

Kretzschmaria quercicola sp. nov., an Undescribed Fungus from Living Oak in Mt. Daeryong, Korea

Ji Ho Yun¹, Jong Won Jo^{1,2}, Jin Heung Lee¹, Sang Kuk Han², Dae Ho Kim^{1,*} and Jong Kyu Lee^{1,*}

¹Tree Pathology and Mycology Laboratory, College of Forest and Environmental Sciences, Kangwon National University, Chuncheon 24341, Korea

²Forest Biodiversity Division, Korea National Arboretum, Pocheon 11186, Korea

Abstract We encountered an unfamiliar ascomycete fruiting body, fitting characteristics of the genus *Kretzschmaria*, which features in a stipitate ascigerous stroma with carbonaceous interior and disintegrating perithecia. In this study, we report and characterize a new species of the decaying fungus. Compared to other species, one of the notable features of this specimen (TPML150908-046) is its stromatal size (up to 15 cm). Although TPML150908-046 is morphologically similar to *K. milleri* and *K. sandvicensis*, it differs sharply from both species in apical ring size (TPML150908-046, 6.5~10.5 μ m; *K. milleri*, 11~16 μ m) and ascospore width (TPML150908-046, 10.5~17 μ m; *K. sandvicensis*, 8.5~11.5 μ m). Phylogenetic trees based on β -tubulin, ITS, and RPB2 sequences showed that our collection clustered with *K. sandvicensis*, with the respective similarities for these sequences being 95.6%, 91.3%, and 97.7%, signifying it as another species. With these results, we report it as a new species, which we call *Kretzschmaria quercicola* sp. nov.

Keywords Ascomycota, *Kretzschmaria*, Morphology, Phylogeny, Taxonomy

Kretzschmaria species are found throughout the temperate and tropical regions of the world, and are known to cause white rots and root rots [1]. These fungi are also the key agents of wood decomposition, playing a central role in the ecology of forests, and are likely to have a tremendous impact on regulating energy and nutrient fluxes [2, 3]. The genus *Kretzschmaria* was well revised by Rogers and Ju [1]. According to their study, this genus is divided into the taxa *Kretzschmarioid* and *Ustulinoid*. Stromata of the former are stipitate or sessile, with their fertile parts and/or stipes often fused, usually with entire margins, while stromata of the latter are more or less sessile, but often attached by rhizoid-like processes or narrow connectives, usually with

crenate margins. A detailed molecular taxonomic study of the genus *Kretzschmaria* has not yet been reported. The studies on family Xylariaceae [4, 5] showed that the genus *Kretzschmaria* has been considered closely related to the genus *Xylaria*. Although 43 species of *Kretzschmaria* have been recorded worldwide, the genus was recently revised by Rogers and Ju [1] to include some taxa previously placed under *Ustulina*, and 16 taxa were recognized and described. Subsequently, two additional species have been reported [6, 7]. In the study on wood decay fungi, one *Kretzschmaria* species could not be assigned to any known species; in this study, we report this species as a new species and also discuss its molecular taxonomy.

The morphological character of TPML150908-046 (Fig. 1) belongs to the *Ustulinoid* taxa. The species most similar to TPML150908-046 are *K. milleri*, having almost identical stromata shapes and overlapping sizes of ascospores, and *K. sandvicensis* which has similar stromata shapes and a slightly overlapping length size of ascospores (Table 1) [1, 6, 7]. However, TPML150908-046 differs sharply from *K. milleri* and *K. sandvicensis* in the size of its apical ring, which does not overlap at all (TPML150908-046, 6.5~10.5 μ m; *K. milleri*, 11~16 μ m), as well as in having different ostioles. Additionally, TPML150908-046 differs sharply from *K. sandvicensis* in having a much broader width of ascospore (TPML150908-046, 10.5~17 μ m; *K. sandvicensis*, 8.5~11.5 μ m) (Table 1) [1]. Sizes of apical ring and ascospore are important keys in *Kretzschmaria* taxonomy [1, 6-8].

