, cotton-blue in lactic acid and distilled water were used for mounting media of microscopy. Dried materials were rehydrated in 3% aqueous KOH. Photographic works were carried out with the aid of a differential interference contrast microscope (Olympus BX51) equipped with a digital camera (ARC-CM13c).

Extraction of genomic DNA was undertaken according to the method outlined by Lee and Tayler (1990). The complete ITS rDNA regions were amplified by PCR using primers ITS1 and ITS4 (White *et al.*, 1990). The success of the amplification was monitored by electrophoresis on 1% agarose gels, and purified using a QIAquick gel extraction kit (Qiagen, Hilden, Germany). Purified DNAs were directly sequenced on an automatic sequencer (ABI Prism TM 377 DNA Sequencer), with primers identical for PCR.

Sequences were introduced and edited with DNASTAR (DNASTAR, Inc., Madison, Wis.). Phylogenetic analysis

Table 1. ITS sequence data used in this study

Species	Source/Geographic origin	GenBank No.
<i>Daldinia concentrica</i>	CBS 139.73/The Netherlands	AF163021
<i>Xylaria acuta</i>	ATCC 56487/USA	AF163026
<i>Xylaria apiculata</i>	CBS 365.81/Colombia	AF163027
<i>Xylaria arbuscula</i>	Unknown/Unknown	AY183369
<i>Xylaria arbuscula</i>	CBS 452.63/USA	AF163029
<i>Xylaria castorea</i>	ATCC 76020/New Zealand	AF163030
<i>Xylaria cornu-damae</i>	CBS 724.69/Canada	AF163031
<i>Xylaria enteroleuca</i>	CBS 651.89/USA	AF163033
<i>Xylaria fioriana</i>	CBS 486.61/South Africa	AF163034
<i>Xylaria hypoxylon</i>	CBS 590.72/The Netherlands	AF163036
<i>Xylaria longipes</i>	CBS 148.73/The Netherlands	AF163038
<i>Xylaria longipes</i>	SFC 960725-6/Korea	AF163039
<i>Xylaria mali</i>	CBS 385.35/Unknown	AF163040
<i>Xylaria persicaria</i>	ATCC 42766/USA	AY909021
<i>Xylaria polymorpha</i>	IFO 9780/Unknown	AF163041
<i>Xylaria</i> sp.	KUS-F50693/Namhae, Korea	EF584496
<i>Xylaria</i> sp.	KUS-F50694/Namhae, Korea	EF584497
<i>Xylaria</i> sp.	KUS-F50748/Jinju, Korea	EF584498

CBS - Centraalbureau voor Schimmelcultures; ATCC - American Type Culture Collection; SFC - Seoul National University Fungus Collection; IFO - Institute for Fermentation Osaka; KUS - Herbarium of Korea University, Seoul.

was performed according to the neighbor-joining (NJ) method in PAUP* ver. 4b10 and relative robustness of the branches was estimated by bootstrapping using 1000 replicates. *Daldinia concentrica* (AF163021), member of the Xylariaceae was selected as outgroup.

Results and Discussion

According to the morphological observation the fungus collected on the *Liquidambar* fruits was determined as *Xylaria persicaria* (Schwein.) Berk. & M. A. Curtis, that was characterized by its ascospores with a long spiral ger-

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mination slit and ecological habit. Since Schweinitz (1822) originally described *Sphaeria persicaria* (= *X. persicaria*) as an inhabitant of buried peach seeds, it has been more frequently collected on *Liquidambar* fruits (Rogers, 1979; San Martin and Rogers, 1989). *Xylaria carpophila* (Pers.) Fr. is externally similar to the present species, but it has ascospores with a straight germination slit and occurs on fallen *Fagus* fruits (Rogers, 1979).

In phylogenetic tree inferred from NJ method, our materials formed a segregated clade with *X. persicaria* (AY909021) and this grouping was supported by high bootstrap value of 100% (Fig. 2). Phylogenetic relationship of some *Xylaria* spp. was studied by Lee *et al.* (2000), in which *Xylaria* spp. were classified into three groups based on the morphological and molecular similarity, viz. *X. apiculata*, *X. arbuscula* and *X. mali* in Group A; *X. acuta*, *X. castorea*, *X. cornu-damae*, *X. enteroleuca*, *X. fioriana* and *X. longipes*, in Group B; *X. hypoxylon* and *X. polymorpha* in Group C. *X. persicaria* clade was nested in the Group A with 57% bootstrap supporting in NJ analysis. But morphological common features of Group A such as a straight germ slit of ascospores were not observed in our materials.

Seed and fruit inhabiting *Xylaria* species have been generally reported to be highly host-specific. Rogers *et al.* (2002) separated *Xylaria* on *Liquidambar* fruit from *X. persicaria* and proposed a new species *X. liquidambaris* J. D. Rogers, Y. M. Ju & F. San Martin. However, They

did not provided appreciable morphological differences between *X. liquidambaris* and *X. persicaria* because the type specimen of the latter was immature (Ellis and Everhart, 1892) and lacking ascospores (Rogers *et al.*, 2002). The validation of *X. liquidambaris* remains uncertain until further morphological and molecular work is carried out. We decided the Korean materials to place in *X. persicaria* (Schwein.) Berk. & M. A. Curtis, non Rogers *et al.* (2002).

Description

Xylaria persicaria (Schwein.) Berk. & M. A. Curtis, 풍나무콩포투리버섯 (신칭) (Fig. 1).

