Phytotaxa 175 (4): 216–224
www.mapress.com/phytotaxa/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

http://dx.doi.org/10.11646/phytotaxa.175.4.3

Poculum pseudosydowianum, sp. nov. (Rutstroemiaceae, Ascomycota) from Japan and its endophytic occurrence

TSUYOSHI HOSOYA[1*], YAN-JIE ZHAO[2], YOUSUKE DEGAWA[3]
[1] *Department of Botany, National Museum of Nature and Science, 4-1-1 Amakubo, Tsukuba, Ibaraki 305-0005, Japan*
[2] *Faculty of Life and Environmental Science, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 386-2204, Japan*
[3] *Sugadaira Montane Research Center, University of Tsukuba, Sugadaira, Ueda, Nagano 386-2204, Japan*
** corresponding author: hosoya@kahaku.go.jp*

Abstract

Sequences of several isolates obtained from fresh leaves of *Quercus crispula* in Japan showed high similarity with those of *Poculum sydowianum* in NCBI. Apothecia of *P. sydowianum*-like fungus were found from *Q. cripula* leaves as a result of field survey. A detailed morphological examination elucidated the difference between *P. sydowianum* and this fungus, including the remarkable pigmentation prior to the ascospore germination. Sequences obtained from isolates from *Q. crispula* leaves, those from ascosporous isolates, and those from isolates of *P. sydowianum* preserved in CBS was compared. The ITS sequences were almost identical within the Japanese isolates and European isolates, respectively, but differed up to 10% between the groups, supporting that they belong to different species. A new species *P. pseudosydowianum* is described.

Key words: Endophyte, Foliicolous, Helotiales, Sequence, Taxonomy

Introduction

During the study of endophytic fungi in *Quercus crispula* in Japan, four isolates obtained from fresh leaves were tentatively referred to *Poculum sydowianum,* hitherto unreported fungus in Japan as a result of Basic Local Alignment Search Tool (BLAST) search. This discovery motivated us to find the apothecia of the corresponding fungus from the field. As a result of field survey, we successfully obtained the fungus from the field in wide areas in Japan. The fungus resembled in fact *P. sydowianum,* but differed in sequences from those previously obtained for *P. sydowianum* to some extent. Here, we provide the detailed description of this fungus as a new species.

Materials and methods

Isolation

For endophytic isolates, fresh leaves of *Quercus crispula* was collected in September, 2009 in Obora, Ueda-shi, Nagano Pref., central Japan from the forest dominated by *Fagus crenata*. Fresh leaves were surface sterilized by immersion in 70% EtOH for 1min, followed by immersion in 1% sodium hypochlorite solution (Wako Pure Chemical Industries, Osaka) for 2 min, washed in water for several minutes, then dried overnight. Several pieces of leaves,1 cm square, were obtained from the leaves. The leaf pieces were inoculated onto potato dextrose agar (PDA, Nissui, Tokyo) and some 50 isolates were obtained from 75 leaf pieces obtained from 15 leaves.

Collection of apothecia from the field, isolation, and observation techniques followed Hosoya and Otani (1997). Description of the color codes followed the Pantone color code adopting CYMK system referring to a Pantone color bridge (Anonymous, 2005). Isolates used in the present study was deposited to Biological Resource Center, National Institute of Technology and Evaluation (NITE-BRC).

Isolates of *Poculum sydowianum* (Rehm) Dumont CBS 115928 and CBS 115975 were purchased from CBS-KNAW Fungal Biodiversity Centre.

 Accepted by Kevin Hyde: 3 May 2014; published: 13 Aug. 2014

Endophytic occurrence

Endophytic occurrence of fungi previously known as saprophytes are known in many examples (e.g. He *et al.* 2012; Osono *et al.*, 2013), but examples of Helotiales are still not infrequent in aerial part of trees. Recent discovery reports frequent occurrence of helotialean fungi in roots as root endophytes (Toju *et al.* 2013). The present study clearly showed the endophytic occurrences of saprophytic or parasitic fungus, and suggested continuous roles of fungi from symbiosis to saprophytism. This is also a remarkable case that discovery of endophytic occurrences of fungi motivated the discovery of the field survey of fruiting bodies. Molecular detection and barcoding is the powerful tool in ecological bioprospecting.

References

Anon. (2005) Pantone color bridge/coated. Pantone Inc, New Jersey.