The specimen (TPML150908-046) used in this study

Mycobiology 2016 June, 44(2): 112-116
<http://dx.doi.org/10.5941/MYCO.2016.44.2.112>
pISSN 1229-8093 • eISSN 2092-9323
© The Korean Society of Mycology

***Corresponding author**

E-mail: daehokim@kangwon.ac.kr (D.H. Kim),
jonglee@kangwon.ac.kr (J.K. Lee)

Received June 1, 2016

Revised June 8, 2016

Accepted June 13, 2016

©This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

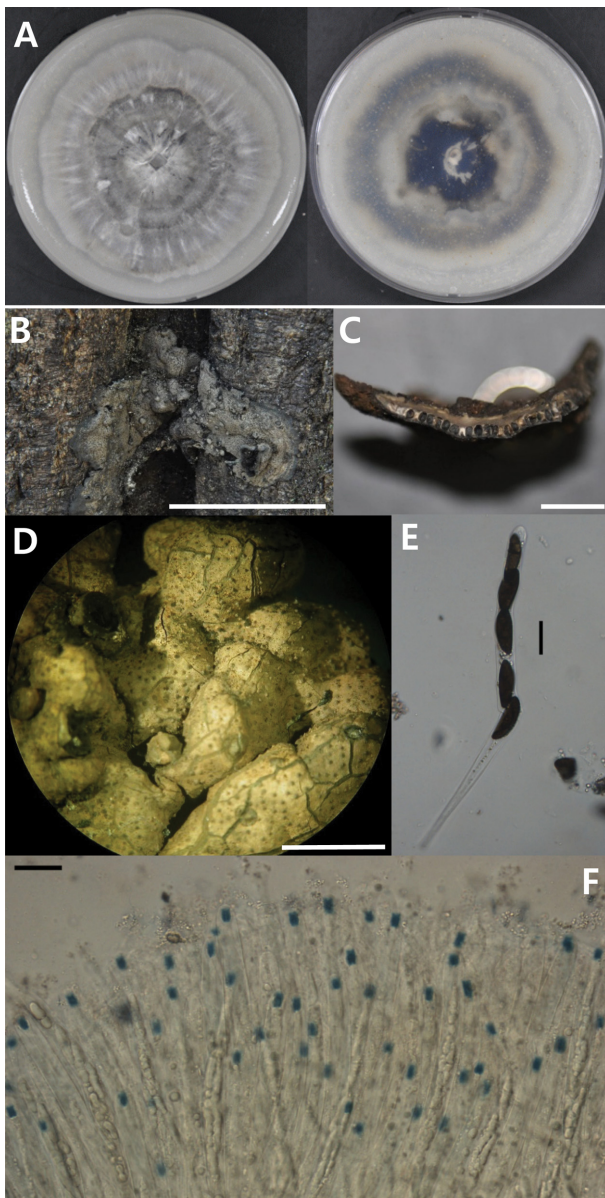


Fig. 1. Colony and morphological characters of *Kretzschmaria quercicola* sp. nov. TPML150908-046. A, Colony on oat meal agar (2%) after 31 days of inoculation; B, Stomata on substrate; C, Vertical section of a stroma showing several immersed perithecia; D, Stromatal surface showing polygonal scales and ostiolar openings; E, Asci with ascospores; F, Apical rings bluing in Melzer's iodine reagent (scale bars: B = 8 cm, C, D = 8 mm, E, F = 26.1 μ m).

was collected on September 8, 2015 from the Mt. Daeryong in Chuncheon. Morphological characters were examined by stereo (SMZ 1500; Nikon, Tokyo, Japan) and optical (COOLPIX 4500; Nikon) microscopy, following the methods described by Rogers and Ju [1]. For observations of cultural characteristics, the pure culture was isolated from specimens and was incubated on oatmeal agar at 20°C for more than 20 days. The isolate has been deposited in the Korean agricultural culture collection (KACC) as KACC 54398 (exo-

type). To extract genomic DNA, the isolate (TPML150908-046) was cultured in potato extract broth. After harvesting the mycelium, genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen 69106; Qiagen, Hilden, Germany) according to the manufacturer's instructions. β -Tubulin (using T1 and T22) [9], partial nuclear internal transcribed spacer (ITS) (ITS1 and ITS4 primers) [10], and the second largest subunit of RNA polymerase II (RPB2) (fRPB2-5F and fRPB2-7cR) [11] regions were amplified under conditions described in references [9-11] and sequenced to determine the phylogenetic position of this species. Reference sequences (Table 2) were obtained from the study of Hsieh *et al.* [12]. The data were analyzed using the Tamura-Nei parameter distance calculation model, and the distances were used to construct the Neighbor-Joining tree with MEGA ver. 7.0.14 [13].