Stromata erect, up to 9 cm high, exterior black to brownish black, solid, divided into fertile head and sterile stalk. Head parts clavate to cylindric-clavate, 0.4–2.8 cm high and 0.1–0.3 cm wide, with longitudinally fine wrinkles, apex commonly pointed like a needle. Stalk parts irregularly twisted and crooked, often thickened toward the base, glabrous to pubescent, somewhat flattened on one side, deeply furrowed in longitudinal direction, 0.5–3.0 cm high, concolorous or paler than head parts, occasionally branched. Perithecia embedded in a stroma, black. Interior flesh white. Growing singly or in clusters. Asci 8-

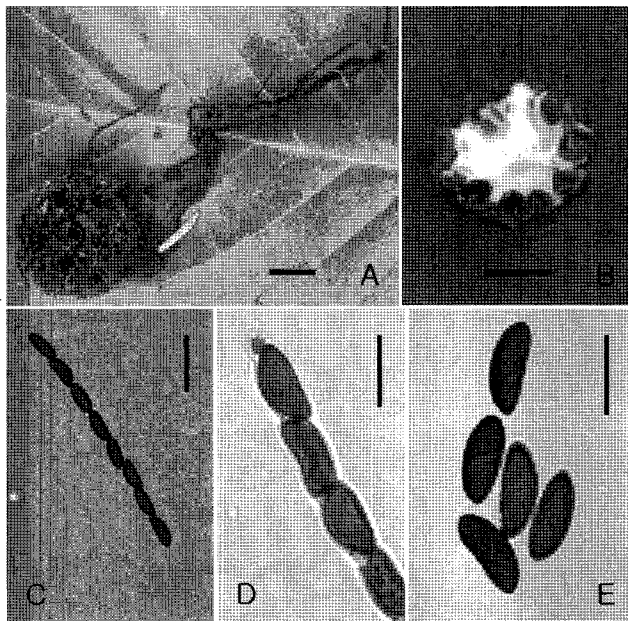


Fig. 1. *Xylaria persicaria* (KUS-F50694). A. Stromata on the fruit of *Liquidambar styraciflua*. B. Cross-section of fertile head part of a stroma. C. 8-spored ascus. D. Bluish apical pore in Melzer's reagent. E. Ascospores with a spiraling germination slit. Scale bars: 1 cm for A; 1 mm for B; 20 μ m for C; 10 μ m for D and E.

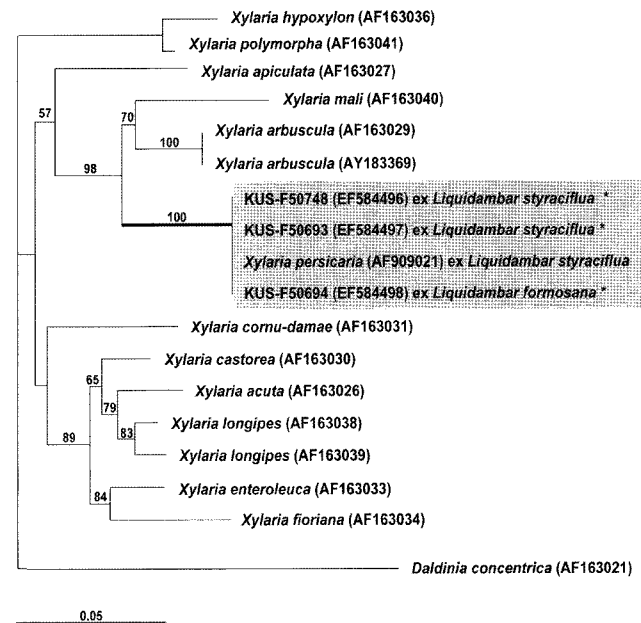


Fig. 2. Phylogenetic tree of *Xylaria* species inferred from NJ method on the basis of complete ITS region (ITS1-5.8S-ITS2). Bootstrap values are represented above the branches (1000 replication, values smaller than 50% not shown). The numbers of nucleotide changed among taxa are represented by branch length and scale bar equals the number of nucleotide substitution per site. Asterisks indicate the sequences obtained from the present study.

spored, cylindric, long stipitate, hyaline, apical pore bluing in Melzer's reagent, $135\sim 150 \times 5\sim 6 \mu\text{m}$. Ascospores elliptical to bean-shaped, commonly flatten on one side, with spiraling germination slit, uniseriate, hyaline when immature stage, becoming dark brown with one distinct oil-drop, $12\sim 15 \times 4\sim 6 \mu\text{m}$. Paraphyses cylindric, hyaline, abundant.

Distribution: Florida, USA (Rogers *et al.*, 2002), Mexico (Rogers, 1979; San Martin and Rogers, 1989), China (Rogers, 1979), and Korea.

Specimens examined: Korea, Namhae, Mt. Geumsan, 3 Jun 2004, on the fruits of *Liquidambar styraciflua* (KUS-F50693) and *L. formosana* (KUS-F50694); Korea, Jinju, Gajwa Arboretum, 17 Oct 2004, on the fruits of *L. styraciflua* (KUS-F50748) and *L. formosana* (KUS-F50749); Korea, Jinju, Gajwa Arboretum, 29 Jun 2006, on the fruits of *L. formosana* (KUS-F51475).

Acknowledgment

This research was supported by a grant (no. 052-052-040) from the Core Environmental Technology Development Project for Next Generation funded by the Ministry of Environment of the Korean Government.

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