Buchwald, N.F. (1947) Sclerotiniaceae Danicae. *Friesia* 3: 235–330.

Dennis, R.W.G. (1956) A revision of the British Helotiaceae in the herbarium of the Royal Botanic Gardens, Kew, with notes on related European species. *Mycological Papers* 62: 1–216.

Dennis, R.W.G. (1980) New or critical fungi from the Highlands and Islands. *Kew Bulletin* 35: 343–361. http://dx.doi.org/10.2307/4114582

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95–98.

Harrington, F.A. (1997) New species of *Sarcoscypha* (Sarcoscyphaceae, Pezizales). *Harvard Papers in Botany* 10: 53–64.

He, X., Han, G., Lin, Y., Tian, X., Xiang, C., Tian, Q., Wang, F. & He, Z. (2012) Diversity and decomposition potential of endophytes in leaves of a Cinnamomum camphora plantation in China. *Ecological Research* 27: 273–284. http://dx.doi.org/10.1007/s11284-011-0898-0

Hosoya, T. & Otani, Y. (1997) Hyaloscyphaceae in Japan (1): Non-glassy-haired members of the tribe Hyaloscypheae. *Mycoscience* 38:171–186. http://dx.doi.org/10.1007/bf02460853

Hosoya, T., Sasagawa, R., Hosaka, K., Hirayama, Y., Yamaguchi, K., Toyama, K. & Kakishima, M. (2010) Molecular phylogenetic studies of *Lachnum* and its allies based on the Japanese material. *Mycoscience* 51:170–181. http://dx.doi.org/10.1007/s10267-009-0023-1

Huhtinen, S. (1983) Finnish records of discomycetes: *Pseudorhizina sphaerospora* and *Poculum sydowianum*. *Karstenia* 23: 10–12.

Kohn, L.M. (1982) A prelminary discomycete flora of Macaronesia: Part 5, Sclerotiniaceae. *Mycotaxon* 16: 1–34.

Nilsson, R.H., Kristiansson, E., Ryberg, M., Hallenberg, N., Larsson, K.-H. (2008) Intraspecific ITS variability in the kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification. *Evolutionary Bioinformatics* 4:193–201.

Osono, T., Tateno, O., Masuya, H. (2013) Diversity and ubiquity of xylariaceous endophytes in live and dead leaves of temperate forest trees. *Mycoscience* 54: 54–61. http://dx.doi.org/10.1016/j.myc.2012.08.003

Queloz, V., Grünig, C.R, Berndt, R., Kowalski, T., Sieber, T.N. & Holdenrieder, O. (2011) Cryptic speciation in *Hymenoscyphus albidus*. *Forest Pathology* 41: 133–142. http://dx.doi.org/10.1111/j.1439-0329.2010.00645.x

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W. & Fungal Barcoding Consortium (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences of the United States of America* 109: 6241–6246. http://dx.doi.org/10.1073/pnas.1117018109

Spooner, B.M. & Dennis, R.W.G. (1985) New or interesting ascomycetes from the Highlands and Islands. *Sydowia, Annales Mycologici Series II.* 38: 294–316.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731–2739. http://dx.doi.org/10.1093/molbev/msr121

Toju, H., Yamamoto, S., Sato, H., Tanabe, A.S., Gilbert, G.S. & Kadowaki, K. (2013) Community composition of root-associated fungi in a *Quercus*-dominated temperate forest: "codominance" of mycorrhizal and root-endophytic fungi. *Ecology and Evolution* 3: 1281–1293.

http://dx.doi.org/10.1002/ece3.546

White, W. (1941) A monograph of the genus *Rutstroemia* (Discomycetes). Lloydia 4: 153–240.

White, T.J., Bruns, T., Lee, S., Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In:* Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.) *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, pp 315–322.

Zheng, H-D., Zhuang, W-Y. (2013) *Hymenoscyphus albidoides* sp. nov. and *H.pseudoalbidus* from China. *Mycological Progress* http://dx.doi.org/10.1007/s11557-013-0945-z

Zhao, Y.-j., Hosoya, T., Baral, H.O., Hosaka, K. & Kakishima, M. (2012) [2013] *Hymenoscyphus pseudoalbidus*, the correct name for *Lambertella albida* reported from Japan. *Mycotaxon* 122: 25–41.
http://dx.doi.org/10.5248/122.25