Each species was well distinguished in individual phylogenetic trees generated by using sequences of β -tubulin, ITS, and RPB2 (data not shown). The combined phylogenetic tree (Fig. 2) based on the β -tubulin-ITS-RPB2 sequences dataset showed that TPML150908-046 was closely related to *K. sandvicensis*, belonging to the same clade. The homologies of TPML150908-046 with *K. sandvicensis* JDR 113 were 95.6% (β -tubulin), 91.3% (ITS), and 97.7% (RPB2), respectively. No significant incongruence was detected among β -tubulin, ITS, and RPB2 datasets in each phylogenetic tree, hence we present a combined dataset. When we consider homology values of individual comparisons, the homology values of TPML150908-046 with *K. sandvicensis* JDR 113 can also signify a different species. Based on morphological and molecular characters, TPML150908-046 represents a new species in the genus *Kretzschmaria* and it is named as *Kretzschmaria quercicola* sp. nov. We describe its taxonomic characteristics below.

Taxonomic description.

Kretzschmaria quercicola J. H. Yun & D. H. Kim, sp. nov. (Table 1, Fig. 1).

Etymology: *quercicola* (Lat.), living on oak.

Mycobank No.: MB 817221.

Stromata pulvinate, discrete, densely aggregated or fused, up to 15 cm \times 1–5 mm, with narrow connectives, usually with crenate, sloped blackish margins; surface dark brown, copper brown to blackish brown, with black ash like spots, often with reticulate cracks and polygonal scales, interior blackish, carbonaceous; tissue between and beneath perithecia fused with substrate, black. Perithecia obovoid to globose, 0.6–0.8 \times 1.2–1.8 mm, ostioles finely papillate to papillate. Asci fragmentary, ca. 275 μ m \times 11–15 μ m, with apical ring, 6.5–10.5 μ m \times 4–5.5 μ m, bluing in Melzer's iodine reagent. Ascospores brown to dark brown, unicellular, fusoid-inequilateral or more or less subellipsoid with smooth ends, 35–46 μ m \times 10.5–17 μ m, with straight germ slit less than spore-length.

Anamorph not observed on stromata. Cultures on 2% oatmeal agar (Difco, Detroit, MI, USA) plate at 20°C in

Table 1. Morphological characters of the genus *Kretzschmaria* [1, 6, 7]

Scientific name	Perithecia (mm)	Ostioles	Asci (µm)	Ascospores (µm)	Apical rings (µm)
<i>Kretzschmaria cetrarioides</i>	0.8~1.2 × 1~1.5	Conical to conic-papillate	170~210 × 10~14	26~33 × 8.5~11	6~7 × 4.5
<i>K. clavus</i>	0.5~1 × 1~1.5	Finely papillate	270~310 × 9~12	27~36 × 7~10	5~7 × 3.5~4.5
<i>K. curvirima</i>	1~1.7 × 1.5~2	Sharply conical	Fragmentary	42~52 × 13.5~16	13~15 × 8
<i>K. deusta</i>	0.7~1.5 × 1~2	Papillate to coarsely papillate	410~480 × 10~14	27~35 × 7~9	4~6 × 3~4
<i>K. eriodendri</i>	0.8~1 × 1.5~2	Conic-papillate	Info none	50~67.5 × 18~22	Info none
<i>K. guyanensis</i>	0.8~1.2 (diam only)	Coarsely papillate to conical	260~310 × 16~18	50~65 × 14.5~17	15~16 × 9~10
<i>K. lucidula</i>	0.5~0.9 (diam only)	Finely papillate	230~270 × 9~12	27~36 × 7~9.5	5.5~7 × 3~4
<i>K. macrosperma</i>	2.5~4 × 2.5~5.5	Obtusely conical	Fragmentary	45~67 × 12.5~17.5	9~12 × 6~8
<i>K. megalospora</i>	1.2~2.5 × 2~3	Obtusely conical	Fragmentary	70~95 × 27~45	10~12 × 11~13
<i>K. micropus</i>	0.7~1.8 × 1.2~2	Sharply conical	Fragmentary	30~40 × 8~12	7~8 × 5
<i>K. milleri</i>	0.5~1.5 × 1.5~3.5	Coarsely papillate to somewhat conical	Fragmentary	40~50 × 11~15	11~16 × 7~8.5
<i>K. neocaledonica</i>	0.7~1.2 × 1.7~2.5	Conspicuously conical	Fragmentary	28~37 × 6.5~8	4.5~5.5 × 3~3.5
<i>K. pavimentosa</i>	0.5~1.2 × 0.8~2	Papillate	290~370 × 11~15	35~54 × 7.5~11	6~9 × 4.5~6
<i>K. sandvicensis</i>	(= <i>K. deusta</i>)	Finely papillate to papillate	290~340 × 11~15	33~46 × 8.5~11.5	6~10 × 3.5~5.5
<i>K. zelandica</i>	0.4~0.5 (diam only)	Finely papillate	210~230 × 6~7	12~15 × 4.5~6	2 × 2
<i>K. zonata</i>	(= <i>K. deusta</i>)	Papillate to coarsely papillate	(= <i>K. deusta</i>)	21~34 × 8.5~12	Info none
<i>K. varians</i>	Up to 2 (diam only)	Finely papillate	235 × 17	32~40 × 13~16	Up to 15 × 7.5
<i>K. parvistroma</i>	0.8 (diam only)	Finely papillate	218~295 × 11~15	37~38 × 12~13	9 × 5
<i>K. quercicola</i> sp. nov.	0.6~0.8 × 1.2~1.8	Finely papillate to papillate	Fragmentary	35~46 × 10.5~17	6.5~10.5 × 4~5.5

Table 2. Sequences used in this study and their information

Taxa	Specimen voucher	GenBank accession No. ^a		
		β-Tubulin gene	ITS gene	RPB2 gene
<i>Kretzschmaria neocaledonica</i>	HAST 94031003	GQ478213	GU300078	GQ844788
<i>K. lucidula</i>	JDR 112	EF025610	EF026125	GQ844790
<i>K. clavus</i>	JDR 114	EF025611	EF026126	GQ844789
<i>K. pavimentosa</i>	JDR 109	GQ478212	GU300077	GQ844787
<i>K. sandvicensis</i>	JDR 113	GQ478211	GU300076	GQ844786
<i>K. megalospora</i>	JDR 229	EF025609	EF026124	GQ844791
<i>K. guyanensis</i>	HAST 89062903	GQ478214	GU300079	GQ844792
<i>K. deusta</i>	TPML160112-001	KX260113	KX260115	KX260117
<i>K. quercicola</i> sp. nov.	TPML150908-046	KX260112	KX260114	KX260116
<i>Xylaria cranioides</i>	HAST 226	GQ478210	GU300075	GQ844785
<i>X. tuberoides</i>	HAST 475	GQ478209	GU300074	GQ844784
<i>X. hypoxylon</i>	HAST 95082001	GQ487703	GU300095	GQ844811

^aEF, GQ, and GU numbers [11] were obtained from the NCBI GenBank homepage (<https://www.ncbi.nlm.nih.gov/genbank/>) and KX numbers were sequenced in this study.

darkness covering 7.5 cm after a month incubation, seeming never reach the edge of the agar plate even after prolonged incubation times; at first white to gray, velvety, zonate, furrowed, distorted, and tend to separate from the plate, turning blackish from center outwards. Reverse becoming blackish from center. Anamorph not observed on culture.

Habitat: Oak-dominated deciduous forest on *Quercus mongolica* Fisch trunk.

Holotype: Korea, Kangwon Province, Chuncheon-si, Mt. Daeryong, coll. Yun & Lee, 8 Sep 2015, deposited to Korea National Arboretum Herbarium (KA15-0891-1).

Isotype: Korea, Kangwon Province, Chuncheon-si, Mt. Daeryong, coll. Yun & Lee, 8 Sep 2015, deposited to Korea

National Arboretum Herbarium (KA15-0891-2).

Notes: *K. quercicola* sp. nov. and *K. sandvicensis* have similar lengths of ascospore but *K. quercicola* sp. nov. has a much broader width of ascospore (*K. quercicola* sp. nov., 10.5~17 µm; *K. sandvicensis*, 8.5~11.5 µm). Although *K. quercicola* sp. nov. has ascospore size more or less similar to that of *K. milleri* in terms of both length and width, the apical ring size of *K. quercicola* sp. nov. does not overlap with the that of *K. milleri* (*K. quercicola* sp. nov., 6.5~10.5 × 4~5.5 µm; *K. milleri*, 11~16 µm × 7~8.5 µm) and their ostiolar papillae are very different.

Currently, taxonomic studies on *Kretzschmaria* dealing with phylogenetic analysis have not yet been conducted.

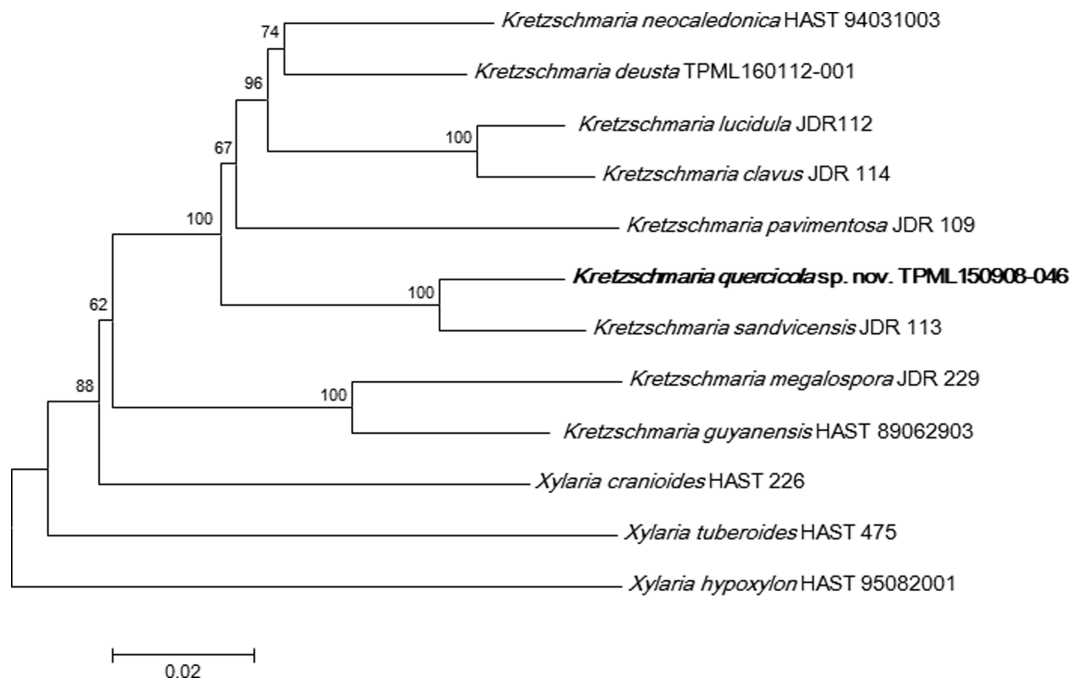


Fig. 2. A phylogenetic tree generated by using the neighbor-joining method for the β -tubulin-ITS-RPB2 dataset. *Xylaria cranioides*, *X. tuberosoides*, and *X. hypoxylon* were used as outgroups. Numbers at the nodes indicate bootstrap values (> 60). Bootstrap analysis was performed with 1,000 replications.

DNA sequences of *Kretzschmaria* that are available online are not from studies focused only on *Kretzschmaria*, but are only part of a study [12] on the overall Xylariaceae family. Hence, we used the sequences from the study of Hsieh *et al.* [12] for our comparisons. Ideally, sequences of *K. milleri*, if available, would provide clearer phylogenetic relationships with our specimen, but morphological characteristics were sufficient to divide the two species of *K. quercicola* sp. nov. and *K. milleri*. According to the study of Rogers and Ju [1], a number of these species seem to be morphologically distinguishable mainly on ascospore size, and the ascospores have ranges of lengths that only slightly overlap. Besides, judging by the fact that apical ring and ostioles were used to distinguish between *K. sandvicensis* and *K. milleri*, they also serve as identification keys [1]. The genus *Kretzschmaria* is not a large family, and has not been studied in detail. Until date, most species have been largely distinguishable based on their microscopic features. Recently, Mugambi *et al.* [7] reported a new species of *Kretzschmaria*, and Recio Herrera and Maldonado Gonzalez [8] revised the genus *Kretzschmaria* in Cuba, based on only morphological characteristics. In short, in taxonomy, data from morphology has been recognized to be the only key of identification. Our study is the first to use genetic information of *Kretzschmaria*, and we believe it should be further utilized in taxonomic studies of the genus.

ACKNOWLEDGEMENTS

This study was supported by the Basic Science Research

Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (NRF-2014R1A1A2009008), the Korea National Arboretum (Project No. KNA 1-1-10, 12-3) and 2013 Research Grant from Kangwon National University (No. 120131830).

REFERENCES

1. Rogers JD, Ju YM. The genus *Kretzschmaria*. Mycotaxon 1998;68:345-93.
2. Boddy L. Fungal community ecology and wood decomposition processes in angiosperms: from standing tree to complete decay of coarse woody debris. Ecol Bull 2001;49:43-56.
3. White NA. The importance of wood-decay fungi in forest ecosystems. In: Arora DK, editor. Fungal biotechnology in agriculture, food and environmental applications. New York: Marcel Dekker; 2003. p. 375-92.
4. Peláez F, González V, Platas G, Sánchez-Ballesteros J, Rubio V. Molecular phylogenetic studies within the Xylariaceae based on ribosomal DNA sequences. Fungal Divers 2008;31:111-34.
5. Tang AM, Jeewon R, Hyde KD. A re-evaluation of the evolutionary relationships within the Xylariaceae based on ribosomal and protein-coding gene sequences. Fungal Divers 2009;34:127-55.
6. Rogers JD, Ju YM. *Kretzschmaria varians* sp. nov., *Xylaria coremifera* sp. nov. and *Xylaria umbonata* sp. nov. from Costa Rica. Mycol Prog 2004;3:37-40.
7. Mugambi GK, Huhndorf SM, Rogers JD. Two new xylariaceous species from Kenya. Mycotaxon 2009;108:499-504.
8. Recio Herrera GM, Maldonado González SG. A revision of the genus *Kretzschmaria* (Ascomycota, Xylariaceae) in Cuba.

- Willdenowia 2014;44:57-64.
9. O'Donnell K, Cigelnik E. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Mol Phylogenet Evol* 1997;7: 103-16.
 10. White TJ, Bruns T, Lee S, Taylor J. 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*. San Diego (CA): Academic Press; 1990. p. 315-22.
 11. Liu YJ, Whelen S, Hall BD. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Mol Biol Evol* 1999;16:1799-808.
 12. Hsieh HM, Lin CR, Fang MJ, Rogers JD, Fournier J, Lechat C, Ju YM. Phylogenetic status of *Xylaria* subgenus *Pseudoxylaria* among taxa of the subfamily Xylarioideae (Xylariaceae) and phylogeny of the taxa involved in the subfamily. *Mol Phylogenet Evol* 2010;54:957-69.
 13. Kim DH, Kim SH, Kwon SW, Lee JK, Hong SB. *Aspergillus cumulatus* sp. nov., from rice straw and air for *Meju* fermentation. *J Microbiol Biotechnol* 2014;24:334-6.